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Preface

The quest to preserve and extend the consumable longevity of foods derived from meat, poultry or seafood is one which is pursued with as much enthusiasm and energy today as it has been over the last several hundred years. While many approaches have been used to accomplish these objectives, packaging is paramount among them. In fact, most of the approaches used to bring about muscle food preservation are quite limited in the absence of utilizing suitable packaging technologies. This is particularly true when considering the challenges presented by the modern-day retailing of such food products.

Food chain distribution systems designed for the commercial movement of food products from the point of manufacture through to their retail display and sale are both complex and demanding. This equally applies to all meat, poultry and seafood products.

Muscle-based products that appear on supermarket shelves today may have their origins many thousands of miles away from where they are being sold, they may have been processed either minimally or fully to meet market demands, they may be required to meet specialized storage conditions and deliver upon expected shelf life and they will need to meet all of the expectations that markets specify in terms of addressing food safety and traceability issues. Modern-day consumers, of course, understand much of this and take it as a given that when purchasing muscle-based food products in their local supermarket that they are buying quality, safety and stability as integral product components, but consumers will have their own specific product demands which will be comprised of issues such as: value-for-money, nutritional requirements, information and convenience. Consequently, packaging plays the pivotal role in coping with all of these situations and demands.

Many packaging systems currently exist for use with muscle-based food products, each one with its own unique attributes and potential for application, from short-term storage (about one week) employing overwrapping, to longer-term

modified atmosphere packaging (MAP) storage (about two to six weeks), to very long-term storage (weeks to months) using a host of approaches to providing gasless packaging systems often employing vacuum to do so. These packaging systems are usually employed singly, but can be combined in different ways (like overwrapped products being held under bulk gas flushed conditions, a commercial approach called mother packing). While the formats described above might suggest regimented and set approaches to packaging muscle food products in centralized meat, poultry and seafood packaging plants, nothing could be further from the truth. The packaging of muscle-based food products is a dynamic process which is constantly evolving as we learn more about the product–package interaction.

The product–package interaction is quite complex and affected by numerous factors. On the muscle-based food product side of the interaction, factors such as pre- and post-slaughter factors (from farming or catching through to chilling following slaughter), further processing (from reforming or restructuring of muscle foods with ingredient manipulation through to cooking or the use of novel processing technologies) and final product composition all play a role in creating a unique set of challenges which the ultimate packaging system will have to contend with. Additionally, from the package side of the interaction, factors such as alterations in packaging materials and constructions, developments in material sciences, selection and use of gas mixes, compatibility of packaging materials with muscle food production processes, capacity to deliver safety, quality and shelf-life throughout the chill chain, utilization of smart packaging technologies, environmental concerns and issues pertaining to sustainability, evolving legal issues, unit costs and market demands and compatibility with consumer aspirations and expectations equally present their own difficulties.

The packaging of muscle-based foods is a dynamic process and it needs to be in order to meet the challenges of our various global food markets. It is for this reason that it was decided upon to develop this publication. All of the issues highlighted above, as part of the product–package interaction, have been raised as issues within this book in one way or another. The information gathered and the case studies presented by each of the contributing authors will adequately highlight the progress that we have made in the area of muscle food packaging over a great many years, will show the current status of muscle food packaging and the developments being made in packaging technologies presently and will illuminate the passages within which we must travel to address the packaging issues that may confront us within the packaging arena tomorrow.

J. P. Kerry

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Major microbiological hazards associated with packaged fresh and processed meat and poultry

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Abstract: This chapter discusses major microbiological hazards associated with packaged fresh and processed meat and poultry, including survival and growth of microorganisms in meat and poultry products, as well as the role of product composition and intrinsic and extrinsic factors (water activity, pH, oxidation-reduction potential, atmosphere, temperature, etc.) affecting microbial growth in these products. Additional information addresses foodborne outbreaks related to packaged fresh and further processed meat and poultry caused by pathogenic *E. coli*, *Campylobacter* spp., *Listeria monocytogenes*, *Salmonella* spp., *Yersinia* spp., *Clostridium* spp., *Staphylococcus aureus* and *Aeromonas* spp. and novel packaging techniques that may be employed to control these pathogens.

Key words: foodborne pathogens, pathogenic *E. coli, Campylobacter* spp., *Listeria monocytogenes*, *Salmonella* spp., *Yersinia* spp., *Clostridium* spp., *Staphylococcus aureus*, *Aeromonas* spp.

1.1 Introduction: survival and growth of microorganisms in meat and poultry products

The many properties of meat and poultry, including intrinsic, as well as extrinsic variables, will determine the survivability of pathogenic and spoilage microorganisms. Microorganisms require plentiful sources of water; adequate carbon sources (sugars, alcohols) for energy, nitrogen (amino acids), B vitamins, related growth factors and various minerals, in order to survive. Other factors, such as water activity (a_w) and oxidation-reduction potential (ORP) of meat and poultry

products can influence significantly the growth and proliferation of organisms. Additionally, extrinsic parameters of meat and poultry, including pH, relative humidity, temperature and gaseous atmosphere affect microbial growth as well as survivability (Cutter, 2002).

1.1.1 Product composition

The composition of meat and poultry products can affect the growth of pathogenic bacteria. Generally speaking, fresh meat or poultry that has undergone rigor is composed of macromolecules such as protein, fat, as well as trace minerals. Compositionally speaking, most meat and poultry is made up of 18% protein, on average, but can range from 12% to 20%, depending upon the type of meat or animal source. Meat products are typically low in carbohydrates (0–6%), except those that have been supplemented for fermentation purposes, for flavor or purely for the creation of specific appearances. For fat content, meat and meat products average 3% fat (w/w), but fat levels can vary widely and can range from 3% to 45% (Jay *et al*., 2005), depending upon the product and how it is processed. Low molecular weight soluble components, including creatine phosphate to glycogen to amino acids and dipeptides, minerals and vitamins, also contribute to the composition of these food products (~3.5%; Jay *et al*., 2005). Additionally, the presence of water in muscle tissue also affords microorganisms with another necessary component to support microbial growth. In fact, muscle is composed of approximately 75.5% water, but again, levels can range from 42% to 80% (Jay *et al*., 2005).

As described above, water is an important component of meat and poultry and, consequently, its presence supports microbial growth. Moisture content, or water activity (a_w), are terms used interchangeably when describing the amount of water in a food product or system. However, a_w is defined as 'the energy status of the water in the system. It is equal to the relative humidity of the air in equilibrium with a sample in a sealed chamber. It is defined as the vapor pressure of water in a sample divided by the vapor pressure of pure water held at the sample temperature … therefore, water activity and moisture content together provide a complete moisture analysis' (Decagon, 2010). a_w measurements range from 0 to 1.0, with moisture-laden products having an a_w of 0.90 or greater, while products with an a_w of < 0.50 are intermediate moisture products, typically described as dry, and are relatively shelf stable.

From a microbiological perspective, the a_w of meat and poultry is an important intrinsic property that will influence the growth of pathogenic microorganisms. Fresh meats and poultry typically exhibit a_w values of > 0.95 (Jay *et al*., 2005). Processing can also influence significantly the ultimate a_w, depending upon the type of parameters (heating, cooling, drying, etc.) or compounds (marinades, salt concentrations, carbohydrates, etc.) employed.

Various microorganisms have varying a_w requirements. For example, Gram-negative organisms (such as *E. coli* O157:H7, *Salmonella* spp., *Campylobacter* spp., etc.) have a minimum a_w requirement of 0.96 to 0.93 for growth, whereas Gram-positive, non-spore-formers (*Listeria monocytogenes*, *Staphylococcus*

aureus, etc.) can grow to a lower a_w of 0.90 to 0.94 (Farkas, 1997). By lowering the a_w of a muscle food, one can increase the lag phase of bacterial growth and, ultimately, decrease the growth rate (Farkas, 1997). Additionally, factors such as pH, temperature, nutrient content, presence of antimicrobials or oxidation-reduction potential, work synergistically with a_w (Jay *et al*., 2005). For example, when stored at a specific temperature, the ability of microbes to grow on meat and poultry is reduced as the a_w is lowered. Similarly, addition of salts or solutes in a marinade to a muscle food and storage under refrigeration will hamper the ability of the pathogenic organism to grow. The difference in a_w limits for microbial growth may be reflected in osomoregulatory capacities since mechanisms of tolerance to low a_w are different in bacteria and fungi (Farkas, 1997). The strategy employed by microorganisms to protect against osmotic stress under extreme conditions of low a_w appears to be the intracellular accumulation of salts, polyols, amino acids or compatible solutes such as potassium ions or amino acids in bacteria (Cutter, 2002; Farkas, 1997; Jay *et al*., 2005). In conjunction with a_w, the relative humidity of the storage environment is also important in determining the growth of microorganisms in foods (Jay *et al*., 2005). Careful consideration should be given when storing low a_w foods in environments where the relative humidity is high since moisture will transfer from the environment to the food. The change in the a_w of the muscle food has the potential to affect the growth of microbes. Conversely, high a_w foods held in packaged environments with low relative humidity tend to lose moisture in the transfer of moisture from the food to the environment. In this case, microbial growth may be slowed by the loss of available water, but undesirable quality changes in the food may occur. It is possible that by altering the gaseous environment, microbial growth can be minimized without lowering the relative humidity (Jay *et al*., 2005).

1.1.2 Storage temperature and oxidation-reduction potential

Another property that influences the growth of microorganisms is storage temperature. While microorganisms grow over a wide range of temperatures, the general temperature ranges for microbial growth are: psychrophiles (−15°C to 20°C with an optimum of 10°C); psychrotrophs (−5°C to 35°C with optimum of 20°C to 30°C); mesophiles (10°C to 35°C with optimum of 30°C to 40°C); and thermophiles (40°C to 90°C with optimum of 55°C to 65°C) (Cousin and Rodriguez, 1987). Above the optimal growth temperature, the growth rates decrease precipitously; below the optimum, growth rates also decrease, but do so gradually (Montville and Matthews, 2008).

The influence of temperature on microbial growth and physiology is obvious; yet the influence of temperature on gene expression is equally important (Montville and Matthews, 2008). For example, psychrophilic organisms not only grow slower under refrigerated conditions, but they also express different genes and are physiologically different than mesophilic organisms (Montville and Matthews, 2008). Temperature also influences the expression of other genes and their representative proteins, such as toxin production in *Yersinia enterocolitica*,

internalin production by *L. monocytogenes*, or production of heat shock proteins involved in thermal resistance in *Escherichia coli* O157:H7 (Montville and Matthews, 2008).

The oxidation-reduction potential (ORP) of a substrate may be defined as how easily a substrate loses or gains electrons (Jay *et al*., 2005). When a substance is oxidized, it loses electrons and these electrons must be accepted by another substance, which then becomes reduced (Jay *et al*., 2005). Aerobic microorganisms require positive ORP values (i.e., oxidized) and therefore, can lower the ORP of their environment. Anaerobes require negative ORP values (i.e., reduced) and therefore, cannot lower the ORP of their environment (Jay *et al*., 2005). The ORP of a muscle food is determined by the resistance to change the potential of the food, the oxygen tension of the atmosphere around the food, and the access which the atmosphere has to the food (Jay *et al*., 2005). Substances, such as sulfide groups, help in maintaining reduced conditions in foods (Jay *et al*., 2005). Thus, it is the presence or absence of appropriate quantities of oxidizing and reducing compounds in muscle foods that are important to the growth and activity of microorganisms (Cutter, 2002; Jay *et al*., 2005).

1.1.3 Atmospheric composition

Perhaps the major technical role played by packaging during containment of meat and poultry is its influence on the water vapor, gas composition and partial pressure in the headspace or atmosphere of the packaged food (Cutter, 2002). Oxygen (O_2), carbon dioxide (CO_2) and nitrogen (N_2) are the three gases used primarily in commercial modified atmosphere packaging (MAP) of muscle-based products. Oxygen generally stimulates the growth of aerobic bacteria, while inhibiting strictly anaerobic bacteria. Oxygen also is necessary to maintain the bright red color of fresh red meat, but, concurrently, it will contribute to lipid oxidation. Nitrogen is an inert, tasteless gas that exhibits low solubility in water and lipids. It is used to replace O_2 in some packaging regimens in order to delay oxidative rancidity and inhibit the proliferation of aerobic microorganisms. Additionally, nitrogen is used as a filler-gas to prevent collapse of packaging (Davies, 1995; Stiles, 1991). Carbon dioxide, which is both a water- and lipid-soluble gas, primarily exerts bacteriostatic effects on selective groups of microorganisms in modified atmospheres. The presence of CO_2 not only prolongs the lag phase of bacterial growth, but also decreases the growth rate during the logarithmic phase (Davies, 1995). Any bacteriostatic effect exerted by carbon dioxide is determined by gas concentration used, gas to product ratio, age and load of the initial bacterial population, temperature and composition of the muscle food product. The modes of CO_2 action on certain bacteria may include: an alteration of the cell membrane function such that nutrients and absorption are affected; inhibition or decrease in enzymatic reactions; intracellular pH changes and/or changes in the physicochemical properties of proteins (Farber, 1991). Under some packaging conditions, carbonic acid may be formed when CO_2 interacts with water, lowering the pH and inhibiting or interfering with microbial growth on the surface of

muscle foods (Gill, 1986). Another gas, carbon monoxide (CO), has been used to preserve muscle foods and to overcome color deterioration of packaged meats (Gill, 1986). However, carbon monoxide has a limited effect on spoilage microorganisms (Gill, 1986; Stiles, 1991).

1.2 Vacuum packaging (VP) and modified atmosphere packaging (MAP) to control microbial populations associated with meat and poultry products

When discussing gas and/or surrounding atmospheres and the effect on microbial populations associated with muscle foods, it is important to include some information about vacuum packaging (VP) and MAP. VP is accomplished by evacuating the air from within a package and ensuring that it continues not to possess an atmosphere prior to heat-sealing (Brody, 1989; Cutter, 2002; Davies, 1995). In the process of VP, a pressure differential exists between the package exterior and interior. This pressure differential can cause package collapse in some rigid packages, but may be very well suited for some types of flexible packaging (Brody, 1989). The gaseous atmosphere surrounding the meat or poultry product is likely to change during storage due to respiration of the muscle food itself or via the metabolism of microorganisms found on the food surface (Davies, 1995). Conversely, modified atmospheres are generated by the initial alteration of the gaseous environment in the immediate vicinity of the muscle food. The gaseous environment within MAP is altered in order to slow down the respiration rate of the muscle foods as well as microbial growth, and to reduce enzymatic degradation resulting in an extension in the shelf life of the food (Cutter, 2002; Stöllman *et al*., 1994). Because fresh foods may be naturally respiring or contain microorganisms that respire, O_2 is consumed and CO_2 and water vapor are produced, reaching a steady-state composition within the package (Brody, 1989; Ooraikul, 1991). Depending upon the type of packaging material(s) used, the package may also transmit oxygen, carbon dioxide and water vapor, resulting in changes to the gaseous environment surrounding the product. VP can be considered a variation of MAP in that the removal of air is an atmospheric modification and not carried out specifically by the introduction of gases (Brody, 1989; Cutter, 2002).

The absence or reduction of oxygen in VP foods may permit conditions suitable for the growth and toxin production by anaerobic pathogens such as *Clostridium botulinum* (Brody, 1989). Additionally, the suppression of aerobic spoilage organisms may create conditions favorable for the growth of pathogenic aerobic bacteria such as *L. monocytogenes*, *Yersinia enterocolitica*, *Aeromonas hydrophila* and enterotoxigenic *Escherichia coli* (Brody, 1989). While the presence of CO_2 in VP products inhibits the growth of some Gram-negative spoilage organisms, lactic acid spoilage bacteria are less affected by elevated levels of CO_2 and grow well. Based on this information, VP and the atmosphere it creates in and around muscle foods may selectively favor the growth of obligate and facultative anaerobic

pathogens (Cutter, 2002). As mentioned previously, several factors are known to influence the antimicrobial effect of CO_2 in MAP. Specifically, the initial microbial load, concentration of gas, temperature and film permeability affect microbial growth (Cutter, 2002; Ooraikul, 1991; Stiles, 1991). MAP can be inhibitory to some microorganisms, including a number of Gram-negative organisms; yet, Gram-positive organisms can grow slowly under modified atmospheres (Brody, 1989; Farkas, 1997; Ooraikul, 1991) Additionally, since O_2 is removed and replaced by other gases in MAP, there is the potential for outgrowth of pathogens such as *L. monocytogenes*, *Bacillus cereus* and *Clostridium botulinum* (Farkas, 1997). Furthermore, the effect of VP and MAP against microorganisms associated with fresh or further processed meat and poultry decreases as the temperature increases. Therefore, elevated temperatures that may be seen with temperature abuse throughout storage, transport, or distribution could permit the growth of once-inhibited microorganisms and create food safety issues (Brody, 1989). Despite these limitations, it has been proposed that the risks of foodborne pathogens associated with modified atmospheres are no greater, and are frequently less than, those from aerobically stored foods (Cutter, 2002; Davies, 1995).

It has been well established that most microorganisms grow at pH values around 7.0, with some molds growing between pH 0 and 11; bacteria growing between pH 3 and 11; and yeast growing between pH 1.5 and 8.50 (Jay *et al*., 2005). In some instances, pH fluctuations on meat and poultry occur as a result of metabolites (e.g., organic acids) produced by the microbes associated with the product over time (Genigeorgis, 1985). In other instances, pH changes can occur due to the amount of gas and/or atmosphere applied. In cases of MAP in which carbon dioxide is used, the conversion of carbon dioxide to carbonic acid on the surface can result in a pH drop that has the potential to affect microbial growth (Genigeorgis, 1985). Along these lines, high levels of carbon dioxide in a packaged product could result in higher levels of carbonic acid on the surface, resulting in lower microbial levels. When microorganisms encounter environments exhibiting pH values above or below their optimal pH, they must adjust by expelling or importing hydrogen ions in an effort to maintain an internal pH near neutrality (Jay *et al*., 2005). Adverse pH conditions also may affect respiring microorganisms by denaturing DNA, altering cellular enzymes or disrupting the transport of nutrients into the cell (Jay *et al*., 2005). Ultimately, these changes can result in cellular death.

1.3 Notable foodborne outbreaks related to packaged fresh and further processed meat and poultry

In addition to the effects of intrinsic and extrinsic factors, as well as packaging materials, on microbial growth, it is important to understand the biological hazards associated with the presence of pathogens which are specifically involved with, and relate to, muscle foods: their properties, mechanisms of infection, incidents of foodborne illness and potential control measures. Of these pathogens,

Salmonella spp., *Campylobacter* spp., *L. monocytogenes* and Shiga-toxin producing *E. coli* are considered the most important bacteria with regard to public health. Additional concerns associated with *Yersinia* spp., *S. aureus*, *C. botulinum*, *C. perfringens*, *B. cereus*, *Aeromonas* spp. and *Shigella* spp. also will be discussed. Some notable foodborne outbreaks, packaging type (if known) and other outbreak statistics related to fresh and further processed meat and poultry products (1988–2010) are presented in Table 1.1. Major microbiological hazards associated with packaged fresh and further processed meat and poultry are discussed in the following sections.

1.3.1 *Salmonella* spp.

Salmonella spp. are Gram-negative, small, non-spore-forming, motile and facultative anaerobic rods (Jay *et al*., 2005). *Salmonella* spp. are commonly distributed in nature, with humans and domestic animals being the primary reservoirs of the organism. *Salmonella* spp. are differentiated serologically by their somatic (O) antigen, flagellar (H) antigen or capsular antigen (K) and classified by group A, B, C, D, etc. The most prevalent serotypes are *Salmonella* Enteritidis (SE), *S.* Typhimurium and *S.* Heidelberg, accounting for the majority of human salmonellosis cases. *Salmonella* spp. can grow between 5°C and 47°C, with an optimum temperature of 35–37°C, and can survive in a pH range of 4.5–9.0, with the ideal pH being 6.5–7.5. Most *Salmonella* spp. grow aerobically at a_w of 0.945–0.999 (Jay *et al*., 2005).

Salmonella spp. cause the gastrointestinal disease known as salmonellosis. The minimum infective dose in humans ranges from 10^7 to 10^9 cells/gram of food (Jay *et al*., 2005). The general symptoms of salmonellosis in humans occur within 12–36 h after the bacteria are ingested and may include diarrhea, vomiting, abdominal cramps or fever, generally persisting for one to seven days. The virulence of *S.* Typhimurium in humans is attributed to several mechanisms. Lipopolysaccharide components of the cell wall and endotoxins have been suggested as the cause of fever in salmonellosis (Jay *et al*., 2005). *Salmonella* spp. do not possess enterotoxins, but are capable of penetrating the ileum and the colon of the gastrointestinal tract in humans, causing inflammation. In some instances, the organism may establish a systematic infection following penetration of blood and lymphatic vessels, causing bacteremia in infected individuals (Flowers *et al*., 1988a, 1988b). In some salmonellosis cases, severe dehydration can lead to death of infected individuals. Other symptoms may include muscular weakness, faintness, restlessness and drowsiness (Jay *et al*., 2005). Another clinical manifestation of *Salmonella* spp. infection is enteric fever, more commonly known as typhoid fever, which is caused by *S.* Typhi (Gonzalez-Escobedo *et al*., 2011).

Salmonella spp. have been implicated in meat borne illnesses for several decades. *Salmonellosis* represents a serious problem worldwide, with billions of dollars spent each year due to the illness, including on: lost wages, medical expenses, lawsuits and product recalls (Buzby and Roberts, 2009; Todd, 1989).

Table 1.1 Notable foodborne outbreaks related to packaged fresh and further processed meat and poultry (1988–2010)

Organism responsible for outbreak	Year (month/months) outbreak occurred	Meat and poultry vehicle(s)	Type of product and/or packaging type	Approximate number of confirmed illnesses	Affected country	Number of affected states (USA) or countries	Reference
Salmonella spp.	2007 (Jan–Oct)	Frozen pot pie products with chicken and turkey	RTE	65	USA	35	CDC Database[1]
	2009 (Nov)–2010 (Jan)	Italian sausage, salami	RTE	272	USA	44	CDC Database
	2009 (May–June)	Ground beef	MAP	14	USA	1	CDC Database
	2010 (April)	Chicken and rice; frozen entrée	RTE	44	USA	18	CDC Database
Campylobacter spp.	2005 (June)	Chicken salad	Undercooked chicken	77	Denmark	1	Mazick *et al.*, 2006
Clostridium perfringens	1998 (April)	Beef, brisket; sausage, unspecified	VP and MAP	50	USA	1	FIO Database[2]
Escherichia coli O157:H7	1992 (Nov)–1993 (Feb)	Beef, ground beef	Unknown	500	USA	1	FIO Database
	1998 (April)	Roast beef, sausage, Genoa salami	VP and MAP	39	Canada	1	FIO Database
	1999 (Nov)	Roast beef, sausage, salami	VP and MAP	143	Canada	1	MacDonald *et al.*, 2004
	2000 (July)	Ground beef	Unknown	4	USA	1	FIO Database
	2000 (Nov)	Ground beef	Retail tube/chub packages	46	USA	1	FIO Database
	2002 (June–July)	Ground beef	MAP	35	USA	7	Vogt and Dippold, 2005

2002 (Aug)	Ground beef	MAP/Chub/ Boxed	57	USA	2	FIO Database
2003 (Aug)	Tenderized steak	VP	12	USA	3	Laine *et al*., 2005
2004 (June)	Beef sirloin steaks	MAP/Boxed		USA	1	FIO Database
2005 (April)	Ground beef	MAP	3	USA	1	FIO Database
2007 (March)	Beef: steak, sirloin, beef tips, tenderized beef	Boxed	8	USA	1	
2007 (May)	Beef: tri-tip, bladed, tenderized	Unknown	124	USA	1	FIO Database
2007 (Aug)	Ground beef, hamburger	MAP	12	USA	2	FIO Database
2007 (Aug)	Ground beef, hamburger	Boxed/ Unknown	47	USA	4	FIO Database
2007 (Dec)–2008 (Jan)	Ground beef	Boxed/ Unknown	6	USA	2	FIO Database
2008 (May)	Ground beef	VP/MAP/ Retail Tube	32	USA	13	FIO Database
2008 (May)	Ground beef	VP/MAP/ Retail Tube	35	USA	9	FIO Database
2009 (April)	Ground beef	VP/MAP/ Retail Tube	23	USA	6	FIO Database
2009 (May)	Ground beef	Unknown	6	USA	1	FIO Database
2009 (May)	Luncheon meat: turkey, ham	RTE	42	USA	1	FIO Database
2009 (Sep)	Ground beef, hamburger	MAP/ Chub	26	USA	4	FIO Database
2009 (Oct)	Ground beef	MAP/Boxed	20	USA	1	FIO Database
2010 (Aug)	Beef: tenderloin, chunks, ground	Unknown	5	Canada	1	FIO Database

(*Continued*)

Table 1.1 Continued

Organism responsible for outbreak	Year (month/months) outbreak occurred	Meat and poultry vehicle(s)	Type of product and/or packaging type	Approximate number of confirmed illnesses	Affected country	Number of affected states (USA) or countries	Reference
Escherichia coli O111:NM	1994 (Dec)–1995 (Feb)	Semi-dry, fermented sausage	Unknown	81	South Australia	1	FIO Database
Non-*Escherichia coli* O157:H7 STEC	1999 (Dec)	Ground beef, hamburger	VP/MAP/ Retail Tube	10	USA	1	FIO Database
	2000 (March)	Beef: seemerrolle, bottom round steaks	Unknown	11	Germany	3	FIO Database
Listeria monocytogenes	1988	Luncheon meat, sausage, turkey franks	RTE	1	USA	1	FIO Database
	1998 (Jan)	Sausage, frankfurters, turkey deli meats	VP/MAP -RTE	108	USA	22	FIO Database
	2000 (May)	Turkey luncheon meat, sausage	VP/MAP -RTE	29	USA	11	FIO Database
	2002 (July)	Luncheon meat, sausage	VP/MAP -RTE	54	USA	3	FIO Database
	2005 (July)	Turkey luncheon meat	VP/MAP -RTE	13	USA	Unknown	FIO Database
	2008 (June)	Luncheon meat	VP/MAP -RTE	57	Canada	Unknown	FIO Database
	2009 (May)	Chicken luncheon meat wraps	Boxed luncheons	5	Australia	Unknown	FIO Database

	2010	Luncheon meat, sausage, salami, ham (cooked, cotto)	Unknown	2	Canada	Ontario	FIO Database
Staphylococcus aureus	2008	Ham and pasta dishes	Unknown	11	USA	1	FIO Database
	2010			17	USA	1	

Notes: RTE = ready-to-eat; MAP = modified atmosphere packaged/packaging; VP = vacuum packaged/packaging.
Commercially available RTE products may be purchased in VP from store shelves or obtained sliced to order from the delicatessen and transported home in aerobic packaging materials.
[1] Centers for Disease Control (CDC) and Prevention Database (2010). Available at: http://wwwn.cdc.gov/foodborneoutbreaks/
[2] Foodborne Illness Outbreak (FIO) Database. Available at: http://www.outbreakdatabase.com/

Outbreaks caused by *Salmonella* spp. have been associated with foods derived from animals, including pork, eggs, poultry, low fat and whole milk, raw ground beef, ice cream, cheese, sausages and cured meats (ham, bacon and tongue). In 1994, a meat-related outbreak was linked to the consumption of raw ground beef contaminated with *S.* Typhimurium. This outbreak yielded a total of 158 confirmed cases experiencing symptoms (diarrhea, abdominal pain, fever and nausea) and 17 hospitalizations. The source of contamination was linked to improper sanitation of utensils used to mince meat (Roels *et al*., 1997). In an outbreak involving improperly cooked beef jerky that occurred in 1995, individuals exhibited symptoms of salmonellosis, with 59 of the infected individuals requiring hospitalization. Strains which were isolated from 18 out of 59 patients proved positive for *Salmonella* Agona (Taylor *et al*., 1998).

As few as 100 cells of *Salmonella* spp. can cause illness, while the average infectious dose can be 10^5–10^6 CFU/mL (Jay *et al*., 2005). Strains with a low infective dose may be responsible for most human disease. In fact, a low infective dose is especially problematic in foods that have a high fat content that can buffer bacteria, such as meat and poultry products, since *Salmonella* attached to these foods may be protected during gastric digestion (Humphrey, 2001).

Of the 2300 *Salmonella* serotypes, *S.* Typhimurium and *S.* Enteritidis are believed to cause almost half of the human cases of salmonellosis (CDC, 1998b). From these, *S. Typhimurium* is most frequently isolated from livestock (36.1%; Sarwari *et al*., 2001), as well as from the fecal matter and rumen contents of livestock (Gay *et al*., 1994; McEvoy *et al*., 2003). All livestock may be susceptible to *Salmonella* infection by the fecal-oral route when contaminated feedstuffs, water or pasture are consumed on the farm (Wray and Davies, 2000). Additionally, *Salmonella* is problematic in veal calves. Calves that do not receive adequate colostrum shortly after birth tend to have weaker immune systems. As such, they are more prone to diarrhea caused by *Salmonella* (McDonough *et al*., 1994). Edel *et al*. (1970) isolated 16 different serotypes of *Salmonella* spp. from calves in slaughterhouses; *S.* Typhimurium comprised 52% of the strains that were isolated. *S*. Newport also was isolated from 4% of bob veal carcasses in the northeastern United States, with a total *Salmonella* prevalence determined at nearly 12% in bob veal (Flowers, 2002). *Salmonella* spp. also have been isolated from fresh veal, albeit many years ago. For example, Buchanan and Bevan (1939) reported an outbreak of *S.* Typhimurium in South Africa that was traced to roasted veal. Anderson and others (1961) reported a series of incidents of foodborne salmonellosis as the results of handling and consumption of undercooked veal and calf products in southeast England.

Contamination of meat and poultry carcasses with *Salmonella* occurs via fecal contamination and largely due to the extent of infection in the live animal and the handling of carcasses by slaughter employees (Hulebak and Schlosser, 2002). Despite efforts by the industry to remove *Salmonella* from meat and poultry carcass surfaces during slaughter, small populations of the pathogen may be embedded in tissues and/or crevices or deposited on surfaces during fabrication and further processing. A study of retail meats from grocery stores demonstrated that

Salmonella is present on most poultry, fresh pork and fresh beef (Zhao *et al*., 2001). The incidence of Salmonella ranges from 12% to 91% in fresh and frozen poultry products (Jay *et al.*, 2005). Several comprehensive studies have evaluated the points of entry of Salmonella onto poultry carcasses during grow out and/or slaughter. Adequate control measures such as chlorination, adequate chilling or other antimicrobial applications (trisodium phosphate, acidified sodium chlorite, etc.) can reduce the incidence of the pathogen in poultry carcasses destined for human consumption.

Even though *Salmonella* spp. is not psychrotrophic, it can survive on low temperatures with high levels of CO_2. Poultry inoculated with *S*. Enteritidis have survived at 3°C with VP as well as with three types of MAP (100% CO_2; 100% N_2 or 20% CO_2/80% air). At 10°C, this organism can grow in the presence of 100% N_2, and 20% CO_2/80% air packages as well as in VP (Garcia de Fernando *et al*., 1995; Kakouri and Nychas, 1994; Nychas and Tassou, 1996).

Similarly, ground beef inoculated with *Salmonella* spp. and stored at 10°C with high levels of CO_2 and low CO mixture resulted in increases of 1.5 $\log_{10}$ CFU/g; with high O_2 concentrations, there was no increase (Nissen *et al*., 2000). In another similar study, when *S*. Typhimurium, *S*. Dublin and *S*. Enteritidis were inoculated into ground beef and stored at 10°C for two days with MAP, there was no significant increase of these organisms. In research using high levels of CO_2 or low CO in ground beef chubs, bacterial populations increased, while high O_2 levels resulted in only slight growth of *S*. Enteritidis (Nissen *et al*., 2000). In another study, *S*. Enteritidis and *S.* Typhimurium were unable to grow in VP, cured and cooked meat products without considerable mishandling (Nielsen and Zeuthen, 1984). Ready-to-eat sausage inoculated with the organisms and VP was inhibited during storage at 8°C and 10°C.

Adding lactic acid bacteria (LAB) is another way to prevent *Salmonella* spp. growth in packaged meats. When LAB was added to raw ground beef, growth of *Salmonella* spp. was reduced to nondeductible levels after five days (Smith *et al*., 2005). Combining LAB with high O_2 and MAP also can reduce the growth of *Salmonella* spp. in meat (McCormick *et al*., 2003).

1.3.2 *Campylobacter* spp.

In the early 1900s, *Campylobacter* spp. was considered a veterinary disease and a causative agent of diarrhea in cattle and abortions in cattle and sheep (Moore *et al*., 2005). Originally, *Campylobacter* was classified in the genus *Vibrio* due to its spiral morphology. In 1963, *Campylobacter* became a genus distinct from *Vibrio*. In the last 40 years, *Campylobacter* spp. has gained importance as a human pathogen (Moore *et al*., 2005).

Campylobacter spp. are very small (1.5–5 μm), Gram-negative, oxidase-positive organisms that do not ferment carbohydrates. The organism is a spiral-shaped rod with corkscrew motility due to a single polar flagellum at one or both ends of the cell (Beumer *et al*., 1988). The genus *Campylobacter* is composed of 20 species and subspecies (Nachamkin *et al*., 2000).

Campylobacter spp. can grow between 25°C and 43°C with an optimum temperature of 42°C (Stern *et al*., 2001), under microaerophilic conditions of 5% O_2, 10% CO_2 and 85% N_2 (Stern *et al*., 2001). The organism also can grow over a pH range of 4.9–9.5, with a minimum water a_w of 0.987 (Doyle and Roman, 1982). While *Campylobacter* spp. is known to survive better at refrigeration temperatures (5–7°C), rather than at room temperatures (25–27°C) (Beumer *et al*., 1988), this organism is sensitive to the stresses of chemicals (e.g., chlorine), freezing, drying, heating, high salinity, as well as other environmental conditions (Palumbo, 1984). For example, when exposed to an environment outside of the host animal, the survival and growth of *Campylobacter* spp. can be inhibited by atmospheric oxygen concentrations, competition with other microflora for nutrients and suboptimal temperatures (>30°C; Doyle, 2004). Furthermore, routine sanitation of slaughter equipment and facilities prevents cross-contamination of carcass surfaces with *Campylobacter* spp. (Gill and Harris, 1982a, 1982b). Additionally, *Campylobacter* spp. are not considered 'environmental organisms', but rather, organisms found in the intestinal tract of many warm-blooded mammals and birds, including those animals used for food (Blaser, 1986). The carriage rate varies between 60% and 80% in swine, 30% and 100% in poultry and 40% and 60% in cattle for *C. jejuni* or the closely related species, *C. coli* (Doyle and Roman, 1982). When subjected to stressors mentioned previously, the organism may transform into a spherical or coccoid form, which has proven very difficult to culture. Because of the slow growth and the microaerophilic atmosphere, recovery of this pathogen from food samples requires specialized selective and enrichment procedures (Corry *et al*., 1995).

Campylobacter spp. is acknowledged as the primary cause of acute bacterial gastroenteritis illnesses and considered one of the most common causes of food borne illness (Nachamkin and Yang, 1992; Stern *et al*., 2001; Tauxe *et al*., 1988). Due to the difficulty in isolating these organisms, *Campylobacter* spp. has only been identified recently as a major meat borne pathogen. Many cases of campylobacteriosis have been linked to the cross-contamination of prepared foods with raw or undercooked foods and with infected food handlers (Doyle, 2004). Outbreaks of food borne illness associated with *Campylobacter* spp. are generally associated with drinking raw milk or unchlorinated water, mishandling during preparation of raw poultry or consumption of undercooked poultry or poultry products (Altekruse *et al*., 1999; Gill and Harris, 1982b).

Campylobacter spp. are highly infective, causing the gastrointestinal disease campylobacteriosis. Disease symptoms are typically less severe than illnesses caused by *E. coli* O157:H7 or *Salmonella* spp., although a victim of campylobacteriosis would likely show symptoms that are very similar to salmonellosis (Skirrow and Blaser, 2000). Depending upon the strain, the level of injury caused by environmental stresses to the cells, and the vulnerability of the host, the minimum infective dose may range from 500 to 10 000 cells (Blaser, 1986; Tee *et al*., 1987). The general symptoms of campylobacteriosis in humans usually occur within two to ten days after the ingestion of the bacteria. The symptoms may range from mild gastrointestinal distress to profuse or bloody diarrhea,

abdominal pain, cramps, fever, headache and nausea. In rare instances, meningitis, urinary tract infections, reactive arthritis, Guillain-Barré syndrome (GBS) or Reiter's syndrome, may occur following initial infection. GBS is a neurological disease that can result in neuromuscular paralysis or reactive arthropathy in humans (Stern *et al*., 2001), which can develop within one to three weeks of infection (Nachamkin *et al*., 2000).

The level of incidence of campylobacteriosis (in the United States) has been estimated to be about 21 diagnosed cases per 100 000, resulting in approximately 2.4 million infections each year. Campylobacteriosis also causes an estimated 500 fatalities each year (Samuel *et al*., 2004). Some outbreaks caused by *Campylobacter* spp. have been associated with retail pork chops, ground pork, loin and sausages. Similar contamination found in beef included fresh retail ground beef, with the contaminating organism traced back to a dairy cattle source (Bae *et al*., 2005; Inglis *et al*., 2004; Sato *et al*., 2004). Evidence from a number of sources indicates that poultry also is a frequent cause of sporadic campylobacteriosis (Samuel *et al*., 2004). The prevalence and frequency of distribution of the serogroups found on poultry are often similar to those obtained from infected humans (Samuel *et al*., 2004). The consumption of rare or undercooked poultry has been implicated in a number of small outbreaks (Blaser *et al*., 1984; Istre *et al*., 1984; Rosenfield *et al*., 1985; Skirrow, 1982).

Campylobacter can be isolated from the intestinal tracts of poultry and resulting poultry carcasses. Luangtongkum *et al*. (2006) evaluated the intestinal contents of organic broilers and organic turkeys, as well as from broilers and turkeys raised conventionally. Conventionally produced broilers had a significantly lower prevalence of the pathogen than organic birds, with a 66% and 89% prevalence, respectively. Conventional birds were 97% positive for *C. jejuni* and 3% were positive for *C. coli*. Organic birds were 72% positive for *C. jejuni* and 27% positive for *C. coli* (Luangtongkum *et al*., 2006).

In one study, whole poultry carcasses were rinsed after various types of chilling procedures. Carcasses subjected to: air chilling were 3–12% positive; evaporative chilling were ~8% positive; immersion chilling were 45% positive; a combination of immersion and air chilled carcasses were 25% positive (Lindblad *et al*., 2006). In another study, fresh and frozen retail chicken samples were obtained and evaluated for *Campylobacter* prevalence, which ranged from 70% to 74% for fresh carcasses and significantly decreased to 58.4–72.6% for frozen carcasses, respectively (Meldrum *et al*., 2006).

Campylobacter also has been isolated from lamb intestines at slaughter. After collecting the intestinal contents of 360 lambs from a processing facility in central England, 91.7% of lambs were determined to carry *Campylobacter* in the small intestine (Stanley *et al*., 1998). These authors concluded that the incidence of *Campylobacter* in lambs previously had been underestimated because prior studies usually examined fecal matter from the colon and not from the more proximal sections of the intestinal tract. While lambs may commonly harbor *Campylobacter* in the small intestine, good manufacturing practices and carcass hygiene may be the key to preventing contamination of lamb carcass surfaces.

There is limited information addressing the effects of packaging and/or gas concentrations on *Campylobacter* spp. survival in fresh or further processed meat and poultry. It has been documented that *C. jejuni* and *C. coli* are unable to grow at temperatures below 30°C, but can survive at temperatures greater than 30°C. Under microaerophilic conditions, as may be seen with MAP, the organism can survive on product, even when subjected to packing with ice (Wesley and Stadelman, 1985). In another study, *C. jejuni* associated with turkey rolls and saturated with 100% CO_2 and VP survived to a greater extent than when subjected to MAP with 80% CO_2 20% N_2, 40% CO_2 60% N_2 or 100% N_2 (Phillips, 1996). Finally, survival of *C. jejuni* in ground beef was found to be greater in VP or atmospheres consisting of 100% N_2 than with 5% O_2 10% CO, 85% N_2 or 80% CO_2 20% N_2 (Stern *et al*., 1986).

1.3.3 *Listeria monocytogenes*

Listeria monocytogenes is one of six species in the genus, *Listeria* that includes *L. innocua*, *L. ivanovii*, *L. seeligeri*, *L. welshimeri* and *L. grayi*. *L. monocytogenes* is a Gram-positive rod, non-spore-forming, facultative anaerobic pathogen that is ubiquitous in nature (Rocourt, 1999). *L. monocytogenes* is commonly found as a saprophyte in the environment (water, soil, mud, sewage, etc.), feeding on dead and decaying vegetation. It has been commonly isolated from fecal specimens of healthy animals and man, as well as from sewage, silage, soil, fertilizer and vegetable matter.

Normally non-mobile, *L. monocytogenes* has the ability to express tumbling motility by way of a few flagella when grown between 20°C and 25°C (Seeliger and Jones, 1986). The optimum growth temperature for *L. monocytogenes* is between 30°C and 37°C; however, it may grow and survive between 1°C and 45°C (Ryser and Donnelly, 2001; Seeliger and Jones, 1986). *L. monocytogenes* is also a fairly osmotolerent foodborne pathogen, as it can grow in media containing 10% salt, or survive in up to 20% salt. In addition, *L. monocytogenes* can grow within a pH range of 4.4–9.6 and to a minimum at an a_w of 0.90, depending on the type of solute used (Miller, 1992). Typical colonies appear bluish-gray in color, displaying a blue-green sheen under obliquely transmitted light when plated on nutrient agar (Seeliger and Jones, 1986). As a result of these diverse conditions in which growth and survival can occur, this intracellular pathogen poses serious food safety issues.

As a facultative anaerobe, the growth of *L. monocytogenes* is only slightly affected by a gaseous atmosphere. Similar generation times have been observed under aerobic, microaerophilic and anaerobic conditions with no evidence of an inhibitory effect exerted by high levels of CO_2, except at low temperatures (Ingham *et al*., 1990). The organism has a water activity (a_w) limit of approximately 0.90°C at 30°C when glycerol is present (Farber *et al*., 1992).

L. monocytogenes causes the disease listeriosis. *L. monocytogenes* is an opportunistic pathogen, capable of surviving and multiplying outside animal hosts and in simple nutrient media. The pathogen has a virulence mechanism that consists

of invading the gastrointestinal epithelium and allowing the bacteria to survive and multiply within the macrophages. Once the bacteria enter macrophages, replicated organisms transfer from cell to cell, infecting intestinal tissue and susceptible cells (Tilney *et al*., 1990).

Despite the rarity of listeriosis in the general population, the pathogen has the highest hospitalization (90.5%) and second highest mortality rate (21%) in the United States according to the Food and Drug Administration (FDA; FDA-CFSAN, 2003). Infections of the central nervous system (CNS) resulting in encephalitis or meningitis are the most common manifestations of listeriosis. Symptoms of meningitis (high fever, nausea, neck stiffness and severe headache) commonly appear before symptoms of CNS infection (Farber and Peterkin, 1991). The minimum infective dose ranges from 10^3 to 10^9 CFU, depending on the type of strain and immunological state of the infected individual. The infective dose is lower for immunocompromised individuals, such as infants, pregnant women, cancer patients and those undergoing chemotherapy, autoimmune deficiency syndrome (AIDS) patients, and the elderly (Farber *et al*., 1996; McCarthy, 1991). Listeriosis often is associated with the consumption of high-risk, ready-to-eat (RTE) foods such as sliced delicatessen meat and poultry products, frankfurters, soft cheese and RTE products with extended refrigerator storage (Rocourt, 1999).

Mild symptoms of listeriosis in humans, which usually occur within two to ten days after the bacteria are ingested, include fever, abdominal cramps, diarrhea, vomiting and nausea. In severe cases, the infection can lead to septicemia, meningitis, encephalitis and intrauterine or cervical infections in pregnant woman, resulting in spontaneous abortion. The duration of the above symptoms varies from two to 21 days, depending on the degree of the infection (Anonymous, 2000).

Due to the serious nature of the disease, and the prevalence of the pathogen in RTE foods, regulatory agencies in the United States have issued a zero tolerance policy toward the presence of this organism in foods (USDA-FSIS, 2003, 2006). According to the FDA-CFSAN (2003), 'Other countries … have different policies for dealing with *L. monocytogenes* contamination. Countries such as Canada and Denmark have a "non-zero tolerance" for *L. monocytogenes* for some classes of foods.' For example, in Canada, RTE foods that have not been associated with an outbreak and do not allow any growth of *L. monocytogenes* during a ten-day period of refrigerated storage, may contain up to 100 organisms per gram of food. Denmark has six classes of foods with various criteria for *L. monocytogenes*. In other RTE foods, for example, two of five samples can contain between 10 and 100 organisms per gram, but no sample can exceed 100 organisms per gram. Unfortunately, there is no epidemiological evidence that demonstrates whether a zero or non-zero tolerance policy leads to a greater rate of listeriosis. Estimates of disease rates between different countries must be considered with caution because of different surveillance and reporting systems (FDA-CFSAN, 2003).

Within the last several years, multistate outbreaks of listeriosis have occurred in the United States. In 1998, a four-month multistate outbreak caused by a strain

of *L. monocytogenes* serotype 4b was reported (CDC, 1998a). Forty listeriosis cases, with a 10% mortality rate, occurred with VP hot dogs implicated as the source of infection (CDC, 1998a). Another outbreak of listeriosis occurred in the United States during 2000. Twenty-nine patients were hospitalized with listeriosis symptoms; four of the patients died and three miscarriages/stillbirths occurred. The source of infection was epidemiologically linked to the consumption of deli turkey meat (Wing and Gregory, 2002). The consumption of pork tongue in aspic also was linked to a listeriosis outbreak resulting in 279 confirmed cases with 63 deaths. In each of the cases cited above, the meat was contaminated in the food processing plant after cooking, before packaging and stored at refrigerated temperatures for extended periods of time prior to consumption.

Although the presence of *L. monocytogenes* and other *Listeria* spp. in fresh, uncooked meat and poultry products may be attributed to fecal and/or environmental contamination during slaughter, *L. monocytogenes* can be effectively reduced and eliminated during adequate thermal processing (Jay, 1996; Johnson *et al*., 1990; Rocourt, 1999; Rocourt *et al*., 2003; Swaminathan, 2001). Within the last two decades, the association between *L. monocytogenes* and fully cooked meat products has become very evident. While thermal processing is effective in eliminating this pathogen, the occurrence of *L. monocytogenes* in processed products suggests that post-processing handling is the main source of recontamination (Johnson *et al*., 1990). Grau and Vanderlinde (1992) surveyed 15 retail stores over a 12-month period, sampling various processed VP meat products. A total of 53 and 44.6% of the samples were contaminated with *L. innocua* and *L. monocytogenes*, respectively, with 4% of the samples containing $> 1 \times 10^3$ CFU/g of sample of *Listeria* spp. In addition, *L. monocytogenes* was found in 7.3% of retail frankfurters from 19 different brands (Wang and Muriana, 1994). One particular brand contained *L. monocytogenes* at a 71% prevalence rate. Additional testing was conducted to determine the source of contamination. These results revealed the presence of *L. monocytogenes* in six of nine (66.67%) exudates, but none was found in the internal portions. These findings indicate that contamination was a result of post-process contamination (Wang and Muriana, 1994).

The US Department of Agriculture Food Safety and Inspection Service (USDA-FSIS) conducted a survey of pathogens in RTE meat and poultry products (Levine *et al*., 2001). The survey was limited to approximately 1800 USDA-FSIS inspected facilities, with the prevalence of *L. monocytogenes* ranging from 0.52% in jerky to 5.16% in sliced ham and luncheon meats combined. The higher prevalence rates were seen in those products in which additional handling was needed. In contrast, the National Food Processors Association (NFPA) conducted a survey of 31 500 packaged RTE meat and poultry samples tested from retail stores in Maryland and California and determined that *L. monocytogenes* was present in only 0.89% of sliced luncheon meats (Gombas *et al*., 2003). The majority of the contaminated RTE food samples (92.6%) possessed < 10 CFU/g *L. monocytogenes*. Interestingly, RTE products subjected to in-store handling and packaging, were up to 6.8 times more likely to contain the pathogen, as compared to commercially packaged products (Gombas *et al*., 2003).

When present in packaged RTE meat products, there is the potential for pathogen growth during storage. Glass and Doyle (1989) demonstrated that, depending on the type of meat product (composition, pH, nitrite level, etc.) and initial inoculation levels (2–5 $\log_{10}$ CFU/package), *L. monocytogenes* could remain constant or increase during storage. In fact, growth of the pathogen reached > 10^4 CFU/g within nine weeks of VP, refrigerated storage (4.4°C). The greatest population increases (3–5 $\log_{10}$ CFU/mL of sample) were observed in turkey and chicken products within four weeks of refrigerated storage, regardless of an initial inoculum level (Glass and Doyle, 1989). Another study screened over 32 500 frankfurters for the presence of *L. monocytogenes* following 150 and 30 d VP, refrigerated storage at 4 and 10°C, respectively. These data not only indicated that the presence of *L. monocytogenes* in RTE meat products was dependent on the product (1.65% initial prevalence rate), but it also revealed the possibility for low levels of initial contamination to increase to high levels during refrigeration.

The US FDA Center for Food Safety and Applied Nutrition (FDA-CFSAN), in conjunction with USDA and Centers for Disease Control and Prevention (CDC), conducted a large-scale risk assessment of *L. monocytogenes* in selected RTE foods (FDA-CFSAN, 2003). The risk assessment used scientific data to estimate the relative risk of listeriosis infections in certain RTE foods and three age-based population groups, in addition to the estimated level of exposure to these RTE foods. The risk assessment identified high-risk VP RTE food products, such as deli meats, frankfurters and pâté, as well as unpasteurized fluid milk, that are more commonly associated with *L. monocytogenes* infections. Conversely, low-risk RTE food products such as packaged hard cheeses, cultured milk products, fermented meats, processed cheeses and frozen dairy products were identified.

The impact of atmosphere and packaging on the pathogen survival and/or growth in RTE muscle foods also has been explored. In one study (Manu-Tawiah *et al*., 1993), *L. monocytogenes* was isolated from VP pork and reinoculated onto fresh pork chops. Pathogen survival and growth were determined under different atmospheres at 4°C during 35 days of storage. Atmospheres included 20% CO_2 0% O_2/80% N_2, 40% CO_2 0% O_2/60% N_2 and 40% CO_2 10% O_2/50% N_2, VP and air. When exposed to air, *L. monocytogenes* grew slower than psychrotrophic spoilage bacteria. Under gas atmospheres, *L. monocytogenes* grew even more slowly. When 10% O_2 was included in the 40% CO_2 mixture, growth of the pathogen was reduced significantly. VP was no more effective than gas mixtures in retarding growth of *L. monocytogenes.* MAP provides an environment in the package that would allow minimal growth of the pathogen and potentially compromise the safety of RTE meat products.

The behavior of *L. monocytogenes* associated with VP beef also depends on the type of tissue (lean or adipose), as well as factors such as storage temperature and pH. For example, *L. monocytogenes* was found to grow better on adipose than on lean beef surfaces (Grau and Vanderlinde, 1990) under VP conditions. In this study, the pathogen grew 4 $\log_{10}$ CFU/cm^2 in 16 days on adipose surfaces versus a 3 $\log_{10}$ increase on lean meat during the same time. When evaluated for pH and

VP, the pathogen grew better on lean surfaces with pH 6.0–6.1 than those with pH 5.5–5.7 (Gill and Newton, 1979; Phillips, 1996).

Methods to control *L. monocytogenes* associated with packaged RTE meat products may include, but are not limited to, post-packaging pasteurization, alone or in combination with food grade antimicrobials. Post-packaging pasteurization has been shown to inactivate bacterial populations of *L. monocytogenes* on the surface of RTE meat products (Britton, 2004; McCormick *et al*., 2003). During in-package pasteurization, a RTE meat product is packaged with a heat-stable polyethylene material. The packaged product is submerged into hot water, thereby allowing the packaging material to shrink and conform to the product surface. Extended times in the hot water have been shown to reduce levels of *L. monocytogenes* on surface-inoculated RTE products (Britton, 2004). However, the convoluted surface of the RTE meat product may affect the efficiency of the heat. Therefore, combinations of post-packaging pasteurization (65°C for 30 sec) with pre-surface application of food grade antimicrobials, such as nisin and/or lysozyme, can reduce *L. monocytogenes* immediately following treatment and during long-term refrigerated storage (Mangalassary *et al*., 2008).

1.3.4 Shiga toxin-producing *E. coli*

Escherichia coli are Gram-negative, non-spore-forming, facultative, anaerobic, rod-shaped microorganisms. They are derived from the family of Enterobacteriaceae (Janda and Abbott, 2006). These versatile mesophiles grow optimally at 37°C; however, they may grow and survive temperatures up to 49°C and as low as 7°C, which is an indication that they are well adapted to environmental habitats (Bell, 2002; Fotadar *et al*., 2005; Sousa, 2006). In addition to temperature adaptation, the organisms have the ability to survive a number of other environmental stimuli such as pH (minimum of 4.4 to a maximum of 9.0), osmolarity and various salt concentrations (≤ 6.5%; Bell, 2002; Jay *et al*., 2005; Sousa, 2006).

Three groups of *E. coli* can be defined in relation to clinical characteristics. The first group comprises avirulent strains that are found in the normal intestinal microbiota, and are also known as commensal organisms. The second group is opportunistic *E. coli* that is only involved in disease when inhabiting the elderly, immunocompromised and children as their host. The last group is the pathogenic *E. coli*, which has caused many worldwide outbreaks of severe human diseases and is a major public health concern (Beutin, 2006; Lupp and Finlay, 2005).

Pathogenicity is the ability of microorganisms to cause disease in a susceptible host (Sousa, 2006). Most *E. coli* are non-pathogenic since they do not carry virulent elements and genetic factors that would illicit disease in healthy individuals (Kaper *et al*., 2004). Conversely, there are strains that do infect humans and cause foodborne illnesses (Kaper *et al*., 2004; Nataro and Kaper, 1998). These strains rely on a multi-step scheme of pathogenesis, similar to that used by other mucosal pathogens, consisting of colonization of a mucosal site, evasion of host defenses, multiplication and host damage (Kaper *et al*., 2004).

Human infections caused by pathogenic *E. coli* can occur within one to six days after ingestion, but can occur up to 13 days after exposure in hosts (Janda and Abbott, 2006). Infections are usually self-limiting, and usually resolved within eight days (Clarke, 2001). Pathogenic *E. coli* can cause infections in virtually every organ and anatomical site, such as the urinary tract, bloodstream, cerebral spinal fluid, respiratory tract, peritoneum, pharynx, abdomen and the pelvis (Russo and Johnson, 2003).

Pathogenic *E. coli* are classified based on serological characteristics, distinct epidemiology, clinical features and virulence properties (Russo and Johnson, 2003). Diarrheagenic *E. coli* can be divided into six categories based on specific virulence determinants and association within certain serotypes. These pathotypes are classified as enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enterohemorrhagic *E. coli* (EHEC; including Shiga toxin-producing *E. coli* [STEC]), enteroaggregative *E. coli* (EAEC), enteroinvasive *E. coli* (EIEC) and diffuse-adherent *E. coli* (DAEC) (Bettelheim, 2007). Virulence elements acquired by these pathogenic *E. coli* include pathogenicity islands, transposons, integrated bacteriophages, or plasmids where transfer events occurred independently of each other and with different sets of virulence genes (Bell, 2002; Clarke, 2001; Kaper *et al*., 2004). The various pathotypes of *E. coli* are characterized by shared somatic O, which corresponds to the external lipopolysaccharides (LPS) that are found on the outer membrane of Gram-negative bacteria, and the somatic H that refers to the flagellar antigens.

Enterohemorrhagic *E. coli* (EHEC) are identified as a subgroup of Shiga toxin-producing *E. coli* (STEC) or verocytotoxigenic *E. coli* (VTEC) that share similar clinical, epidemiological and pathogenic features that occur in patients, such as hemolytic uremic syndrome (HUS) and severe bloody diarrhea (Levine, 2007). Generally speaking, all EHEC are STEC, but not all STEC are EHEC.

The transmission of STEC is mediated by the ingestion of contaminated foods, person-to-person contact, or by zoonotic transmission. Cattle appear to be the primary reservoirs from which STEC are transmitted to the human population. Up to 30% of all cattle are asymptomatic carriers of serogroup O157 because they lack the vascular receptors for *E. coli* O157:H7 (Callaway *et al*., 2009; Pruimboom-Brees *et al*., 2000).

Meat (sausage, ground beef), milk, vegetables (lettuce), sprouted vegetables, fruits (melons, apple juice) and foods exposed directly and indirectly to animal fecal contamination are all potential vectors for STEC (Bell, 2002; Jenkins *et al*., 2008). Outbreaks of STEC demonstrate seasonal variation, with peaks in the summer months to early autumn (CDC, 2010). STEC-infected patients acquire illness via contamination of foods rather than person-to-person transmission (Tauxe, 1998). Although infection can pass via person-to-person or the fecal-oral route, this transmission is limited to a few cycles at most (Tauxe, 1998).

STEC do not grow below 7°C, as was evaluated in beef products (Rhoades *et al*., 2009). STEC can infect the large intestine (in piglet models) and are associated with a range of symptoms that include mild fever, watery to bloody diarrhea, vomiting, dysentery, abdominal pain, hemorrhagic colitis (HC), hemolytic-uremic

syndrome (HUS) and thrombotic thrombocytopenic purpura in humans (Clarke, 2001; Jay *et al*., 2005). Between 2% and 7% of infected individuals develop HUS, which is more common in young children, with mortality rates of 10%, followed by the elderly (Clarke, 2001; Rhoades *et al*., 2009). HUS is defined by a triad of clinical features that include: acute renal failure, thrombocytopenia and microangiopathic hemolytic anemia (O'Brien and Kaper, 1998). The pathogenic bacteria may be more aggressive in children, causing a larger amount of Shiga toxin to be released into the bloodstream than in adults (Monnens *et al*., 1998).

Direct infection from food is the main route of human exposure to foodborne pathogenic bacteria. Other potential routes include: routes from animals to humans by direct contact with infected animals; the contamination of food crops by manure that is used for fertilizer; water contamination through irrigated field crops where fruits and vegetables are at risk of being contaminated; contamination of carcasses during slaughter and evisceration processes; and consumption of contaminated raw milk, or raw products derived from it (Bell, 2002). Furthermore, grazing herds on lands adjacent to freshwater sources can lead to accidental contamination of rivers, lakes or by heavy rains (Janda and Abbott, 2006).

Beef is the pre-eminent food source of pathogenic *E. coli*, with the list of other foods constantly evolving. Beef contamination can occur during the processing of slaughtered animals at abattoirs, by improper storage at refrigerated temperatures, or inadequate cooking, especially in the case of ground beef. In Canada, surveys of ground meats have demonstrated detection rates of 15–40% for non-O157 STEC, while in most studies STEC O157 was not detected (Johnson *et al*., 1996). Other muscle foods implicated in human illnesses due to these organisms include ground beef (Doyle and Padhye, 1989), roast beef (Rodriguez *et al*., 1995), venison jerky (Keene *et al*., 1997), sausage (Banatvala *et al*., 1996) and salami (CDC, 1995). As for other reservoir sources, *E. coli* has been isolated from the gastrointestinal tract of a large number of animals including dogs, cats, sheep, goats, horses, poultry, lambs, cattle, pigs and birds, or from their environment, feed and water sources (Bell, 2002; Janda and Abbott, 2006; Vidotto *et al*., 1990).

Worldwide, there are over 60 EHEC serotypes that have been implicated in human diarrhea. As such, EHEC are considered an important cause of gastrointestinal diseases with complications in humans, even though infections are extremely rare in developing nations (Bettelheim, 2007; Janda and Abbott, 2006). *E. coli* O157:H7 is well recognized as the predominant serotype in the United States, Canada, Japan and the United Kingdom. Other non-O157 serogroups are more common in Europe, India, Chile and Australia (Janda and Abbott, 2006). The other STEC serogroups that have been implicated in foodborne illnesses include O26, O103, O111, O91, O118, O166 and O145 (Bell, 2002; Kaper *et al*., 2004; Srinivasan *et al*., 2007).

Serotype O157:H7 causes approximately 50–80% of all STEC infections worldwide. Since EHEC serotypes, other than O157:H7, are not routinely screened, the overall incidence of EHEC infections is difficult to estimate. Caprioli *et al*. (1997) calculated 10–30% of HUS in patients from Germany, Italy and United Kingdom resulted from non-O157 STEC infection, while estimates suggest that

25% of HUS from non-O157 STEC occurs in the United States (Johnson *et al*., 1996). Outbreaks due to O111 also caused HUS in patients from Italy and France (Johnson *et al*., 1996). There have been additional cases in Germany and Italy that were linked to O103 and O26 (Beutin *et al*., 1998). In 1996, there were 15 different typable non-O157 O-serogroups that were reported from 89 STEC-infected patients (Beutin *et al*., 1998). Compared with *Salmonella* and *Campylobacter*, which are major causes of human gastroenteritis, reported incidence of EHEC is generally low. In 1999, approximately two cases per 100 000 individuals were identified in England and Wales and one case per 100 000 individuals were identified in Denmark. In the United States, 2.8 cases per 100 000 individuals were documented in 1998 and 1.6 cases per 100 000 individuals in 2007 (OSHD, 2008). In 1996, 4.1 cases per 100 000 individuals from Canada were identified (Bell, 2002).

E. coli O157:H7 has been isolated from large foodborne outbreaks as well as sporadic cases (Erickson and Doyle, 2007). In 1993, ground beef was responsible for the largest documented outbreak of *E. coli* O157:H7 infection in the United States (Bell *et al*., 1994). The strain was isolated from undercooked hamburger patties and amplified by a secondary spread via person-to-person transmission. In total, 195 people were hospitalized, 55 developed HUS and four children died (Bell *et al*., 1994). In 2008, another multistate outbreak related to ground beef occurred with 49 confirmed cases. The organism was linked both epidemiologically and by molecular fingerprinting to the ground beef, resulting in the recall of approximately 5.3 million pounds of product (CDC, 2009). It is important to note that there are no reported cases of STEC O157 causing disease in animals other than humans (CDC, 2009).

Human and animal infections due to STEC O26 have been documented over the last 25 years. Serotype O26:H11 was the first STEC strain to be described in the literature (Jenkins *et al*., 2008). Recently, the organism was implicated in several food-related outbreaks causing HUS (Jenkins *et al*., 2008). STEC O26 is frequently found in cattle herds, but rarely in the food supply (Jenkins *et al*., 2008). It has been linked to meat (lamb, minced meat) and dairy products made with unpasteurized cows' milk. In reference to its reservoir, O26 has been detected in calves and cattle since the early 1980s (Sherwood *et al*., 1985). A unique feature of STEC O26 (that is not found in STEC O157) is its ability to cause disease in both humans and animals. The incidence of STEC O26 is underestimated because of diagnostic limitations. It is assumed to have a low incidence in the United States, but a higher incidence in other countries (Caprioli *et al*., 1997). Recently, a US firm recalled approximately 8500 pounds of ground beef products due to possible *E. coli* O26 contamination – the first case of a non-O157:H7 strain linked to beef in the United States (USDA-FSIS, 2010). In Italy, STEC O26 surpassed STEC O157 as the major cause of HUS and HC (Tozzi *et al*., 2003). In other countries, the incidence of O26 is similar to that of O157 in the United States (Caprioli *et al*., 1997). Isolates have been obtained from the feces of cattle, calves, pigs, lambs and goats, humans, animals, chickens, pigs and primates and from their environment (Jenkins *et al*., 2008; Srinivasan *et al*., 2007). Transmission of

drug-resistant *E. coli* O26 to humans may also result from antimicrobial therapy, use of drug supplements in foods originated from cows and pigs or contamination from workers (Srinivasan *et al*., 2007).

In the 1940s, serogroup O111 was also among the first strains implicated as the main cause of infantile diarrhea from infant nurseries in the United Kingdom. In the United States, from 1934 to 1987, 28% of the 50 outbreaks reported also were associated with severe enteric diseases in infants (Bray, 1945; Moyenuddin *et al*., 1989). In 1990, an *E. coli* O111 strain caused an extensive community outbreak of diarrhea involving more than 700 schoolchildren and adults in Finland (Viljanen *et al*., 1990). The source of the organism remains unknown. In 1995, South Australia experienced an outbreak in which 23 cases of HUS were reported among children after eating an undercooked, semi-dry fermented sausage product. This outbreak of O111 also was the first in which the vehicle of transmission was identified (Meng and Doyle, 1998). In 2008, the largest STEC O111 outbreak in the United States occurred in Oklahoma, where 341 people became ill, 70 people were hospitalized, 17 people received kidney dialysis and one person died from an unknown source (Marler, 2008). According to Bettelheim's analysis (2007), non-motile O111: H- and motile O111:H8 have been isolated from diseased humans and cattle from associated outbreaks from 1989 to 2005. While cattle are considered the reservoir for these pathogens, other sources of O111 include diseased chickens found in Canada and wild deer found in Japan (Bettelheim, 2007).

Among the serotypes of the O103 serogroup, there are two which predominate, according to Bettelheim's (2007) worldwide review: O103:H- and O103:H2. These serotypes represent 82% of all reports of STEC cases from 1988 to 2005. In 2006, fermented sausage was the vehicle of transmission for an outbreak that occurred in Norway where 17 people became ill, nine developed HUS and one died (Sekse *et al*., 2009). In the same year, an outbreak of STEC O103:H2 occurred at a nursery in Japan where three children fell ill (Muraoka *et al*., 2007). The original source remains unknown. The main reservoirs are thought to be cows or sheep. To date, the serogroup has not been isolated from any other animals.

In the case of serogroup O113, three serotypes predominate: O113:H-, O113:H4 and O113:H21, of which the latter is the most prominent, comprising nearly half of all reports from this serogroup between 1992 and 2005. STEC O113 was one of the first STEC serogroups to be associated with HUS (Karmali *et al*., 1985). In 1998, an outbreak occurred in Southern Australia where three children developed HUS from an unknown source (Paton and Paton, 1999b). The serotype has been isolated from venison, a horse and a sheep, while the rest have been isolated from cattle (Bettelheim, 2007). STEC O113 also has been isolated from ground beef, cheese, game, pork, raw milk and raw spreadable sausage in Germany (Werber *et al*., 2008). STEC O113:H21 was found in cattle and was found to cause HUS (Paton and Paton, 1999a, 2002, 2005).

Serotypes O145:NM, O145:H-, O145:H8, O145:H16, O145:H25 and O145:H28 have been associated with cases of bloody and non-bloody diarrhea, HC and HUS worldwide (Fratamico *et al*., 2009). Serogroup O145 has been isolated in Japan, Germany and in the United States from patients with bloody

diarrhea, HC, HUS, cattle and from food such as ground beef (Feng *et al*., 2005; Jelacic *et al*., 2003). In 2007, there was a STEC O145 and O26 outbreak involving 12 cases, with five individuals developing HUS linked to ice cream (Schrijver *et al.*, 2008). Cattle also have been designated a potential reservoir of this pathogen for humans (Sonntag *et al*., 2004).

Bettelheim (2007) identified four serotypes of O91. From 1992 to 2005, 91% of cases were linked to serotypes O9: H-, O91:H10, O91:H14 and O91:H21. The serogroups have been isolated from healthy and diseased humans, sheep, pigs and from both healthy and diseased cattle. Between 1996 and 2007, STEC O91 was isolated from raw spreadable sausage, ground beef, raw milk, game and raw sausage meat in Germany (Werber *et al*., 2008). Strain O91:H21 has been isolated in many parts of the world including Canada, France and Finland. In 1996–1997, Pradel *et al*. (2002) conducted a study in France to determine the prevalence of non-O157:H7 STEC in a particular geographical area. STEC O91:H21 was isolated from bovine feces, cheese, beef and children, with 12 isolates among the 203 typable strains obtained. In addition, two STEC O91 isolates were isolated from French dairy products among 34 STEC strains isolated (Fach *et al*., 2001). This strain has the ability to produce Shiga toxin 2 and intimin (Pradel *et al*., 2002).

Of all the food vectors contributing to STEC diseases worldwide, beef has been implicated more often than any other food (Rhoades *et al*., 2009). Between 1996 and 2000, England and Wales had 7% of 1.7 million cases of foodborne illnesses that were attributed to beef products, resulting in 67 deaths from *E. coli* O157:H7 and other non-O157 isolates (Adak *et al*., 2005). In 2007, a multistate outbreak in the United States resulted in a recall of 21.7 million pounds of contaminated frozen ground beef patties, which resulted in at least 40 people becoming ill and two people developing HUS (CDC, 2007). In 2008, the United States issued its largest recall of 143 million pounds of beef contaminated with *E. coli* O157:H7, some of which was used in school lunch programs (Martin, 2008). Recently, a multistate outbreak of *E. coli* O157:H7 infections in the United States was associated with beef that resulted in a recall of 41 280 pounds. Two cases of HUS occurred from the 20 persons infected (CDC, 2009). Aside from the outbreaks, there have been many beef product recalls. In 2007, California recalled 5.7 million pounds of ground beef after 14 people fell ill (USDA-FSIS, 2007a). Minnesota recalled 188 thousand pounds of beef trim following a suspected O157 infection (USDA-FSIS, 2007b). Wisconsin recalled approximately 845 000 pounds of frozen ground beef patties (USDA-FSIS, 2007c). Finally, a nationwide recall of 95 898 thousand pounds of ground beef products was reported for possible contamination by *E. coli* O157:H7 after illnesses occurred that were reported in Ohio, Pennsylvania and Illinois (USDA-FSIS, 2009).

Currently, there is only limited information addressing the effects of atmosphere and/or packaging on survivability of non-O157 STEC. However, survivability of *E. coli* O157:H7 under various packaging and antimicrobial regimens has been documented. For example, there were no significant changes in the numbers of *E. coli* O157:H7 during storage either at −1.5°C or 4°C subjected to VP or MAP with CO_2 (Dykes *et al*., 2001). However, adding LAB with VP reduced

E. coli O157:H7 significantly in cooked, sliced meat. The addition of 4–5 $\log_{10}$ CFU/g of LAB also had an inhibitory effect on the growth of *E. coli* O157:H7 in cooked ham stored at 10°C for four weeks (Bredholt *et al*., 1999). And finally, growth of *E. coli* O157:H7 at 10°C in ground beef was inhibited in packaged meats treated with the high CO_2/low CO mixture and the high O_2 mixture (Nissen *et al*., 2000).

1.3.5 *Yersinia* spp.

The genus *Yersinia* is comprised of Gram-negative facultative anaerobes which are ovoid to rod-shaped in appearance. These bacteria belong to the family Enterobacteriaceae. Of the 11 species identified in this genus, there are three primary *Yersinia* species which are pathogenic to mammals: *Y. pestis*, *Y. enterocolitica* and *Y. pseudotuberculosis* (Brubaker, 1972, 1991). Similar to the other intracellular bacterial pathogens, *Yersinia* spp. are able to penetrate epithelial cells and cause cellular invasion with an infectious process that ultimately results in systemic illnesses (Isberg and Leong, 1988).

Yersinia pestis is a non-motile, coco-bacillus approximately 1–3 μm long. The pathogen can grow over a wide pH and temperature range. For example, *Y. pestis* grows in a pH range of 5–9.5 (optimum pH is 7.2–7.6) and in temperatures from 4°C to 40°C (optimum temperature is 28–30°C). This organism is known to be lethal to a wide range of mammals, including primates and rodents. The infectious dose for most rodents is approximately ten cells, which is less than needed to infect primates (Brubaker, 1991; Sebbane *et al*., 2009). Hence, humans can be infected zoonotically through indirect contact with rodents and/or their fleas. As such, this organism was responsible for several pandemic outbreaks, including the Bubonic plague or Black Death during the fourteenth century in Europe in which one third of the population in Europe died. Additional outbreaks occurred in Asia, India, South America, Australia and the United States. While large pandemics have not occurred in over 100 years, sporadic cases (10–15 people/year) have been reported (Perry and Fetherston, 1997).

A close cousin of *Y. pestis* is *Yesinia enterocolitica.* It is a motile aerobic asporogenous rod to coco-bacilli-shaped bacterium. Over the last 50 years, this organism has been responsible for more than a thousand sporadic human infections and hospitalizations each year in the United States. Pigs are the main reservoir of pathogenic *Y. enterocolitica.* Both wild and farmed pigs naturally harbor serotypes of O:3, O:8, O:9 and O:5 which are similar to clinical isolations of *Y. enterocolitica. Y. enterocolitica* outbreaks tend to be associated with undercooked pork or contaminated post-pasteurized milk, water and vegetables that have come into contact with hog feces (Gemski *et al*., 1980; Tuohy *et al*., 1999; Wesley *et al*., 2008). This organism also has been isolated from other meat sources such as lamb, beef and chicken (Fukushima, 1985).

Outbreaks of *Y. enterocolitica* have been reported in cooler climates or during winter months. The main symptoms of *Y. enterocolitica* infection in humans are severe diarrhea, fever and abdominal pain. Cases of yersiniosis have been

identified in Canada, Europe and the United States. Most cases of yersiniosis in the United States are reported in urban cities and mainly affect African-Americans (Abdel-Haq *et al*., 2000, 2006). The most common serotype related to outbreaks in the United States is O:8, but O:3 and O:9 serotypes have been recognized in other countries (Cover and Aber, 1989).

Y. enterocolitica is of concern in the food industry with particular regard to meat packaged using low oxygen concentrations, such as MAP with low O_2, chub packs (stuffed in plastic casings) or VP meats (Gill and Newton, 1979). The three factors effecting the growth of *Y. enterocolitica* in commercial packaging systems are: gas composition of the modified atmosphere, initial pH and the storage temperature. Saturated CO_2 atmosphere packing is more effective in controlling growth of *Y. enterocolitica* than VP at a storage temperature of 3°C (Hudson and Mott, 1993). When pH was 5.8, the growth of *Y. enterocolitica* was inhibited with 100% CO_2 at 4°C. However, if the pH was higher than 6.0, the growth of this organism was not suppressed (Strotmann *et al*., 2008). Under similar atmospheric conditions, *Y. enterocolitica* could be inhibited by storing the meat below 2°C (Phillips, 1996). Hence, growth suppression of *Y. enterocolitica* in meat requires that the initial pH be less than 6.0 or the meat be kept below 2°C with 100% CO_2.

With a combination of 20% CO_2:80% O_2 atmosphere, the growth of *Y. enterocolitica* in minced beef was hindered at 4 and 1°C, but not at elevated temperatures of 11 and 15°C (Kleinlein and Untermann, 1990). Furthermore, packaging with high CO_2 with low CO mixture with inert N_2 (0.4% CO/60% CO_2 40% N_2) was able to suppress the growth of *Y. enterocolitica* at 4°C and 10°C. On using a high oxygen mixture (70% O_2/30% CO_2) or with chub packs, the growth of *Y. enterocolitica* was not observed at these temperatures (Nissen *et al*., 2000).

Yesinia pseudotuberculosis is the third pathogenic organism in this genus. It can cause similar symptoms to infections with *Y. enterocolitica* (fever and right-sided abdominal pain, mimicking appendicitis without diarrhea), making the infection difficult to diagnose (Lamps *et al*., 2001). *Yersinia pseudotuberculosis* can also cause a necrotizing granulomatous disease involving the liver, spleen and lymph nodes (Bottone, 1977).

Several strains of *Y. pseudotuberculosis* have been detected in retail pork and in healthy swine. This organism is well recognized in Europe, Canada and Japan as an animal pathogen which causes zoonotic infections in humans (Shiozawa *et al*., 1988). *Y. pseudotuberculosis* is also an important organism as a sporadic and epidemic human enteric disease (Fukushima *et al*., 2001).

Y. pseudotuberculosis also has been isolated from pork (Bailey *et al*., 2003; Shiozawa *et al*., 1988). Hence, packaging pork in MAP, VP or chub packing with low O_2 may allow for growth of *Y. pseudotuberculosis*. Using the same MAP condition, which suppresses *Y. enterocolitica*, may be useful in controlling *Y. pseudotuberculosis* in pork.

Outbreaks of *Y. pseudotuberculosis* gastroenteritis have been reported recently from Russia, Japan, Canada and Finland and have resulted from pork consumption (Frimodt-Moller and Hammerum, 2006; Jalava *et al*., 2006). In these instances,

the pathogen could have been introduced onto pork through hog manure (Kangas *et al*., 2008). Furthermore, *Y. pseudotuberculosis* could transmit easily through contact with infected animals or through human fecal-oral contact.

1.3.6 *Clostridium botulinum*

Clostridium botulinum is a Gram-positive, obligate anaerobe, spore-forming and rod-shaped neurotoxin-producing bacterium; it is commonly isolated from terrestrial and marine sediments throughout the world (Hinderink *et al*., 2009). According to the genetic and phenotypic characteristics, *C. botulinum* strains can be categorized into four genotypically and phenotypically distinct groups, I through IV. The organism can be further divided into seven subgroups, A through G, according to the serological properties of the neurotoxins (Hinderink *et al*., 2009; Holdeman, 1970; Lindstrom and Korkeala, 2006).

The group I and II *C. botulinum* strains can cause human botulism, which is a serious condition that can cause paralysis and even death. Group III is involved with animal botulism and group IV poses no identifiable threat. The proteolytic group I strains are able to produce toxin types A, B and/or F and non-proteolytic group II strains are able to produce toxin types B, E or F (Lindstrom and Korkeala, 2006). The neurotoxin A tends to be more potent than B and E. Toxin F is rarely reported as a cause of foodborne human botulism.

When neurotoxins enter the body, either through the gastrointestinal tract or through other mucous membranes, they reach motor nerve endings through the blood and lymphatic circulation. As soon as this occurs, they start to block the neurotransmitter release system, resulting in muscle paralysis. This muscle paralysis can be fatal if not treated promptly and recovery time may vary from several weeks to months, depending on the amount of toxin ingestion.

Even though group I strains are mesophilic and grow optimally at 35–37°C, they can produce heat-resistant spores. The optimum growth pH is 4.3–4.5 and cells can tolerate sodium chloride concentrations as high as 10%. Hence, group I *C. botulinum* have been identified as a problematic organism in the food industry, especially with regard to canning and home preservation of meat (Blake *et al*., 1977; Pinchuk and Filiptosov, 1986; St Louis *et al*., 1988; Swaddiwudhipong and Wongwatcharapaiboon, 2000; Tukhaeva, 1971). Group II strains of *C. botulinum* are psychrotrophic, with an optimum growth temperature of 26–30°C, but with the ability to grow at temperatures as low as 3°C with somewhat lower spore heat resistance (Graham *et al*., 1997).

Botulism is derived from the Latin word ‘botulus’, meaning sausage. The first reported outbreak occurred in Germany, in the eighteenth century (1793), with contaminated sausage. *Clostridium botulinum* was first isolated in the late nineteenth century (Erbguth, 2004). After the twentieth century, mortality rates for botulism declined significantly due to commercial canning. Symptoms of botulism occur within 12–72 h after ingestion of the contaminated food. In some instances, it can take up to ten days before symptoms appear. Early symptoms include those seen with general foodborne illnesses, such as nausea, general weakness, vomiting,

constipation or dry mouth. If untreated, symptoms can progress to include cranial muscle paralysis resulting in double vision and dilated pupils, slurred speech, difficulty in swallowing and speaking and facial paralysis (Lindstrom and Korkeala, 2006; Lindstrom *et al*., 2006; Meunier *et al*., 2002). Although intravenous antitoxins of A, B and E are available to neutralize the toxin, treatments depend on the time of administration.

Outbreaks of *C. botulinum* are rare, as compared to other foodborne pathogens. Several sporadic cases reported in Europe and Asia have been attributed to home-canned meat (Aureli *et al*., 1996; Delbos *et al*., 2005; Meusburger *et al*., 2006; Swaddiwudhipong and Wongwatcharapaiboon, 2000; Tseng *et al*., 2009). Globally in 1995, 52% of botulism cases were due to type B neurotoxin, 34% of them by A and 12% by type E (Lindstrom and Korkeala, 2006).

The evidence indicates that a low incidence of *C. botulinum* spore contamination in meat and meat products has occurred in North America and Europe (Dodds, 1993). Contamination of meat and poultry with *C. botulinum* could occur via soil and the intestinal tract of infected animals during slaughter and handling of meat carcasses. Meat packaging by MAP and VP may allow for the absence or reduction of oxygen, which are conditions suitable for the growth of *C. botulinum* (Brody, 1989). Hence, reducing microbial contamination by the pathogen is very important in safety and shelf-life extension of packaged meat and poultry products. The growth of *C. botulinum* in packaging can be inhibited by altering the gas mixture of MAP to 1–3% O_2 (Ozturk *et al*., 2010). It also can be inhibited by strategically adding food preservatives, such as nitrites, sorbic acid, ascorbates, pabans, phenolic antioxidants, polyphosphates, lactic acid bacteria and/or bacteriocins, to the meat. Combined with packaging systems, these food additives can be used to exert control over the growth of *C. botulinum* (Hauschild and Dodds, 1993; Mazzotta *et al*., 1997; Okereke and Montville, 1991).

C. botulinum type E is prevalent in aquatic environments and type E human botulism is often reported in connection with processed fish, such as VP hot-smoked fish (Hyytia *et al*., 1998, 1999). During the 1960s, several fatal cases of type E botulism involving VP smoked fish were reported in the United States, Canada and France (Ager and Dolman, 1964; Ball *et al*., 1979; King *et al*., 2009). During the hot-smoking process, temperatures reach 60–80°C, which may not eliminate spores of *C. botulinum* Type E. While in storage, spores germinate and actively growing cells produce toxins. Recent findings indicate that spores can be reduced by a factor of 6 $\log_{10}$ when applying temperature combinations/ranges of 90°C for 10 min, 85°C for 36–52 min or 80°C for 129–270 min, prior to packaging (Lindstrom *et al*., 2003).

Because of the morbidity and mortality of botulism toxin, it may be used as a weapon in bioterrorism by nefarious parties (Arnon *et al*., 2001).The estimated death toll by one aerosolized gram is 1 million (Irwin and Rippe, 2003). If botulism were released into the national food supply, it could be difficult to differentiate it from unintentional contamination. Hence, workers within food industry need to be educated about the complexities of such possible threats through training and education.

1.3.7 *Clostridium perfringens*

Clostridium perfringens is a non-motile, encapsulated Gram-positive, anaerobic, endospore-forming bacterium. It is widely distributed in sewage and soil. Although this organism is commonly found in the gastrointestinal tract of animals and humans, it can become an opportunistic pathogen when a large number of cells are ingested in food (Chakrabarti *et al*., 2003; Niilo, 1980; Petit *et al*., 1999). Spores and vegetative cells are heat-resistant and aerotolerant, with the ability to form protein toxins (Czeczulin *et al*., 1993). Based on the toxin type, *C. perfringens* strains are classified into five groups, A through E. The main toxin types which demonstrate pathogenesis are alpha-, beta-, epsilon- and iota-toxins (Songer, 1996). Strain A is commonly associated with human foodborne illnesses in which intense abdominal cramps and diarrhea occur.

The CDC estimated that around 250 000 Americans get sick every year from *C. perfringens*. It is the third most common foodborne pathogen in the United States (Miliotis and Bier, 2003). Most of these outbreaks are associated with large quantities of food prepared in advance or improperly cooled, such as is required for banquets or buffets (Roach and Sienko, 1992).

Vegetative cells or spores of *C. perfringens* can enter the body through wounds or ingestion and can grow rapidly in the gut and/or body tissues. Bacteria can produce various toxins and enzymes that cause massive destruction with systemic toxemia, shock or death if antibiotic or surgical treatments are not feasible (Rood, 1998).

The optimal growth temperature for *C. perfringens* is in the range of 43–47°C and germination time is around 8 min (Doyle, 2002) under ideal conditions. Vegetative cells of *C. perfringens* can tolerate heat (10–54°C) and spores can survive under refrigeration and freezing temperatures (Li and McClane, 2006). Unlike other clostridial organisms, significant amounts of enterotoxin are not produced during vegetative growth, but rather during spore formation (Duncan, 1973). Under optimal conditions, sporulating cells of *C. perfringens* produce heat-resistant enterotoxin (CPE), yet no significant release of CPE into the intestinal lumen can be seen until sporulation is completed (Paredes-Sabja and Sarker, 2009). CPE binds to a mammalian cell receptor and then enters into the cell, and complexes with mammalian membrane proteins. After formation of this large complex, plasma membranes become permeable to small molecules, such as ions and amino acids (McClane, 1994, 1996, 2000). Using this mechanism, symptoms of *C. perfringens* infection include diarrhea and/or flatulence with severe abdominal pain; some patients report a fever, chills and headache. However, vomiting and nausea have not been reported. These symptoms occur within 6–24 h after ingesting contaminated food. Usually, the accompanying diarrhea associated with this condition flushes out both the enterotoxin and sporulating cells from the small intestine, leading to persistent conditions lasting up to a day, or as long as one to two weeks. Most incidents of *C. perfringens* are under-reported, especially for symptoms which are often mild and last for only short durations. In severe cases, especially with infected immunocompromised individuals, dehydration and other complications can result in death.

C. perfringens can be seen in high-protein foods of animal origin such as meat and poultry. Contamination of meat and poultry carcasses with *C. perfringens* can occur via soil from hides or feathers, or fecal contamination from intestinal tracts of infected animals during slaughtering. Such contamination can be minimized or avoided by following appropriate sanitation procedures and/or good manufacturing practices.

Surveys have demonstrated that up to 50% of all raw or frozen meat contain 3–4 $\log_{10}$ of *C. perfringens* CFU/g meat (Novak and Yuan, 2004). During MAP or VP of raw meat, the absence or reduction of oxygen may permit conditions suitable for the growth of *C. perfringens*. Since *C. perfringens* has relatively high heat stability, the organism can grow rapidly at elevated temperatures and forms heat-resistant spores during the cooking of meat products. Many outbreaks of *C. perfringens* occur as a result of inadequate cooling practices in food operations, allowing for the organism to proliferate to very high levels. During a slow cooling process, spores can germinate. Therefore, foods should be cooked to an internal temperature of 74°C or higher to inactivate the pathogen's vegetative cells. Additionally, the cooked food must be chilled rapidly to 4°C or less, or kept at hot holding temperatures of 60°C or higher to prevent any activation and/or growth of *C. perfringens* spores. Other studies have demonstrated that *C. perfringens* exhibits faster multiplication in food (4–5 logs within 18 h) than other spore-forming organisms such as *B. cereus* and *C. botulinum* (Doyle, 2002; Juneja *et al*., 1994).

Currently, little research has addressed the effect of packaging and atmosphere regimens for controlling *C. perfringens.* Therefore, during the packaging of muscle-based RTE food products, such as red meat, pork, poultry, sausages, bean and other protein-containing foods, including gravies, soups and sauces, special concern must be exercised to prevent *C. perfringens* outgrowth. Even though high oxygen levels in MAP can inhibit the growth *C. perfringens*, there is a possibility of growth in VP RTE meat and poultry products. US regulatory agencies recommend rapid cooling methods during preparation of RTE beef, poultry or bean products. For example, USDA-FSIS guidelines recommend cooling food from 49 to 13°C within 6 h, followed by further cooling to 4°C before packaging. The FDA Food Code recommends cooling food from 57°C to 10°C within 2 h, followed by further cooling to 4°C within 6 h or less; the total allowable cooling time is not more than 8 h (Juneja *et al*., 1994). According to USDA-FSIS, the standards for growth of *C. perfringens* in cooled RTE meat and poultry products should be no more than 1 $\log_{10}$ CFU/g sample (Doyle, 2002).

1.3.8 *Staphylococcus aureus*

Staphylococcus aureus is a facultative anaerobic non-motile, non-spore-forming, Gram-positive grape-like coccus (0.5–1.0 μm), found in clusters. The organism is found in mucous membranes, such as the nose and also on skin (Kluytmans, 1990; Kluytmans *et al*., 1997; Namura *et al*., 1995; Noble and Naidoo, 1978; Rountree *et al*., 1954).

According to the FDA, 'Staphylococci exist in air, dust, sewage, water, milk and food or on food equipment, environmental surfaces, humans and animals. Humans and animals are the primary reservoirs. Staphylococci are present in/on the nasal passages, throats, hair and skin of 50% or more of healthy individuals. Although food handlers are usually the main source of food contamination in food poisoning outbreaks, equipment and environmental surfaces can also be sources of *S. aureus* contamination' (FDA, 2012a).

Some strains of *S. aureus* have the ability to produce staphylococcal enterotoxins (SE) which can cause staphylococcal food poisonings. Eight antigenically distinct SE have been identified (Sandel *et al*., 2003). *S. aureus* has long been recognized as an important human pathogen, with some strains demonstrating resistance to antibiotic therapy (Ayliffe, 1997; Breinl, 1959). First reported in 1961, and known as methicillin-resistant *S. aureus* (MRSA), infections caused by these organisms are creating significant public health problems. Some studies suggest that livestock may be a reservoir of MRSA, prompting research surveys to address the incidence and/or prevalence of these organisms in meat and poultry products.

Growth conditions of the bacterium are complex and vary from strain to strain. Generally, this bacterium is able to grow between 7°C and 47°C, with an optimum range of 30–37°C. The production of SE can begin between the temperature range of 10–46°C, with a pH of approximately 7. It has been observed that SE production is reduced when growth is below 25°C. For growth, the optimum pH is 7.0–7.5, but cells can tolerate a pH between 4.2 and 9.3 and a salinity level up to 15% salt (Le Loir *et al*., 2003).

A total of 30–50% of the human population carry *S. aureus* naturally (Le Loir *et al*., 2003). Hence, RTE foods can be easily contaminated with *S. aureus* during or after preparation by food handlers though perspiration, the aerosol effect from sneezing, saliva, by hand contact or by cross-contamination (Todd *et al*., 2008). A total of 14% of all foodborne outbreaks in the United States have been associated with *S. aureus* contamination (Sandel *et al*., 2003). It is important to note that most outbreaks associated with *S. aureus* are under-reported and self-limiting due to the short duration of the associated illness.

S. aureus causes a food intoxication, rather than a foodborne infection. Issues arise when the food has not been kept hot enough (60°C or above) or cold enough (7.2°C or below), allowing the organism to proliferate and produce SE. The illness has a short incubation time – 2–4 h – with a maximum duration of 18–24 h. The short incubation time is a result of preformed enterotoxin types A and D (Buzby, 1996). Common symptoms of staphylococcal intoxication include vomiting, nausea, cramps and diarrhea; however, no fever has been associated with a staphylococcal intoxication. Death from staphylococcal food intoxication is very rare, though such cases can occur in immunocompromised individuals, the elderly or in infants.

A 1.0 μg quantity of the SE or a bacterial population of 6 $\log_{10}$ CFU/g in contaminated food are capable of causing staphylococcal intoxication. According to the FDA, 'Foods that are frequently associated with staphylococcal food poisoning include meat and meat products; poultry and egg products; salads such as egg,

tuna, chicken, potato, and macaroni … milk and dairy products. Foods that require considerable handling during preparation and that are kept at slightly elevated temperatures after preparation are frequently involved in staphylococcal food poisoning' (FDA, 2012a). Numerous intoxications associated with meat or poultry have been documented over the years. Such products include cooked turkey, eggs and the interior of some salted and/or cured meat products (FDA, 2012a).

Prevention of *S. aureus* is difficult due to the ubiquitous nature of the organism. However, it can be controlled by proper personal hygiene, hand washing and wearing gloves properly. Control of the pathogen in VP RTE meat products, such as cooked bacon, salami, beef jerky, can be controlled by reducing water activity and, in some cases, increasing the salt level in the food (Christiansen and Foster, 1965; Ingham *et al*., 2005, 2006).

Research addressing control of *Staphylococcus* spp. using various packaging materials or gaseous atmospheres is limited. In one study, *S. aureus* was experimentally inoculated onto fresh chicken thighs, dipped in potassium sorbate solutions and packaged in Nylon/Plexar/Surlyn bags under air, vacuum, 20%, 60% or 100% CO_2 atmospheres. Changes in gaseous headspace and microbial populations were observed following storage at 10°C for ten days. *S. aureus* growth was inhibited by exposure to high levels of CO_2. Additionally, increased concentrations of sorbate, in combination with higher concentrations of CO_2 in the packaged environment, provided a more effective inhibitory system against the pathogen on fresh poultry (Elliott *et al*., 1985).

In another study, sliced cooked roast beef was inoculated with *C. perfringens, S. aureus* and *S*. Typhimurium, packaged under air or MAP consisting of 75% CO_2, 15% N_2 and 10% O_2, and stored at 4.4°C for 42 days. The pathogens were unable to grow under MAP, probably due to the combined effects of atmosphere and temperature. *S.* Typhimurium and *C. perfringens* declined more rapidly under MAP than with air, but *S. aureus* numbers did not decrease in either atmosphere. As evidenced by the above information, more research is needed to determine optimal atmospheres and packaging regimens for controlling *S. aureus* associated with meat and poultry products.

1.3.9 *Bacillus cereus*

Bacillus cereus is a spore-forming, facultative anaerobe that is a highly motile, Gram-positive rod (Cardazzo *et al*., 2008; Drobniewski, 1993; Giannella and Brasile, 1979). The vegetative cells of this organism range in size from 1.2 to 1.5 μm in width and 3–5 μm in length. The organism can tolerate a wide range of temperatures, ranging from 10°C to 48°C and a pH range of 4.9–9.3 (Drobniewski, 1993). This organism is closely related to six other organisms in the *Bacillus* genera, including *B. thuringiensis*, *B. anthracis*, *B. mycoides*, *B. pseudomycoides* and *B. weihenstephanensis* (Lechner *et al*., 1998; Tourasse *et al*., 2010).

B. anthracis is commonly found in soil and it also acts as an opportunistic pathogen in humans with soft tissue infections (Cardazzo *et al*., 2008). For example, *B. anthracis* is an obligate mammalian pathogen and causative

agent of anthrax, which is a fatal mammalian disease. Foodborne anthrax incidents have occurred. In these cases, the disease was spread through infected meat (Guillemin, 1999).

B. cereus is able to form ellipsoidal, central or paracentral-shaped endospores, which are highly hydroscopic, dormant spores capable of surviving very long periods of adverse environmental conditions. When conditions become favorable, the spore starts its germination into vegetative cells within two hours (Broussolle *et al*., 2008). Due to its hygroscopic nature and resistance to adverse conditions such as heat, radiation, disinfectants and cleaning agents, *B. cereus* is of significant importance within the food industry.

B. cereus is able to produce two types of toxins during the log phase of vegetative growth: heat-stable emetic and heat-labile diarrheagenic toxin and enzymes (Hobbs, 1974). *B. cereus* causes foodborne intoxication with emetic or diarrheal symptoms. Diarrheal toxins (heat-labile enterotoxin) are formed after ingestion of large numbers of vegetative cells in the intestine, whereas emetic toxins (heat-stable enterotoxin) are formed in food (Ankolekar and Labbe, 2009). The heat-labile enterotoxin-producing *B. cereus* strains grow in a wide variety of foods such as meat, vegetables, milk, milk products, soups, sausages, desserts and sauces. The heat-labile diarrheal toxin acts on the small intestine and results in diarrhea and abdominal pain within 8–16 h after ingestion. The heat-stable, emetic-toxin producing *B. cereus* strains grows in starchy foods such as fried rice, with nausea and vomiting occurring approximately 1–5 h after ingestion. Some *B. cereus* outbreak victims have reported both vomiting and diarrhea (Deshpande, 2002; Haggblom *et al*., 2002).

Enterotoxin production by *B. cereus* in food is dependent on several factors such as pH, water activity, temperature and food composition. For example, at 17°C, hemolysin BL toxin production was observed in rice meal when cell populations reached 7.1 log CFU/g rice. The toxin also was produced in rice flour cakes when vegetative bacterial population reached 6.6 log CFU/g sample after seven days at 22°C. In salmon, non-hemolytic enterotoxin was detected at 22°C when cells reached 8 $\log_{10}$ CFU/g salmon (Ankolekar and Labbe, 2009).

B. cereus was first isolated by Frankland and colleagues in 1887 from air in a cow shed and called it 'cereuse' meaning 'wax colored' in Greek (Larsen and Jorgensen, 1997). During the early twentieth century, the first outbreak associated with *B. cereus* was reported in Europe, but not fully confirmed as the causative agent until the 1950s. At that time, Hauge and his colleagues volunteered for a feeding test and clearly demonstrated the symptoms associated with the organism. Using this information, Hauge investigated four additional outbreaks in Norway (Jay *et al*., 2005; Logan, 1988). From 1993 to 1997, *B. cereus* was linked to 14 outbreaks and 691 reported cases of foodborne illness in the United States. Outbreak incidents have also been reported in Canada, India, Japan and Russia, as well as Europe. In Japan, emetic outbreaks were ten times more likely than the diarrheal outbreaks, as compared to other western countries and attributable to the eating habits of the population (Deshpande, 2002).

The majority of *B. cereus* foodborne outbreaks have been linked to the consumption of slowly cooled, or improperly refrigerated, cooked foods. This organism has been isolated from a variety of food sources such as meat, infant foods, milk products, spices, RTE foods, seafood and rice (Ankolekar *et al*., 2009). According to the FDA, such foods as beef, turkey, and Mexican foods, rice and shellfish also have been reported. Other outbreaks may go unreported or are misdiagnosed because of symptomatic similarities to *S. aureus* intoxication (*B. cereus* vomiting-type) or *C. perfringens* food poisoning.

In food service, bacterial growth by *B. cereus* can be minimized by hot holding foods over 60°C and cold foods under 4°C (Ferrer *et al*., 2009). Antimicrobial agents and food additives also have been used to prevent the growth of *B. cereus* in food. Washing foods with antimicrobials such as enterocin AS-48, cinnamic and hydrocinnamic acids, carvacrol, polyphosphoric acid, peracetic acid, hexadecylpyridinium chloride or sodium hypochlorite can control *B. cereus* (Cobo Molinos *et al*., 2008). By combining food additives (glucono-delta-lactone, sodium erythorbate and citric acid with sodium nitrite and salt), the growth of *B. cereus* could be delayed or inhibited in liver sausage (Asplund *et al*., 1988).

Although vegetative cells of *B. cereus* are easily destroyed by heat, spores are harder to destroy. Adding lactic acid bacteria or organic acids produced by lactic acid bacteria during preparation of meat products, such as sausage, salami or bologna, can inhibit germination of *B. cereus* spores (Abriouel *et al*., 2002; Wong and Chen, 1988). Studies that address the synergistic effects of packaging materials or various atmospheres, alone or in combination with antimicrobials for controlling *Bacillus* spp. in meat and poultry products are lacking. Therefore, presence of *B. cereus* in muscle foods must be prevented by GMPs, sanitation measures, employee training and incorporation of Hazard Analysis Critical Control Point (HACCP) programs.

1.3.10 *Aeromonas* spp.

Aeromonas spp. are motile, Gram-negative, facultative anaerobic, non-spore-forming rod-shaped, ubiquitous, waterborne motile organisms with polar flagella. These organisms are found naturally in both fresh and brackish water (Holmberg and Farmer, 1984). *Aeromonas* spp. range in size from 0.4 to 1.0 μm in width and 1.0 to 4.4 μm in length. These organisms can tolerate a pH range of 4.0–10 and can grow optimally within a wide range of temperatures from 20°C to 35°C (Doyle, 1989). Based on phenotypic characteristics, the genus *Aeromonas* was initially placed under the family *Vibrionaceae*, but later transferred to the family of *Aeromonadaceae* (Abbott *et al*., 2003; Colwell *et al*., 1986). The first identified *Aeromonas* species was isolated from eggs in 1937. During the 1960s, it was recognized as a potential human pathogen. In 1984, *A. hydrophila* was classified officially as a foodborne pathogen (Isonhood and Drake, 2002).

Only certain subgroups of *Aeromonas* spp. are pathogenic, namely: *A. hydrophila*, *A. bestiarum*, *A. caviae*, *A. jandaei*, *A. media*, *A. schubertii*, *A. veronii* and

A. trota (Isonhood and Drake, 2002). *Aeromonas* spp. have been identified as emerging infectious or enterotoxigenic human pathogens with a variety of virulence factors, including cytotoxic and cytotonic enterotoxins, aerolysins, hemolysins, proteases, hemagglutinins and lipases (Bhowmik *et al*., 2009; Isonhood and Drake, 2002). As a result of the virulence factors, *Aeromonas* spp. can cause gastroenteritis (ranging from mild enteritis to cholera-like diarrhea), cellulitis, wound infection, peritonitis, endocarditis, osteomyelitis, HUS, peritonitis, meningitis and suppurative arthritis in patients with leukemia (Vasaikar *et al*., 2002). Of all the clinical isolates, *A. hydrophila*, *A. sobria* and *A. caviae*, are well recognized as contributory agents of human gastroenteritis, wound infections and septicemia. In addition to humans, *Aeromonas* spp. are also important pathogens in amphibians, reptiles and fish. *Aeromonas* spp. infections are a major problem in fish farming, causing an epizootic ulcerative syndrome in humans (Rahman *et al*., 2002).

Most *Aeromonas* spp. gastroenteritis cases are associated with mild and self-limiting diarrhea. Occasionally, patients report abdominal pain, fever and bloody diarrhea (Doyle, 1989). These reported incidents of outbreaks mainly involved seafood, such as oysters, sashimi, prawns and shrimp (Isonhood and Drake, 2002). However, *Aeromonas* spp. also has been isolated from a variety of foods such as vegetables, raw milk, ice cream and meat.

Since *Aeromonas* spp. is psychrotrophic, there are public health risks which may be associated with consumption of RTE refrigerated food products. As a result of these concerns, the psychrotrophic ability of *Aeromonas* spp. has been extensively studied in refrigerated, raw and cooked meat and fish products (Doherty *et al*., 1996; Szabo *et al*., 2000; Velazquez *et al*., 1998). In Switzerland, *Aeromonas* spp. is examined qualitatively, as well as quantitatively, in meat, poultry, fish and shellfish products (Gobat and Jemmi, 1995).

Due to its psychrotrophic nature and growth requirements of low oxygen concentrations, *Aeromonas* spp. are of concern in both MAP-packaged and VP raw meat products such as pork, poultry and lamb. To prevent growth of *Aeromonas* spp. in these products, combined effects of pH, temperature and sodium chloride have been investigated. Growth of *A. hydrophila* can be hampered under VP conditions by lowering the pH below 6.0 or lowering the storage temperature to 5°C (Phillips, 1996). *A. hydrophila* has been controlled for 24 days by lowering the pH to 4.5 at 5°C. Similar control was seen in VP ground pork over a period of 22 days' storage by increasing the sodium chloride level to 3% and storage at 5°C (Beuchat, 1991). Another way to decrease the growth of *A. hydrophila* in food is by adding lactic acid bacteria (LAB) or bacteriocins produced by LAB to the product during processing (Arques *et al*., 2004; Lewus *et al*., 1991; Vescovo *et al*., 1996). Furthermore, spices containing essential oils and herbs, such as sweet basil, eugenol and coriander, clove, oregano and thyme oils can be used to control *Aeromonas* spp. in RTE meat and poultry products such as salami and ham prior to packaging (Hao *et al*., 1998a, 1998b). A synergistic effect was observed against *Aeromonas* spp. when essential oils and/or LAB were combined with VP or MAP at 40% CO_2, 30% N_2 and 30% O_2 (Burt, 2004).

1.3.11 *Shigella* spp.

The genus *Shigella* is a Gram-negative, non-motile, non-spore-forming, facultatively anaerobic rod under the family Enterobacteriaceae. This organism is biochemically and antigenically related to enteroinvasive *E. coli* (EIEC; Ewing, 1949; Pupo *et al*., 2000; Torres, 2004). Humans and closely related primates can be infected by shigellosis, but other mammals are asymptomatic.

Shigella spp. was first identified and described by Kiyoshi Shiga in 1898 as a result of epidemic dysentery which spread across Japan during the nineteenth century (Levine *et al*., 2007). Later, this species was further classified and documented by Ewing and colleagues (Edwards and Ewing, 1986). Four *Shigella* species are pathogenic to humans: *S. dysenteriae* (Group A) with 15 serotypes, *S. flexneri* (Group B) with 14 serotypes, *S. boydii* (Group C) with 20 serotypes and *S. sonnei* (Group D) with a single serotype (Levine *et al*., 2007).

Shigella spp. can cause an acute gastrointestinal syndrome in humans with fever, intestinal cramps and headaches, as well as blood and mucus in diarrheal stools. Shigellosis has a large impact on global health with an estimated 1 million deaths and 163 million cases of dysentery annually (Torres, 2004). *S. sonnei* is responsible for over two-thirds of shigellosis in the United States, followed by *S. flexneri* (Kopecko *et al*., 1980). *S. dysenteriae* has a high fatality rate (5–15%). While *S. boydii* is not common in North America, it is responsible for infections in South America, India and its neighboring countries (Torres, 2004; Warren *et al*., 2006). Transmission of *Shigella* spp. is typically through a direct or indirect fecal-oral route, from person-to-person, and can be spread by contaminated food or water in less developed countries (Kapperud *et al*., 1995). The infectious dose is < 10 CFU for *S. dysenteriae* and 500 CFU for *S. sonnei*. These bacterial cells colonize in human intestinal epithelium and create an intense acute inflammatory response with infiltration by polymorphonuclear leukocytes. The organism can cross colon cells and gastrointestinal-associated lymphoid tissue to invade epithelial cells (Torres, 2004).

Bloody diarrhea due to *Shigella* spp. has been observed mainly in children (66%) and adults (26%). In these cases, the stool consists of 44% blood and 50% mucus. Along with these conditions, respiratory problems and chest pains may occur (DuPont and Pickering, 1980). Dysentery, or watery diarrhea, is common in patients infected with *S. dysenteriae* type 1 and *S. flexneri* organisms. Some strains of *S. sonnei* infections can cause fever without gastrointestinal symptoms. HUS can be caused by *S. dysenteriae* when the resulting toxin attacks small vessels in the kidney. In rare instances, the organism can attack the central nervous system, resulting in meningitis (Janda and Abbott, 2006).

Outbreaks of shigellosis occur in places where there are poor sanitary conditions or poor infrastructure. In developed countries, *Shigella* spp. outbreaks have been associated with banquets, buffets, or community events where the source is contaminated protein- or carbohydrate-rich salads such as ham, chicken, or pasta (Black *et al*., 1978). Several other incidents of shigellosis have been reported with commercially prepared foods (Dunn *et al*., 1995). In 1983, two Texas university campuses, 60 miles apart, experienced outbreaks associated with a meat-based

salad contaminated with *S. sonnei* (Martin *et al*., 1986). In October 1988, the Minnesota Department of Health reported an outbreak of *S. sonnei* associated with commercial airline food that affected over 200 national and international passengers (Hedberg *et al*., 1992). In August 1992, the Michigan Department of Public Health reported *S. flexneri* infections in patrons of a single restaurant chain from multiple states (Dunn *et al*., 1995). In 1998, another multistate shigellosis outbreak was reported (Wu *et al*., 2000). In all of these cases, outbreaks were linked to a single commercial kitchen that prepared and distributed the food.

Since the organism is spread via the fecal-oral route, control of *Shigella* spp. in the food industry can be accomplished by having food handlers undergo frequent and proper hand washing. Additionally, treatment of experimentally inoculated foods with various antimicrobials such as vinegar, chlorinated water or ozone is known to reduce or eliminate the pathogen (Selma *et al*., 2007; Wu *et al*., 2000). Active packaging material applied to meat also can inhibit the growth of *Shigella* spp. on meat surfaces (Cutter, 1999). Additional studies addressing the application of packaging or atmosphere regimens for control of *Shigella* spp. are lacking. However, if foods contaminated with *Shigella* spp., are cooked thoroughly to >77°C, the pathogen will be inactivated. Also, the organism can be controlled adequately by following HACCP procedures in meat and poultry processing plants and by maintaining hygienic practices among food handlers.

1.4 The future of food packaging for controlling pathogens associated with fresh and further processed meat and poultry

Recent developments in the area of packaging for controlling pathogens in the meat and poultry industries has focused on the introduction of active packaging, including antimicrobial films, the development of edible films and the incorporation of nanotechnology into plastic-based or edible packaging materials or biosensors. According to Akbari *et al*. (2007), nanotechnology and nanocomposites are poised to become one of the most powerful forces of innovation in the food packaging industry. Current research is focusing on sources of new or novel packaging materials with nanoparticles, methods for production of the materials and evaluation of the mechanical properties (tensile strength, transparency, etc.), as well as gas permeability properties of these packaging materials. It is thought that the use of bio-based nanocomposites may replace a variety of films currently used in different areas of food packaging (Akbari *et al*., 2007).

These nanocomposite packaging films may not only extend the shelf life of food, but also control pathogenic organisms by blocking gases and moisture from reaching the food. For example, the antimicrobial activity of polymer-layered silicate nanocomposites has been demonstrated against Gram-negative (*E. coli*) and Gram-positive (*S. aureus*) bacteria (Nigmatullin *et al*., 2008; Kang *et al*., 2007). Also a biopolymer/rectorite nanocomposite has been shown to demonstrate an antibacterial

effect against a wide variety of microorganisms, including fungi (Wang *et al*., 2009). It has hypothesized that carbon nanotubes may cause cellular damage in *E. coli*, *Pseudomonas aeruginosa* and *S. aureus*, resulting in death (Li *et al*., 2008).

Nanosensors are another technological innovation that may revolutionize the packaging industry. Nanosensors are made up of nanoparticles that can be used to detect chemicals, pathogens and toxins inside food packages. For example, biosensors have been developed that detect *Staphylococcus* enterotoxin B, *E. coli* O157:H7, *Salmonella* spp. and *L monocytogenes* on packaged food surfaces (Brody *et al*., 2008). In another example, protein G-liposomal nanovesicles also have shown the ability to detect *E. coli* O157:H7, *L. monocytogenes* and *Salmonella* spp. in packaged food matrices (Chen and Durst, 2006).

Despite these innovative approaches and advances in packaging technologies, the application of nanotechnology into food packaging should be undertaken with care as the effects of nanomaterials on humans are still not fully understood and consumer acceptance has not been fully researched.

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2

Major microbial hazards associated with packaged seafood

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Abstract: Seafood is a highly perishable product. Unlike some meats that are aged to enhance tenderness, seafood decomposition begins immediately post-mortem. Pathogens such as *Listeria monocytogenes*, *Salmonella* and *Staphylococcus aureus* are common to seafood and terrestrial muscle foods; however, others such as *Clostridium botulinum* Type E, *Vibrio* species and *Aeromonas* are more commonly associated with or exclusive to marine food products. Packaging of seafood products has historically been passive, or used to protect from oxygen, desiccation and microbial contamination. Tamper-evident packaging became the norm 30 years ago. Around the same time, modified atmosphere packaging (MAP) appeared to be a panacea for shelf-life extension, but is tightly regulated in the United States because of the concern for temperature abuse and toxin production by *Clostridium botulinum* Type E. Time temperature indicators (TTIs) present the obvious solution to MAP but are seldom used due to cost. Nanotechnology is the newest wave in food processing, but its long-term effects on the human metabolism are unknown and there is inertia over defining its properties and its regulatory oversight. Momentum appears largely to be driven by large retailers that prefer case-ready seafood products in standard package sizes for rapid stocking with limited effort.

Key words: spoilage, histamine fish poisoning, *Salmonella*, *Listeria monocytogenes*, *Vibrio vulnificus*, *V. parahemolyticus*, *V. cholera*, *Staphylococcus aureus*, *Clostridium botulinum*, *Aeromonas*, *Giardia lamblia*, modified atmosphere storage, *Carnobacterium*, *Lactobacillus collinoides*, *L. pastorianus*, antibiotic resistance.

2.1 Introduction

Seafood products present unique challenges post-harvest; the appreciable quantity of non-protein nitrogenous compounds are ready bacterial food sources and

species harvested from colder waters tend to carry microbial loads accustomed to low-temperature storage. Some products such as oysters and mussels are sold live. Other products such as crabs and lobsters are cooked live, without evisceration, while yet other product species such as cod, pollock and whiting are processed and frozen into blocks at sea. Different categories of seafood products have unique spoilage patterns based upon innate compositional, chemical biochemical and microbiological differences. Further, seafood species are poikilothermic and have shifts in metabolism and biochemistry based upon their migratory patterns. Species range from burrowing animals (oysters, crawfish and some clams) to bottom dwellers (soles and other flatfish) to shallow water inhabitants (crabs and some shrimp species). Harvest waters may range in temperature from near freezing to higher than room temperatures (90°F/32°C) and water quality may be fresh, brackish or seawater with varying levels of pollution (of either human or animal origin), all of which contribute to bacterial, viral and amoebic contamination. Depth of waters at which the fish live will impact on the microflora present. Greater depth leads to increased hydrostatic pressure, which leads to a different microbial population, type and metabolic activities. Activity of enzymes in deeper water (groundfish) exhibit accelerated activity at ambient pressure and spoilage proceeds at a rapid rate. Species harvested from cold waters (proximity to poles and greater depth) tend to have low levels of saturated and higher levels of unsaturated fatty acids. Such a variety of biochemical and environmental conditions will result in shifts in microfloral population and type. Also, in areas with seasonal temperature changes, bacterial pathogens tend to occur in higher numbers during the warmer summer months. Seafood favors bacterial growth for a variety of reasons: due to low glycogen levels, the post-mortem pH of finfish does not typically decrease to the same extent as it does in terrestrial meat-producing animals (oysters are the exception to this); seafood has a high moisture content and species of marine origin tend to contain appreciable quantities of non-protein nitrogen (Franzetti *et al*., 2003; Gram and Huss, 1996).

Historically, it was thought that the bacterial flora of finfish was confined to the skin, the gills and the alimentary canal. The flesh was thought to be sterile from the aspect of microflora, with the exception of parasites (nematodes, cestodes and trematodes) that may be present in some species. On the other hand, molluscan shellfish are filter feeders, ingesting and concentrating bacteria and viruses from the filtered water. Fish have a number of defense mechanisms, beginning with the surface slime. The slime serves as a substrate in which antibacterial mechanisms (peptides, lysozyme and lectins) act and mucous production is enhanced in response to environmental stresses (Tort *et al*., 2003) and tends to be protective against attack by bacteria. Fish also have both an innate immune system and one which appears to be an adaptive immune response (Tort *et al*., 2003). Although fish are poikilothermic, their immune response is reduced at low temperatures, particularly if there is thermal (low-temperature) shock. This response will be more limited if it occurs during spawning (such as fungal attack on the skin of salmon during the later stages of reproduction). Fish eggs contain an immune system similar to the live animal.

2.2 Seafood spoilage

The enzymatic systems present in seafood products tend to be highly active; seafood begins to undergo autolysis more rapidly than terrestrial animals. This is caused by the fact that pH typically does not decline to the level of terrestrial muscle and autolytic enzymes are compartmentalized within membranes that are easily ruptured by handling, such as crushing in the hold, pressure within a purse seine or by deep packs in ice. With some species, such as the flatfish, rapid chilling followed by evisceration is critical, since the fish will begin to digest itself both outwardly and inwardly, increasing vulnerability to bacteria, with one notable example – the movement of *Clostridium botulinum* from the intestinal area into the flesh. The same is true in heavily feeding species such as sardines. Rough handling of the fish and crushing from very large hauls and/or packs of fish in ice exceeding 30 cm may lead to rupture of the gut and seepage of bacteria into the flesh. Post-mortem energy metabolism proceeds in this order: ATP → ADP → AMP → IMP → INS → Hx, where ATP is adenosine triphosphate; ADP is adenosine diphosphate; AMP is adenosine monophosphate; IMP is inosine monophosphate; INS is inosine; and Hx is hypoxanthine. The rate of hydrolysis is species-dependent (Ehira and Uchiyama, 1987). Gram and Huss (1996) reported that it is the degradation of inosine monophosphate (IMP) that is responsible for fresh fish flavor.

Fish microflora typically include Gram (−) rods of the species *Pseudomonas, Moraxella*, *Acinetobacter*, *Shewanella*, *Flavobacter*, *Vibrio* and *Aeromonas* as well as Gram (+) species of *Bacillus*, *Micrococcus*, *Clostridium*, *Lactobacillus* and *Corynebacteria* (Gram and Huss, 1996; Shewan, 1962). In temperate waters, enteric bacteria are typically present in larger numbers. Hazen (1988) reviewed literature on the presence of enteric organisms in tropical waters. It was shown that there are significantly different numbers of coliforms in temperate versus tropical waters and that coliform bacteria may be present in the absence of fecal contamination (in tropical waters). Alternative microorganisms (to coliforms) to screen for in harvesting waters for safety were suggested to be the polio virus or *Salmonella* species (Hazen, 1988).

Fresh seafood will typically deteriorate through autolysis and lipid oxidation, while bacterial action accelerates degradation (Fletcher and Statham, 1988; Herbert *et al*., 1971), as shown by studies evaluating the differences between deterioration of sterile fillets and those subjected to bacterial spoilage. Many bacteria may be associated with spoilage but are not necessarily the actual organisms responsible for this spoilage (Gram and Huss, 1996). Herbert and Shewan (1976) showed that only 10% of the bacteria present were responsible for producing volatile sulfide spoilage odors. Often seafood is rejected for odor due to the presence of hydrogen sulfide (H_2S); methyl mercaptan (CH_3SH) and dimethyl sulfide ($(CH_3)_2S$) (Herbert *et al*., 1975) and the decomposition products of trimethylamine oxide (TMAO – an osmoregulatory compound). Early reports on the spoilage of cod (Castell *et al*., 1948) and haddock (Chai *et al*., 1968) showed that spoilage bacteria and total microbial flora were unrelated. The more pungent H_2S odors

produced in seafood were attributed to *Shewanella putrefaciens*. Older publications may cite this organism as *Pseudomonas putrefaciens* and/or *Alteromonas putrefaciens*. In cold temperatures, *Pseudomonads* tend to form ammonia via deamination of adenine nucleotides in teleosts but in elasmobranchs, urea is enzymatically degraded to ammonia (Howgate, 2010). The fruity (cantaloupe-like) odors are typical of decomposition mediated by *Pseudomonas* species. It is important to note that decomposition does not necessarily coincide with the presence of pathogens or toxins.

Iced seafood packed tightly in ship holds may develop an offensive off-odor described as 'bilgy' in the absence of obvious physical deterioration. To simulate conditions, Lapin and Koburger (1974) stored 100 g of fresh, headless shrimp under either nitrogen or air. It was shown that *S. putrefaciens* produced 250 μg H_2S in eight days at 5°C under nitrogen, with none produced from air-held shrimp. It was further demonstrated that *S. putrefaciens* produced the H_2S from decomposition of cystine and cysteine, but failed to do so when cultured on a basal medium spiked with methionine, elemental sulfur, bisulfite, sulfite or sulfate. Herbert and Shewan (1975) evaluated the spoilage of iced cod and showed that methyl mercaptan and dimethyl sulfide were produced from methionine and H_2S from cyst(e)ine.

Seafood species contain appreciable levels of non-protein (free amino acids, TMAO), nitrogen that serves as a simple nutrient source for bacteria. TMAO is reduced by some spoilage bacteria to trimethylamine (TMA) which, in limited quantities, is a malodorous compound. Greenman *et al*. (2004), determined the human detection thresholds (mol.dm^{-3}) were: Skatole (7.2×10^{-13}) < methylmercaptan (1.0×10^{-11}) < trimethylamine (1.8×10^{-11}) < isovalerate (1.8×10^{-11}) < butyrate (2.3×10^{-10}) < hydrogen sulfide (6.4×10^{-10}) < putrescine (9.1×10^{-10}) < dimethyl disulfide (5.9×10^{-8}). When TMAO is reduced to TMA, the redox potential is decreased, electrical conductivity is increased (Gram, 1992) and pH is increased (Malle *et al*., 1986). Among the TMAO-reducing organisms are *S. putrefaciens, Photobacterium phosphoreum* and, to a lesser extent, species of *Pseudomonas*, *Alteromonas* and *Alcaligenes* and some species of *Vibrio*. *P. phosphoreum* is reported to produce far more TMA than *S. putrefaciens* (Dalgaard, 1995a). As a rule, *S. putrefaciens* utilizes TMAO in anaerobic respiration on iced fish and *Pseudomonas* spp. for warm water species (Howgate, 2010). Using sterile cod juice as a medium, TMA developed when numbers of *S. putrefaciens* numbers reached 10^7 CFU/mL and the odor was detectable at 10^8 CFU/mL (Jorgensen and Huss, 1989). These researchers also determined that there are strain differences and grouped them as fast versus slow reducers of TMAO. Fast reducers grew in the presence of 6% NaCl at 25°C and had a generation time of 38–49 min; slow reducers would only grow at < 6 % NaCl at 25°C with a generation time of 55–92 min.

Care should be exercised in assuming that a strong odor of TMA indicates bacterial spoilage since cod, pollock, Pacific whiting (Spinelli and Khoury, 1981) and European hake (Rey-Mansilla *et al*., 2004) have an endogenous TMAOase. In these gadoids, TMA is degraded to dimethylamine (DMA) at −5°C to −10°C,

below the freezing point of the flesh (Spinelli and Khoury, 1981). The TMAO content of different families of marine species varies significantly (Howgate, 2010). There is no TMAO in some freshwater species while it is present in appreciable quantities in some species, such as Nile perch and tilapia, and is thought to be a relict since these fish can tolerate brackish water (Anthoni *et al*., 1990).

Marine bacteria are typically cultured in the presence of NaCl since seawater is approximately 3.25% NaCl or 0.55 M and Na is a required growth factor. Incubation temperatures are typically 20–25°C. Detection of *Shewanella putrefaciens* is facilitated if iron is a component of the agar since this organism will form Fe_2S and the colonies will be black. For many years, *Phosphobacterium phosphoreum* was not detected because it would not grow in pour plates.

2.3 Major microbiological hazards associated with fresh seafood

For most pathogens associated with seafood, cooking by the end user to an adequate temperature for a specific time will be suitable control to prevent illness. If, however, there is temperature abuse, heat-stable toxins may be formed or pathogens may have time to reproduce. Further, if seafood is harvested from waters contaminated by sewage (of either human or animal origin) or inadequate sanitation is followed at any of the unit operations from harvest to table, there will be greater opportunity for the development of seafood-borne illness. Obviously, it is undesirable to bring any seafood heavily contaminated by bacteria, viruses or amoeba into a processing facility due to the potential for contamination of 'clean' species and/or the potential for cross contamination. Furthermore, a seafood processor's HACCP plan is written for events that are *reasonably likely to occur* – not any eventuality. There are never any guarantees that a raw seafood will be free of pathogens; however, it is critical to have some assurance from the harvester, receiving dock or wholesaler that species have been harvested from waters of purity acceptable within that locale. In the United States, waters are certified by state officials, while waters further than three miles from shore are overseen by the National Marine Fisheries Service (NMFS), an agency within the National Oceanic and Atmospheric Administration. Waters for growing aquacultured species should have similar assurances of purity, although they are geographically variable and have become a topic of regular reporting in the popular press.

2.3.1 Histamine fish poisoning

Histamine fish poisoning (also referred to as scombrotoxic fish poisoning) occurs in scombroid and non-scombroid finfish species (Table 2.1) that have naturally elevated levels (up to 15 g/kg in tuna; Ijomah *et al*., 1992, as cited by Lehane and Olley, 2000) of the amino acid histidine. Marine bacteria of the family Enterobacteriaceae, especially *Morganella morganii*, some strains of *Klebsiella pneumonia* and *Hafnia alvei*, produce decarboxylases which act on histidine to produce histamine and other

Table 2.1 Species known to cause histamine fish poisoning

Alewife or River Herring	*Alosa pseudoharengus*
Amberjack or Yellowtail	*Seriola* spp.
Anchovy	*Anchoa* spp., *Anchoviella* spp., *Cetengraulis mysticetus, Engraulis* spp., *Stolephorus* spp.
Bluefish	*Pomatomus salatrix*
Bonito	*Cybiosarda elegans, Gymnossarda unicolor, Orcynopsis unicolor, Sarda* spp.
Escolar or Oilfish	*Lepidocybium flavobrunneum, Ruvettus pretiosus*
Gemfish	*Lepidocybium flavobrunneum*
Herring	*Etrumeus teres, Harengula thrissina Ilisha* spp., *Opisthopterus tardoore, Pellona ditchela, Alosa* spp.
Herring or Sea Herring or Sild and roe	*Clupea* spp.
Herring, Thread	*Opisthonema* spp.
Horse Mackerel or Scad	*Trachurus trachurus*
Jack	*Caranx* spp., *C. ignobilis, C. melampygus, C. latus, C. lugubris, C. ruber, Carangoides bartholomaei, Oligoplites saurus, Selene* spp., *Seriola rivoliana, Urapsis secunda*
Jack or Blue Runner	*Caranx crysos*
Jack or Crevalle	*Alectis indica*
Jack or Rainbow Runner	*Elagatis bipinnulata*
Jack or Roosterfish	*Nematistius pectoralis*
Kahawai	*Arripis* spp.
Mackerel	*Gasterochisma melampus, Grammatorcynus* spp., *Rastrelliger kanagurta, Scomber scombrus*
Mackerel, Chub	*Scomber* spp.
Mackerel, Jack	*Trachurus* spp.
Mackerel, Spanish	*Scomberomorus* spp.
Mackerel, Narrow-barred Spanish	*Scomberomorus commerson*
Mackerel, Spanish or King	*Scomberomorous cavalla*
Mahi-mahi	*Coryphaena* spp.
Marlin	*Makaira* spp., *Tetrapturus* spp.
Menhaden	*Brevoortia* spp., *Ethmidium maculatum*
Pilchard or Sardine	*Sardina pilchardus, Sardinops* spp.
Sailfish	*Istiophorus platypterus*
Sardine	*Harengula* spp., *Sardinella* spp.
Saury	*Cololabis saira, Scomberesox saurus*
Scad or Horse Mackerel	*Trachurus* spp.
Shad	*Alosa* spp.
Shad, Gizzard	*Dorosoma* spp., *Nematalosa vlaminghi*
Shad, Hilsa	*Tenualosa ilisha*
Spearfish	*Tetrapturus* spp.
Sprat or Bristling	*Sprattus* spp.
Trevally	*Caranx* spp.
Tuna (small)	*Allothunnus fallai, Auxis* spp., *Euthynnus* spp., *Katsuwonus pelamis, Thunnus tonggol*

Table 2.1 Continued

Tuna (large)	*Thunnus alalunga, Thunnus albacores, Thunnus atlanticus, Thunnus maccoyii, Thunnus obesus, Thunnus thynnus*
Wahoo	*Acanthocybium solandri*
Yellowtail or Amberjack	*Seriola lalandei*

Source: FDA (2011).

biogenic amines such as putrescine (from ornithine), cadaverine (from lysine) and spermidine and spermine (from arginine; Lehane and Olley, 2000). Papadopoulou *et al*. (2007) identified the histamine-producing bacteria *E. coli* (87%); *Proteus mirabilis* (51%); *P. vulgaris* (45%); *Klebsiella ozonae* (40%); *Morganella morganii* (30%); and *Hafnia alvei* (20%) from 360 samples of locally (N.W. Greece) caught freshwater finfish, marine finfish and shellfish sampled within 24 h of harvest. Histamine, and, it is thought, related biogenic amines, act on the cardiovascular system to cause dilation of blood vessels, hypotension, urticaria (itching), flushing and headache. Histamine fish poisoning is insidious because it can take place quickly without any outward indications of fish deterioration since it is quickly formed at 70–90°F (21.1–32.2°C) which are considered abuse temperatures, but common as ambient temperatures in the Gulf of Mexico and more tropical regions (FDA, 2001). Rapid chilling of the affected species is the best means to control the development of the biogenic amines. Once formed, histamine is unaffected by freezing, cooking or retorting. The tolerance level allowed by the FDA in the United States is 50 ppm, while the Codex Alimentarius (1995 and 1981) lists 100 and 200 ppm in raw tuna, sardines and sardine-type products as raw materials and canned goods, respectively.

2.3.2 *Salmonella* spp.

Contamination with *Salmonella* species is the leading cause of seafood-borne bacterial illness in the United States and the leading cause for detention of imports at the border (Wan Norhana *et al*., 2010). Some domestic species, such as alligator and frogs legs, have naturally occurring high levels of this organism. There is a zero tolerance for *Salmonella* in either raw or cooked shrimp in Australia, New Zealand, the EU, Hong Kong or the United States (Wan Norhana *et al*., 2010). Further, the United States has a zero tolerance for *Salmonella* in any raw seafood product. Historically, the presence of fecal coliforms has been considered a presumptive for this organism; however, this is not a reliable indicator. Brands *et al*. (2005) evaluated the incidence of *Salmonella* in Pacific, Atlantic and Gulf of Mexico areas of the United States during the summer and winter. It was demonstrated that it was not related to fecal coliforms, but was correlated to population density. *S. enterica* serovar Newport was isolated more in summer than winter months. It is important to note that this study failed to make meaningful comparisons of test locations with proximity to residential areas, river deltas and agricultural/pasture areas. Further, no statistical design was used nor statistical analyses conducted.

Table 2.2 Growth factors associated with *Salmonella* species

Parameter	Minimum	Optimum	Maximum
Temperature (°C)	5.2	35–37	45–47
pH	3.7	5.5–7.5	9.5
Salt tolerance (%)	—	—	4–5
Water phase NaCl (%)	—	—	8.0
Water activity (a_w)	0.94		0.99

Source: Adapted from Wan Norhana *et al*. (2009); FDA (2011).

Salmonella are Gram (−), non-spore-forming rods and facultative anaerobes. Some key growth factors are listed in Table 2.2. There are more than 2500 serovars that are considered to be pathogens. Many strains of this pathogen can survive freezing for up to nine months. Wan Norhana *et al*. (2010), have recently published an impressive review of *Salmonella* in shrimp aquaculture, processing, distribution and retail.

2.3.3 *Listeria monocytogenes*

Listeria monocytogenes is a ubiquitous, Gram (+), non-spore-forming rod that is made mobile by a peritrichious flagella, is halotolerant, grows at refrigerated temperatures and is a facultative anaerobe (Table 2.3). This bacterium is opportunistic toward pregnant women and is a cause of spontaneous abortion. Mortality overall is estimated to range between 20% and 40% (Wan Norhana *et al*., 2010). *Listeria* is not as prevalent as *Salmonella*, but it is commonly observed to occur in seafoods and may be an environmental inhabitant in processing facilities (Gudbjörnsdóttir *et al*., 2004). Typical value-added processing in the United States includes steps taken within the HACCP plan to assure its destruction through cooking. Post-processing contamination through handling, air or unit operations is a common cause for its reintroduction into a food product. For example, crab is cooked to an end time and temperature to destroy *L. monocytogenes*. It is then cooled to 4°C, picked and kept below 20°C before floating the flesh in a 25% NaCl brine (4°C) to remove any residual bits of shell. The brine is prepared each morning and cycled throughout the day. *L. monocytogenes* could theoretically be introduced during the picking stage or through contact with the brine. If the brine was to be contaminated early in the day, the entire day's production could be in jeopardy.

Another factor to consider with *L. monocytogenes* is the observation by Shineman and Harrison (1994) that this organism grew faster and reached a higher population on shrimp and catfish than when inoculated onto either beef or chicken. The pH differences (pH 5.7 on fresh beef versus 7.6 on fresh shrimp) were first considered to be the reason until the shrimp was acidified and the beef pH raised and the growth rates were unchanged. It could be important to determine if the catfish and shrimp had been treated with sodium tripolyphosphate alone or in combination with NaCl (to control drip loss) to determine if sodium was a contributing factor.

Table 2.3 Growth conditions for *L. monocytogenes*

Parameter	Minimum	Optimum	Maximum
Temperature (°C)	−0.4	37	45
pH	4.4	7.0	9.6
Salt tolerance (%)	—	10	25% @ 4°C
Water activity (a_w)	0.92	0.92	0.97

Source: Adapted from Wan Norhana (2010).

2.3.4 The *Vibrio* species

The *Vibrio* species have long been associated with cholera caused by *Vibrio cholerae*, which is associated with catastrophic events that usually include flood and famine. *V. parahemoylyticus* and *V. vulnificus* are also associated with seafood-borne disease. *V. vulnificus* is the most virulent of the known species. It can lead to fulminating septicemia from ingestion or contact via an open wound. Slight abrasions formed while harvesting shrimp and amorous activity at the beach have been known to cause wounds that have become infected with *V. vulnificus* (Midturi *et al*., 2005).

Vibrio vulnificus

Vibrio vulnificus can cause septicemia in vulnerable individuals, including those with cirrhosis of the liver (primarily middle-age alcoholic men), hemochromatosis, hypochlorhydria and/or immunosuppression. It is most often associated with the consumption of raw or mildly cooked oysters, but can be introduced through an open wound. The fatality rate is estimated to be 60% in those who are susceptible (Linkous and Oliver, 1999). This organism thrives during the warmer, summer months. Since the oyster is a filter feeder, it ingests and concentrates bacteria like *V. vulnificus* (as well as viruses). As a consequence, the National Shellfish Sanitation Program (the US agency that oversees molluscan shellfish) has recommended maximum times (6 h) between oyster harvest and cooling oysters to < 12.7°C to limit reproduction of *Vibrio vulnificus*. Most Gulf of Mexico oysters must be placed under refrigeration within 1 h of dredging during the months of May through October. The time specified is dependent upon the ambient air or water temperature for oysters harvested from other geographic regions. See Table 2.4 for key growth conditions.

Cook (1994) conducted a study of oysters (*Crassostrea virginica*) harvested from Mobile Bay (Alabama, US) during the summer when water temperatures ranged from 28°C to 31°C. One third of the oysters were air cooled within 60 min to 7–10°C and maintained at 10°C in a cooler. The remaining oysters were transported in an insulated carton (to prevent further heating on deck) and within 2 h were divided into three groups and stored at 13 or 18°C or outdoors (in a burlap bag) at ambient air temperature (AAT). *V. vulnificus* counts were determined 12 and 30 h later. The results indicated that counts decreased at 10 and 13°C; there was no change after 12 h at 18°C with nearly a one log increase by 30 h, and greater than one log increase after 12 and 30 h at AAT.

Table 2.4 Growth conditions for *V. vulnificus*

Parameter	Minimum	Maximum
Temperature (°C)	8	43
pH	5	10
Water phase salt (%)		5
Water activity (a_w)	0.96	

Source: Adapted from FDA (2011).

Schwarz (2000) took this one step further and used the same species of oyster harvested off the coast of Texas. Half of each harvest was chilled in an ice bath to a core temperature of 35°C in a time of < 60 min. The second half was packed in 110 pound burlap bags, stacked on pallets and held in a conventional cooler. The rapidly cooled oysters reached a reduction in numbers of *V. vulnificus* of 97.8% while the conventionally cooled oysters took four days to reach the same reduction in numbers. Mortality was similar among treatments and there were no differences in sensory attributes. Freshly shucked oysters show a decrease in numbers of *V. vulnificus* during chill storage while viruses, if present, do not increase since they rely on a host for reproduction.

Vibrio parahemolyticus

Vibrio parahemolyticus is indigenous to the marine environment and is predominant in the warmer months of the year. In the United States, it exists in the sediment during the winter months and moves into the water column as ambient temperature rises (Rodrick, 1991). Incidence of this organism has been shown to fluctuate less in tropical waters and in a two-year study of two bays in Southwest India, it was identified in 98.5% of oysters harvested (Deepanjali *et al*., 2005). *Vibrio parahemolyticus* is reported to be responsible for an estimated 70% of seafood-borne illness in Japan and is attributed to consumption of raw or lightly cooked seafood (Kaneko and Colwell, 1973). DePaola *et al*. (2003) have reported that oysters have the ability to concentrate *Vibrio* species 100-fold over the levels existing in the growing waters. See Table 2.5 for key growth conditions.

Virulence of *Vibrio parahemolyticus* is associated with the presence of a beta-hemolysis (Kanagawa reaction) on high NaCl blood agar (Wagatsuma agar) and pathogenicity is identified in only 1–2% of environmental samples (Kelly and Stroh, 1988; Tada *et al*., 1992). Recently, levels of pathogenic *V. parahemolyticus* have risen in Asia (10.2%) and the United States (12.8%) as reported by Deepanjali *et al*. (2005) and DePaola *et al*. (2003), respectively. This increase in incidence has been attributed to a new clone of the virulent O3:K6 serovar of *V. parahemolyticus* (Matsumota *et al*., 2000). It is a halophile that occurs in estuarine environments on a global basis (DePaola *et al*., 1990).

Vibrio cholerae

There are toxigenic and nontoxigenic serotypes of *Vibrio cholerae*. There has not been a major outbreak of cholera in the United States since 1911. This is not the

Table 2.5 Growth conditions for *V. parahemolyticus*

Parameter	Minimum	Maximum
Temperature (°C)	5.0	45.3
pH	4.8	11.0
Water phase salt (%)		10
Water activity (a_w)	0.94	

Source: Adapted from FDA (2011).

Table 2.6 Growth conditions for *V. cholerae*

Parameter	Minimum	Maximum
Temperature (°C)	10	43
pH	5.0	10.0
Water phase salt (%)		6
Water activity (a_w)	0.97	

Source: Adapted from FDA (2011).

case with other areas of the world. For example, in 1991, there were 46 320 cases of a diarrheal illness in Ecuador, with 36% of stool samples testing positive for *V. cholerae*. There were 697 deaths in this case study (Weber *et al*., 1994). It is important to note that the strain of cholera showed multi-drug resistance.

Seafood and water are the usual vectors of transmission. For key growth conditions, see Table 2.6. Sewage contamination of drinking water sources and shellfish growing areas leads to the presence of *V. cholerae*. Another source of introduction is through ballast water. McCarthy and Khambaty (1994) studied ballast water from 19 cargo ships in Mobile, AL, and Pasagoula and Gulfport, MS, ports on the Gulf of Mexico. Ballast water from five of 19 ships were positive and two of 19 bilge water samples contained *V. cholerae* at levels of log 10^6 or greater. Four of five ships in port tested positive for strains of *V. cholerae* not indigenous to the Gulf of Mexico. Although the FDA recommended that ships traveling to the US dump ballast water in the high seas, not all boat captains complied.

In McCarthy and Khambaty's study (1994), the presence of *V. cholerae* was not synonymous with the presence of fecal coliforms. Murphree and Tamplin (1991) held oysters at 15°C, 19°C or 25°C under controlled conditions (depuration) and showed that *V. cholerae* was present at greater levels than *E. coli* and *S. tallahassee*. Furthermore, *V. cholerae* numbers continued to increase, especially at temperatures > 15°C. This clearly indicates that screening growing waters for *E. coli* is an inadequate predictor for the presence of *V. cholerae*; more suitable alternatives need to be identified.

2.3.5 *Staphylococcus aureus*

Staphylococcus aureus is not an organism normally associated with fresh seafood; it is more often associated with egg, chicken, tuna and potato salads containing

Table 2.7 Growth conditions for *S. aureus*

Parameter	Minimum	Optimum	Maximum
Temperature (°C)	6–7	—	50
pH	4.0	—	10
Salt tolerance (%)	—	7–10	20
Water phase NaCl (%)	—	—	—
Water activity (A_w)	0.83	—	0.99

Source: Adapted from Bremer *et al*. (2004); FDA (2011).

chopped or diced ingredients that may be mixed with the hands. Humans may also be carriers of *S. aureus* in skin cracks adjacent to the fingernails, cuts and wounds. For key growth conditions, see Table 2.7. *S. aureus* is most commonly introduced to seafood as a post-processing contaminant. In India, 168 samples of fish products and 87 samples from factory workers were analyzed for enterotoxigenic *S. aureus*. It was shown that 17% of the seafood and 62% of the workers tested positive for this organism. Incidence here was attributed to delay in processing, inadequate refrigeration, poor personnel hygiene and post-processing contamination (Simon and Sanjeev, 2007).

2.3.6 *Clostridium botulinum*

Clostridium botulinum (Groups I and II) is a spore-forming anaerobe that may form a heat-stable toxin of significant lethality to humans. Non-proteolytic Type E (Group II with non-proteolytic [Group I] types B and F) is the form most often associated with the estuarine environment and its spores are less heat resistant than proteolytic Type B. It has been estimated to occur at a level of one to 2400 spores per kilogram of seafood (Dodds, 1992). Type E is of most concern because it can grow at temperatures exceeding 37.9°F (3.3°C). *C. botulinum* spore germination and outgrowth is not of concern in atmospherically packaged iced or frozen seafood. It is of concern, though, in anaerobic packaging. An anaerobic atmosphere is defined as packaging under either vacuum or modified atmospheres (including carbon monoxide or 'cold smoke' used to fix color in tuna, mahi-mahi and similar species), in hermetically sealed containers, in deep containers from which air is expressed (e.g., caviar in deep containers) or in oil. Packaging film that provides an oxygen transmission rate of 10 000 $cm^3/m^2/24$ h at 24°C may be regarded as an oxygen permeable packaging material for fishery products.

The toxin produced by *C. botulinum* is of great concern because it is heat-stable and the most toxic of bacterial toxins known. The toxin binds to presynaptic nerve ending and blocks acetylcholine secretion to the synaptic cleft, thus causing paralysis. The incubation time is 12–72 h and begins with impaired vision, salivation, slurred speech and difficulty swallowing (Lindström *et al*., 2006). Recovery occurs only with intravenous administration of an antitoxin.

Table 2.8 Growth conditions for *C. botulinum* type E and non-proteolytic types B and F

Parameter	Minimum	Maximum
Temperature (°C)	3.3	45
pH	5.0	9.0
Water phase salt (%)	—	5
Water activity (a_w)	0.97	—

Source: Adapted from FDA (2001).

Graham *et al*. (1996) evaluated 78 combinations of anaerobic conditions (H_2/N_2, 10:90 v/v), a defined growth media, temperatures that varied from 4°C to 30°C, pH levels from 5.0 to 7.3 and NaCl levels ranging from 0.1% to 5.0% on growth and toxin formation of *C. botulinum* (mixed culture). The results indicated that growth resulted under 58 combinations, but no growth occurred at pH < 5.1 or at 5% NaCl. The authors showed elegant Gompertz and Baranyi models for *C. botulinum* growth.

Reddy *et al*. (1996) evaluated tilapia fillets inoculated with spores of *C. botulinum* type E and packaged under MAP (75% CO_2, 25% N_2), vacuum or 100% air. Samples were incubated at 4°C, 8°C or 16°C for up to 90 days. Key results from this study showed that at the abuse temperature (16°C) spoilage occurred either at or before toxin production under all three atmospheres. At storage temperatures of either 4°C or 8°C, spoilage preceded toxin formation. MAP retarded TMA development. Lindström *et al*. (2006) conducted a comprehensive review of literature and showed that the presence of lysozyme in foods imparts heat resistance to *C. botulinum* (Group II) spores. Spores impermeable to lysozyme are less heat resistant than those permeable to the enzyme. This is the reason for the biphasic thermal death time curve. Raw foods contain more lysozyme than heat-processed foods. Moist heat (70% relative humidity) has been shown to be more destructive to these spores. For key growth conditions, see Table 2.8.

Recommendations to inhibit growth and toxin formation of *C. botulinum* for seafood packaged in the absence of oxygen include an NaCl content of 5% (w/v); an $a_w < 0.97$; or pH of < 5.0 (Lindström *et al*., 2006). A storage temperature of 3.3°C or less is always recommended. Sodium nitrite is also an inhibitor of *C. botulinum*, but its use is restricted and varies by country.

2.3.7 *Aeromonas* spp.

Aeromonas hydrophila is the most studied species and is common to seafood and fresh flowing, stagnant and brackish water (Bremer *et al*., 2003). This organism is rod-shaped and has strains that are either mesophilic (35°C, optimum) or psychrotropic (15–20°C), however; it is believed the psychrotrophic strains are the more common pathogens (Bremer *et al*., 2003). Studies have shown 78.4% (Thayumanavan *et al*., 2003) to 98% (Ullmann *et al*., 2005) of isolates produce hemolysin. Illness attributed to Aeromonads range from wound infection and diarrhea to septicemia, meningitis or kidney failure (Thayumanavan *et al*., 2003; Ullmann *et al*., 2005).

Papadopoulou *et al*. (2007) obtained local seafood within 24 h of harvest and showed *A. hydrophila* to be the predominant organism in freshwater fish (38%); 73–86% of shellfish (mussels < octopus < prawns < squid) and 93% of marine finfish. As an aside, in this study it was observed that mussels carried the largest load of pathogens, including *E. coli* (83.3%); *Yersinia enterocolitica* (40%); *S. aureus* (56.6%) and *Listeria* spp. (3.3%). *Pleisimonas shigelloides* was isolated from marine finfish (4%) and freshwater fish (2%), but not from shellfish. It is interesting to note that *Salmonella* spp., *V. parahemolyticus* and C. *perfringens* were not detected in any of the 360 samples. *Listeria* spp. were detected in only mussels and in only 2% of those samples.

Vivekanandhan *et al*. (2005) evaluated 536 fish and 278 prawns (sample sizes not provided) for the presence of *A. hydrophila* from the major seafood market in Coimbatore, South India, over a two-year period. The incidence of *A. hydrophila* was 17.6% in prawns and 33.6% in finfish and the occurrence was highest during the monsoon season. Distribution of *A. hydrophila* was highest in the intestines (38.4%), the body surface (32.5%) and the gills (29.1%). The investigators concluded that these results were of concern since this organism is an emerging pathogen.

Thayumanavan *et al*. (2003) reported on harvests evaluated from four regions of southern India over the period of one year. Of the 514 samples tested, 37.3% of the 410 finfish and 35.6% of the 104 prawns tested positive for *A. hydrophila*. Two hundred and fifty-five strains were isolated and, of these, 78.4% produced hemolysin. The more concerning aspect of this study is the number of strains with antibiotic resistance (Table 2.9).

Table 2.9 Antibiotic resistance (%) among *A. hydrophila* strains isolated from fish and prawn

Antibiotic (↓)	Fish (↓) $n = 213$	Prawn (↓) $n = 42$	Total (↓) $n = 255$
Bacitracin	100.0	100.0	100.0
Chloramphenicol	0	0	0
Erythromycin	90.3	84.6	87.5
Gentamicin	4.2	3.0	3.6
Kanamycin	84.5	96.0	90.3
Methicillin	94.2	90.3	92.3
Nalidixic acid	11.2	10.9	11.1
Neomycin	89.7	80.9	85.3
Novobicin	91.6	93.0	92.3
Polymyxin B	82.4	86.2	84.4
Rifampicin	92.6	89.9	91.3
Streptomycin	5.1	3.9	4.5
Tetracycline	44.6	41.9	43.3
Trimethoprim	56.3	62.4	59.4
Vancomyxin	71.6	80.9	76.3

Source: Thayumanavan *et al*. (2003).

2.3.8 *Giardia lamblia*

Giardia was first discovered by Antonj van Leewenhoek in 1681, yet the first proven water-borne transmission was reported in 1970 (Boreham *et al*., 1990). Van Leewenhoek ground lenses for more than 500 microscopes, which were used to make the first observations of protozoans from loose stools. Vilem Lambl made observations of fecal stools from children with diarrhea and described the flagellated organisms that now bear his name (Boreham *et al*., 1990). *Giardia lamblia* is also known as *G. intesinalis* and *G. duodenalis*. Drinking untreated stream water, presumably contaminated with animal excreta, is the presumptive mode of transmission for hikers; however, the mode of transmission is most commonly the fecal-oral route (Boreham *et al*., 1990).

An outbreak of Giardiasis that exemplifies the mode of transmission occurred in Minnesota. Twenty-nine of 60 employees at a school developed Giardiasis 4–27 days after consuming a dish prepared from home-canned salmon. The preparer fell ill 19 days after making the dish. It was learned that she had changed the diaper of an infant who was asymptomatic of Giardiasis before preparing the food (Hedburg *et al*., 1994; Osterholm *et al*., 1981).

It is estimated that the infective dose ranges from ten to 100 cysts, with an incubation period ranging between 12 and 20 days (Butt *et al*., 2004). Symptoms may include nausea, chills, fever, epigastric pain and foul-smelling diarrhea that may contain mucous and blood (Butt *et al*., 2004). There are few reports of *Giardia*-borne illness attributed to seafood; however, most seafood is consumed away from home. The turnover in restaurants is estimated to be 42% in food service establishments and it is difficult to provide sanitation and personal hygiene training to so many new personnel (Hedburg *et al*., 1994). Further, an organism cannot be the causative factor if the diagnostician does not assay for its presence. This protozoan has now been identified in 11 of 12 growing waters of mussels in Ireland (Lucy *et al*., 2008); their cysts in *Macoma* clams from the Rhode River, a Chesapeake Bay subestuary (Graczyk *et al*., 1999); 11.1% of Spanish oysters (*Oestrea edulis*; Gómez-Couso *et al*., 2004); 3.4% of Dutch oysters (*Crassostrea gigas*; Schets *et al*., 2007) and 41.8% of mussels farmed in northwest Spain (Gómez-Couso *et al*., 2005). There was sufficient concern for parasites in seafood that the province of Ontario (Canada) passed a regulation in 2004 that all 'raw, raw-marinated and partially cooked seafood be frozen before preparation and serving to temperature of −20°C or below for seven days or to a temperature of −35°C or below for 15 hours' (Saul, 2005). The regulation was rescinded later that year. It is speculated that Giardiasis will increase due to the enhanced interest in the consumption of raw and minimally cooked foods (Hedburg *et al*., 1994). Since molluscan bivalves have the propensity to concentrate, their potential for acting as transmission vehicles for outbreaks of protozoan infections in humans has been well reviewed (Robertson, 2007).

2.4 Live animals

Some seafood products, especially oysters, clams and mussels, are shipped live. Of these mollusks, oysters are commonly consumed live. The common test for

determining viability of a molluscan shellfish is to tap a gaping shell with the presumption that a live animal will quickly close its shell and be suitable for consumption. In the United States, live oysters are commonly packed in ca. 110 pound (50 kg) burlap sacks and transported in coolers held at ≤ 50°F (10°C). In Europe, they may be transported, at chilled temperatures, in polystyrene boxes containing freshwater ice with either a thin layer of ice on top or wood wool dampened with seawater.

Aaraas *et al*. (2004) evaluated the quality of live oysters held in seawater at 9°C (control); packed on ice with a thin layer of ice on the surface (1°C) or on ice with wood wool wetted with seawater atop the animals in a cooler (5°C). Mantle fluid was sampled for spoilage indicators such as *S. putrefaciens* at days 0, 7, 12 and 19 and sensory evaluation was conducted at days 2, 5, 9, 12, 16, 19 and 23. Reaction to a pinch test and heart beat were used to determine if the animals were alive; surface pH of the mantle and histology of sacrificed animals were also monitored. The results indicated that most animals were alive after three weeks' storage. Within a few days, soft tissues appeared to be slightly desiccated, with some retraction of the mantle; this progressed throughout the study in the iced and cold-stored animals. In these same treatments, mantle fluid became opaque with mucous accumulation along the gills, which was evident by day 19. The pH of the live animals ranged between 5.6 and 6.3. Black colonies, indicative of spoilage microorganisms, appeared on the petri plates by day 12 from the iced oysters and by day 19 from the cold-stored animals at 10^4 CFU/mL mantle fluid. Histological changes were first evident in the digestive tract. By day 23, iced and cold-stored oysters had developed significant ($P < 0.05$) and objectionable odors described as seaweed, fish and mud. The authors concluded that shell closing and response to pinching did not necessarily indicate that oysters were suitable for consumption. Fresh odor and plumpness of the animal was a far better gauge of freshness.

2.5 Major hazards associated with processed and packaged seafood

This section looks at different approaches to packaging seafood and considers the pros and cons with regard to maintaining quality during storage.

2.5.1 Modified atmosphere storage

In 1930, it was reported that the shelf-life of muscle foods could be extended in CO_2 rich atmospheres (Killeffer, 1930). Modified atmosphere packaging (MAP) is the process of preserving a food in atmospheres other than ambient air. There is regulatory concern over vacuum or anoxic packaging of refrigerated seafood due to the potential for the germination, release of toxin and growth of *Clostridium botulinum*. Type E is the strain most often associated with seafood. To control growth of this organism, vacuum packaged seafood products must be held at ≤ 3.3°C.

Unlike vacuum packaging, atmospheres of mixed gases for seafood frequently contain some oxygen as a mechanism to prevent growth of *C. botulinum*.

Typically, under vacuum and modified atmosphere packaging, the spoilage microflora shifts from Gram (−) to Gram (+) bacteria (Banks *et al*., 1980). Historically, it was thought that this would represent a shift from Pseudomonads to Lactobacilli. Using CO_2 in the gas mixture has long been known to hydrate and form carbonic acid, which was thought to favor the shift to Lactic acid organisms. Fish fillets stored under MAP typically show lower bacterial numbers and extended shelf life when compared against counterparts stored in air.

Dalgaard (1995b) isolated *S. putrefaciens* and *P. phosphoreum* from packed cod fillets stored at 0°C and evaluated the effect of varying levels of CO_2 (0%, 25%, 50%, 75% or 100%) on the growth in cooked fish muscle juice and growth medium broth. Neither organism showed a lag in the growth curve. It is interesting to note that growth of *S. putrefaciens* was inhibited by 35% and 65% in 20% and 60% CO_2, respectively. *P. phosphoreum* was resistant to the inhibitory effects of CO_2.

Dalgaard *et al*. (1998) extended this work to evaluate the effect of varying levels (125, 250 and 500 ppm) of $Na_2CaEDTA$, potassium sorbate or protamine sulfate on *P. phosphoreum* grown in either growth medium broth or on sterile cod muscle blocks, in MAP (50% each N_2 and CO_2) or air at 5°C. The $Na_2CaEDTA$ was selected because *P. phosphoreum* has a high mineral requirement and a chelator may bind required growth factors; potassium sorbate, because it is an antimicrobial with GRAS (generally recognized as safe) status, and protamine sulfate, because it is an inhibitor to some pathogens and spoilage bacteria. Preliminary screening showed $Na_2CaEDTA$ at the 500 ppm level to be the most effective inhibitor to the target organism so it was selected for extended studies with naturally contaminated cod fillets. MAP extended the shelf life of cod fillets up to three days without $Na_2CaEDTA$ and six to eight days longer with the chelator than holding in air. Total bacterial counts were not significantly different and odors of decomposition were not masked, thus indicating that MAP plus a chelator did not represent a safety hazard by inhibiting the development of spoilage odors.

Mejlholm *et al*. (2005) evaluated the shift in microflora of cooked and peeled shrimp (*Pandalus borealis*) in MAP (50% CO_2; 30% N_2 and 20% O_2) held at 2°C, 5°C or 8°C and after four months' freezing of the MAP product. It is interesting to note that the CO_2 concentration decreased (to ca. 25%) rapidly after packaging due to the absorption by the shrimp. As storage temperature increased from 2°C to 8°C, shelf life decreased from 25–26 days to nine to ten days. It is important to note that populations of *L. monocytogenes* increased 1000-fold before the shrimp were judged spoiled from a sensory aspect; however, this was not observed at 2°C. After freezing, the time to reach a 100-fold increase in *Listeria* was extended from 7.4 to 18.8 days. Both Mejlholm *et al*. (2005) and Lyver (1997) observed that MAP storage failed to inhibit growth of *L. monocytogenes* at refrigerated temperatures in shrimp and seafood nuggets, respectively. Total volatile nitrogen (TVN) was significantly inhibited in MAP-stored shrimp held at 2°C, but not at either 5°C or 8°C. TMA was not detected prior to termination of the study due to

sensory spoilage. The predominant spoilage microflora (n = 116) were isolated and identified as *Carnobacterium maltaromaticum* (a genus of lactic acid bacteria; 60%); *Brocothrix thermosphactum* (27%) and *Psychrobacter* spp. (13%). Development of spoilage odors was verified by culturing *C. maltaromaticum* and *B. thermosphactum* alone and in combination and the combined cultures showed the sour, wet dog and chlorine-like aroma that developed in the spoiled shrimp.

Studies of cooked and peeled shrimp inoculated with *Carnobacterium divergens*, *C. maltaromaticum* and *C. mobile* alone or in combination with *B. thermosphactum* and held under MAP (50% CO_2; 30% N_2 and 20% O_2) at 5°C were conducted to evaluate spoilage development and metabolites (Laursen *et al*., 2006). Different species of these bacteria showed markedly different effects on shrimp spoilage odor development. For example, *C. maltaromaticum* cluster H growth resulted in intense off-odor development in shrimp, while cluster L growth resulted in limited spoilage effects. *C. maltaromaticum* cluster H isolates formed 2- and 3-methyl-1-butanal; 2- and 3-methyl-1-butanol and ketones with five or more carbon atoms. These ketones have been described as having the sweet and fruity odors characteristic of bacterial decomposition. Both *C. divergens* and *C. maltaromaticum* cluster H metabolized tyramine from tryptophan, but *C. maltaromaticum* cluster L only did so at significantly ($P < 0.001$) lower levels. Tyramine is of significance to sensitive individuals with innate low levels of monoamine oxidase (MAO) or those on MAO inhibitors. Laursen *et al*. (2006) confirmed the reported wet dog odor arising from mixed cultures of *C. maltaromaticum* and *B. thermosphactum* reported by Mejlholm *et al*. (2005).

Carnobacterium spp. have been shown to synthesize bacteriocins. These have been proposed to be listericidal in cold-smoked salmon (Lebois *et al*., 2004), which was attributed to both low-temperature holding and a synergism with NaCl, but were not listericidal in cooked shrimp (Mejlholm *et al*., 2005). Leisner *et al*. (2007) have published a review including the beneficial effects of the *Carnobacterium* spp. on inhibition of fish pathogens. Poysky *et al*. (1997), showed that the effect of heat and either smoke or liquid smoke was listericidal in smoked salmon. Himelbloom *et al*. (2007), showed the listericidal impact to be greater if liquid smoke was applied before the pellicle formed, which could be a protective barrier to pathogens from the phenolics in hot and cold smoked salmon. Listeria is also identified as an issue in cold-smoked salmon (Nakamura *et al*., 2004).

2.5.2 Dried and pickled seafood

Seafood products inadequately dried will be more prone to spoilage by osmotolerant molds and microbial hazards from toxins produced by *Clostridium botulinum* or *S. aureus.* Shelf-stable dried seafood should be dried to an a_w of 0.85 or lower (FDA, 2001). Pickled products (herring) will be spoiled by *Lactobacillus collinoides* and *L. pastorianus* (Varnam, 2002). Magnússon and Möller (1985) reported characterizing a bacterium that caused ropiness in the brine of sugar-salted herring. Ropiness was caused by a fructose (levan) polysaccharide formed from sucrose.

Characterization of the bacterium showed it to be a halotolerant, Gram (−), non-motile, oxidase positive rod that best compared with the genus formerly known as *Achromobacter*. Optimal growth conditions included 10% NaCl, a neutral pH and an incubation temperature of 22°C. Prevention of ropiness could be achieved by substituting glucose for sucrose in the formulation; adding potassium sorbate to a level of 0.01–0.05%; using either new or thoroughly cleaned (if wood) barrels; prevention of cross contamination and adequate temperature control.

2.6 Future trends

The first steps in producing high quality seafood products are good manufacturing practices, rapid chilling post-harvest, maintaining the cold chain, good sanitation and proper handling practices. In the United States, most seafood packaging materials are passive or act to protect the seafood from oxygen, moisture (Yam *et al*., 2005), desiccation and/or bacterial contamination. Packaging changed dramatically after the Tylenol tampering and cyanide poisoning incident of 1982 that killed seven people in the Chicago suburbs. Almost overnight, most retail food in the United States was sold in tamper-evident packaging. Packaging has continued to evolve into active protection of food. The protection may take the role of shelf-life extension (Miltz *et al*., 1995) and/or to change the environment in the package to improve safety and/or sensory properties of the food (Vermeiren *et al*., 1999). Active packaging has further been defined to be either 'intelligent' or 'smart'. Intelligent packaging is defined as having the ability to communicate the condition and location of the food while smart packaging facilitates communication (bar codes and radio frequency identification tags) on food quality and/or safety indicators as time/temperature abuse, gases and biosensors (Yam *et al*., 2005).

2.6.1 Time temperature indicators

The earliest form of smart packaging is the time/temperature indicator (TTI). This has evolved from cumbersome leads connected to a data logger to small adhesive labels that are affixed to packages or their master carton. TTIs are produced to indicate if a critical temperature has been exceeded and provide either a partial history or a full history (Singh, 2000). These devices are ideally suited for use on refrigerated packages containing histamine-forming fish because histamine formation can easily occur with temperature abuse, as previously noted; they can indicate on vacuum and MAP seafoods whether temperature abuse has occurred, thus allowing germination of *C. botulinum* Type E. TTIs could also be used on frozen foods to indicate temperature abuse and refreezing. Using TTIs would seem like an obvious solution to assure that temperature abuse has not taken place; however, at an estimated cost of $0.10 per package their use in the United States is limited (Kramer, 2009) and there are reports that consumers are confused over TTIs that change from yellow to pink with temperature abuse (Kramer, 2007).

2.6.2 Oxygen-permeable films

As an alternative to MAP, CPT Plastics (Edgarton, WI) introduced a polypropylene tray that uses 30% less energy and waste and is sealed with a film with an oxygen transmission rate of 250 000 cc/m^2/24 h. The breathable package cycles oxygen and results in an eight-day extension of shelf life. The packaging film is estimated to cost $0.30–$0.35 per unit. This was introduced in 2006 but the customer base is low (10–12 users) and is sold into larger outlets as WalMart and Safeway (Kramer, 2007).

2.6.3 Total volatile base nitrogen indicators

Post-mortem biochemical changes take place with time and may be monitored. EU Directive 95/149/EEC (determination of total volatile base nitrogen TVB-N, a combination of TMA, DMA and NH_3) must be used if freshness of seafood cannot be confirmed by sensory evaluation. Byrne *et al*. (2002), described the development of a rapid method for determination of TVB-N. Cresol red, sensitive to color change from yellow to purple when deprotonated, was embedded onto polyethylene tetraphtalate (PET) strips. Pieces of cod and orange roughy were incubated in the presence of the strips and the color change correlated with UV/Vis spectra at 573 nm and log NH_3 concentration in the headspace. Byrne *et al*. (2003) extended this work to orange roughy and black scabbard of known origin and whiting obtained from a local market; it was concluded that color change could be monitored with a simple illumination source, such as a light-emitting source (LED) and photodiode detector. Pacquit *et al*. (2004), developed the LED and verified the sensor to monitor spoilage of cod, whiting, roundnose, brigadier and cardinal. Pacquit *et al*. (2007) further refined the sensor and showed the change in dye color corresponded with a 'best used by' coding concept. Other gas indicators may include oxygen, change in MAP and microbial respiration.

2.6.4 Bacteriocins

Bacteriocins have been extensively reviewed (Calo-Mata *et al*., 2008); however, there are limitations to their effectiveness over time of storage. Many consumers believe that less is better when it comes to added food ingredients. Surveys often show that the consumer wants a safe food supply, but price point will limit the extent of advances that can be made.

2.6.5 Irradiation

Irradiation will result in greater assurance of safety but the radura symbol is often a deterrent to the consumer. It has been estimated that 50% of US consumers will accept irradiated product if it is no more expensive and 80–90% will be accepting if there is assurance that it is safer because of pathogen destruction (Tauxe, 2001). Most consumers do not realize that, globally, 405 000 tons of food were irradiated in 2005 (Kume *et al*., 2009). The irradiated foods consisted of spices and

dry vegetables (46%); sprout inhibition on garlic and potatoes (22%); disinfestation of grains and fruit (20%); pathogen reduction in meat and seafood (8%) and other foods as honey, mushrooms and health foods (4%). Kume *et al*. (2009) also observed that irradiation is increasing dramatically in Asia but decreasing in the EU. Although the World Health Organization has determined that irradiation of any food up to 10 KGy does not represent a toxicological hazard (López-Gómez *et al*., 2009), the practice is slow to gain traction in either the EU or the United States.

2.6.6 High-pressure packaging

High-pressure (40 000–80 000 p.s.i.) pasteurization (HPP) treatments have been shown to be effective to eliminate *V. vulnificus* in oysters and are used by two Louisiana processors. Additionally, HPP effectively separates the oyster meat from the shell. A band is then placed around the shells as an aid to assure closure during transportation and distribution. The oysters are sold for raw consumption. The capital investment (1–2 million US dollars or 0.5–1 million euros) for the equipment and throughput is a limitation for much of the seafood industry. In 2009, the US FDA proposed, but did not finalize, mandatory post-harvest processing (high-pressure, low-temperature pasteurization or cryogenic freezing) of all Gulf of Mexico oysters during the summer months to assure lethality to *V. vulnificus*. It was estimated that there was capacity to treat less than 15% of all Gulf of Mexico oysters harvested by these methods. This method has not been adopted in the EU.

2.6.7 Engineered nanoparticles

Nanotechnology has received a high level of interest in recent years with an estimated $50 billion invested in more than 1300 products (Suppan, 2011). Nanoparticles may be used for microencapsulation for timed release of antimicrobials in packaging or for imparting a bacteriostatic effect on food contact surfaces. Silver nanoparticles have been embedded in conveyer belts in meat-processing facilities and some packaging materials for pathogen reduction and shelf-life extension. Chinese researchers have reported that absorption of silver nanoparticles can interfere with DNA replication and re-route neural networks, creating genetic mutations (Suppan, 2011); they may be an environmental hazard, in addition, in terms of their disposal (Otles and Yalcin, 2008). The United States has no regulatory authority over nanotechnology and Codex may soon consider whether or not to include it in its strategic plan for 2013–2018 (Suppan, 2011). There is international regulatory inertia due to funding and little definition of nanoparticles, aside from size and properties.

2.6.8. Case-ready packaging

Of continued emphasis with seafood will be the case-ready package. Case-ready meats became popular because the in-store butcher could be eliminated; products

could be packed by the manufacturer, meaning that the latter bore the brunt of liability (as well as the onus of receiving expired product). Seafood is highly perishable and the retail ready package (RRP) is likely gradually to replace the retail seafood counter in large, warehouse-style grocery outlets. WalMart is forcing the RRP concept in the EU by limiting and standardizing acceptable package sizes for greater efficiency of stocking and placement (Anonymous, 2011). This should be easier to enforce in the EU than in North America because customer visits to grocery stores are weekly in the United States, whereas they are almost daily in the EU due to the size of household kitchens and appliances. In contrast, the EU is far more progressive in its acceptance of MAP of seafood, the use of TTIs and regulations regarding the use of rapid indicators for quality. Although Mahalik (2009) indicated that the United States is the leader in active packaging systems, it is clearly the follower in its application within the seafood industry. Cost is the driver. More research needs to be conducted into low-cost, effective treatments to extend the shelf life of seafood and prevent pathogen growth therein.

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3

Sensory and quality properties of packaged fresh and processed meats

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Abstract: The primary objective for packaging fresh and processed meats is to limit and delay both the growth of spoilage and pathogenic microorganisms and deteriorative chemical reactions through adequate containment, which is followed by the maintenance of the optimal sensory and other quality characteristics of the product within a specified shelf life through adequate protection and preservation. This book chapter will discuss the sensory quality changes that occur in fresh and processed meat products with respect to colour, flavour and texture, and also the typical packaging conditions that are used to maintain the sensory quality of these products throughout shelf life. Sensory quality changes that are not directly related to packaging conditions are also mentioned briefly for the sake of completeness.

Key words: sensory quality packaging, modified atmosphere packaging, vacuum packaging, vacuum skin packaging, controlled atmosphere packaging.

3.1 Introduction

Fresh and processed meat products present their own unique challenges with respect to maintaining sensory quality and food safety throughout shelf life. The fundamental aspects of all food packaging materials is that, in an economic manner, they must contain, protect, preserve, inform (throughout the entire distribution process from point of manufacture to points of consumer usage) and provide convenience (at many different levels) while acknowledging the constraints placed upon their usage from both legal and environmental perspectives. As these fundamental principles apply to all forms of packaging materials and systems, it follows that, irrespective of the specific level at which the packaging is industrially applied (primary-sales packaging, secondary-collation and handling packaging or

tertiary-transport packaging), all must conform to these same principles (Cruz-Romero and Kerry, 2008).

Meat colour is the first sensory modality that consumers encounter when purchasing both fresh and processed meat products. Only when the product has been taken home and cooked do sensory flavour and texture come in to play. For this reason, the packaging methods by which meats are presented to the consumer have been optimized to maintain the optimum colour of meat and retard the development of unsightly discolourations. This, for the most part, has been achieved through the use of modified atmosphere packaging (MAP). In the fresh meat industry, beef steaks, for example, are routinely packaged under oxygen (O_2) and carbon dioxide (CO_2) concentrations in MAP in order to enhance colour stability (O_2 promotes desirable fresh red meat colour formation) and prolong shelf life (CO_2 selectively inhibits microbiological growth), while nitrogen (N_2) may also be used to maintain the final pack shape, depending on the interactive degree of CO_2 solubility with the meat product in question (Walsh and Kerry, 2002). Typically, fresh red meats are stored in MAP containing 80% O_2:20% CO_2 (Georgala and Davidson, 1970). Another method of packaging meat, vacuum packaging (VP), will be also discussed in this book chapter. Both of these techniques have their own unique qualities for meat shelf-life extension, particularly with respect to sensory and microbiological attributes.

For processed meats, such as cured meats, the addition of nitrite, in the form of sodium nitrite, during manufacture acts as an antimicrobial agent, specifically inhibiting the growth of *Clostridium botulinum*, the organism that causes botulism. Additionally, this added nitrite also produces the characteristic pink colour of cured meat products and is of major importance from a consumer sensory viewpoint.

The breakdown products of lipid oxidation have also been associated with the development of off-flavours and off-odours and loss of colour in meat (Faustman and Cassens, 1989). Furthermore, MAP meat products held in high O_2 atmospheres may result in protein oxidation which may have a negative effect on meat tenderness (Rowe *et al*., 2004; Torngren, 2003; Zakrys *et al*., 2008; Zakrys-Waliwander *et al*., 2009, 2010). As such, the requirements for colour stability must be balanced against the deteriorative action of lipid oxidation.

3.2 Packaging of fresh and processed meats

The cutting and packaging of raw refrigerated meat in air-permeable overwrap packaging at individual stores has been gradually replaced by case-ready or centralized operations in many developed countries (McMillin, 1994). MAP was first used in 1930 by Killefer (1930) using 100% CO_2 to store pork and lamb at 4–7°C. Around the same time period refrigerated beef carcasses were transported from Australia and New Zealand in a CO_2-enriched environment (Floros and Matsos, 2005). VP of retail packs was introduced in the 1950s (Floros and Matsos, 2005) and, in 1981, Marks & Spencer introduced MAP to the United Kingdom (Inns, 1987). MAP is now used ubiquitously across the meat industry for many different

meat products. It is used for fresh meat cuts like beef steaks, lamb and pork chops, as well as beefburgers, sausages and blood puddings. In this chapter, we will discuss three categories of preservative packaging that can be used with fresh and processed meat products. These are VP, high O_2 modified atmosphere packs (high O_2 MAP) and low O_2 modified atmosphere packs (low O_2 MAP).

3.2.1 Use of vacuum packaging (VP) for meat products

VP is extensively used for products such as prime cuts of fresh red meat (during stored aging, transport and retail frozen storage), cured meats (from manufacture through to retail display) and chub-format processed meat products. Vacuum packs are comprised of evacuated pouches or vacuum skin packs, in which a film of low gas permeability is closely applied to the surface of the product. Preservative effects are achieved by the development of an anaerobic environment within the pack (Gill and Gill, 2005). Respiration of the meat will quickly consume the vast majority of residual O_2, replacing it with CO_2, which eventually increases to 10–20% within the package (Gill, 1996; Parry, 1993; Taylor, 1985). Vacuum-packaged fresh meat is unsuitable for the retail market because the lack of oxygen in the package causes a change of meat colour from red to purple due to the conversion of oxymyoglobin to deoxymyoglobin. Drip loss can also occur during prolonged storage of meat in vacuum packs (Jeremiah *et al*., 1992; Parry, 1993; Payne *et al*., 1997). However, vacuum skin packaging (VSP) can counteract this problem as this packaging approach uses a film that fits very tightly to the meat surface (Fig. 3.1), leaving little space for the accumulation of any fluid exudate (Hood and Mead, 1993). Processed meat products are often packaged in the vacuum format. Figure 3.2 depicts a vacuum-packaged whole ham in a heat-shrinkable film. Other types of processed meats that can be vacuum packed include: rashers, black and white puddings, sliced meats like corned beef, pastrami, salami, pepperoni or ham, frankfurters, hard meat terrines or roulades and meat rolls, which will extensively extend shelf life at refrigerated temperatures. Marinaded products can also use the vacuum-pack format and include marinaded barbecue pork spare ribs.

3.2.2 Use of high O_2 modified atmosphere packaging (MAP) for meat products

High O_2 MA packs usually contain mixtures of two or three gases and will typically be comprised of: O_2, to enhance colour stability; CO_2, to inhibit growth of selective spoilage bacteria; and N_2, to reduce the proportions of the other gases or to maintain pack shape. As discussed earlier, colour perception plays a major role in consumer evaluation of meat quality (Lanari *et al*., 1995; Risvik, 1994). By packaging beef in an MA high in O_2 and maintaining the product at an adequately chilled temperature, the colour shelf life of the product can be prolonged considerably (Fig. 3.3) (Gill and Penney, 1988; Young *et al*., 1983). In European countries such as Ireland, the UK and France, beef steaks are commonly displayed in 70 mL O_2 and 30 mL CO_2 per 100 mL pack gas in MAP, whereas the concentrations used

Fig. 3.1 Vacuum skin packaged (VSP) beef steak.

Fig. 3.2 Vacuum-packaged whole ham in a heat-shrinkable film.

in the USA are 80 mL O_2 and 20 mL CO_2 per 100 mL pack gas (O'Sullivan *et al*., 2011). However, high O_2 concentrations in MAP may impact negatively on the oxidative stability of muscle lipids and lead to the development of undesirable flavours (Estevez and Cava, 2004; Rhee and Ziprin, 1987) in both fresh and cooked meats. Oxidation of polyunsaturated fatty acids (PUFA) also affects meat colour, nutritional quality and texture (Kanner, 1994). With respect to processed meats, beefburgers can be seen on supermarket shelves packed in MAP, particularly in the summer months in the UK and Ireland to coincide with the greater prevalence of outdoor barbecue cooking. Also, MAP is widely used by the industry to reduce spoilage of minced meat (Koutsoumanis *et al*., 2008).

Fig. 3.3 Modified atmosphere packed (MAP) beef rib roast.

3.2.3 Use of low O_2 MAP for meat products

Meat products can also be packed in the refrigerated controlled atmosphere packaging (CAP) and low O_2 packaging formats (O'Sullivan and Kerry, 2012). CAP is essentially O_2-free MAP; it has been used commercially for shipment of chilled lamb to distant markets (Gill, 1990). O_2 scavengers may also be incorporated to remove any residual O_2 that may have been included during the manufacturing process. Low O_2 MAP usually consists of CO_2, N_2 and low levels of O_2. The removal of O_2 is particularly important with cooked MAP muscle foods, which are prone to oxidation (Jensen *et al*., 1994). Low O_2 MAP meat discolouration can be prevented by the additional inclusion of low levels of carbon monoxide (CO) in the gas mixture (Luno *et al*., 2000). Carboxymyoglobin (COMb) is more resistant to oxidation than oxymyoglobin, owing to the stronger binding of CO to the iron-porphyrin site on the myoglobin molecule (Wolfe, 1980). However, CO generally is not used now because of negative consumer sentiment regarding this poisonous compound. CO will be discussed in greater detail in a later section of this chapter on meat colour.

The shelf-life extension of bacon by packaging in CO_2-enriched atmospheres was investigated by Callow as early as 1932 (Callow, 1932). The storage life of chilled meat can be extended by packaging the product with N_2 or CO_2 (Gill and Molin, 1991). Typically, cooked meats are stored in 70% N_2:30% CO_2 (Smiddy *et al*., 2002b). Modern trends towards convenience foods have led to an increase in the production of precooked and restructured meat products; low O_2 MAP is widely used for the packaging of these foods (Smiddy *et al*., 2002a). While MAP and VP packaging techniques may extend the shelf life and keeping quality of foods, microbiological spoilage and lipid oxidation may occur, depending on the level of residual oxygen within food packs and microbial numbers present on these foods (Rooney, 1995). High-quality sausages are also sometimes packed

with low O_2 MAP as the selling price justifies the higher packaging cost compared to overwrapped products.

In relation to processed meat products, there is less potential to extend the shelf life of processed meats using both high and low O_2 MAP than with fresh meat since operations such as drying, curing, smoking, fermentation, freezing, cooking and chilled storage, usually in VP format, already help with this (Church, 1993). However, the use of MAP formats offers manufacturers and retailers greater opportunities to promote and market meat products in a more versatile and attractive manner. The application of low O_2 MAP to processed meat has grown considerably in recent years, but optimization of gas composition is critical to ensure both product quality and safety (Moller *et al*., 2000). Colour stability of cured meat packaged in modified atmospheres depends on a complex interaction between headspace O_2 level, product to headspace volume ratio and the level of illuminance (García-Esteban *et al*., 2004). MAP has been used, furthermore, to accelerate ripening of dry-cured boneless hams; it has been observed that it is feasible to ripen them without negative influence on quality (Wang, 2001). Additionally, Rubio *et al*. (2006) reported no significant ($p > 0.05$) change in a* values with increased storage time for a MAP dry-cured beef product (Cecina de Leon). GÖk *et al*. (2008) showed that MAP was the most effective packaging system at preserving pastirma (a popular Turkish dry-cured beef product made from whole muscle, usually loin) quality, especially cured meat colour, over the course of 120 days of storage. MAP produced better colour quality over VP or aerobic packaging as it preserved typical cured meat colour better than either of the other methods (GÖk *et al*., 2008).

3.2.4 Salt and nitrate reduction strategies in processed meats

Consumers are demanding variations of meat products that are low in salt, fat, cholesterol, nitrites and calories in general and contain, in addition, health-promoting bioactive components – for example, carotenoids, unsaturated fatty acids, sterols and fibres (Weiss *et al*., 2010). The use of salt, nitrates and nitrites in preserving processed food products was vital in the past, but the advent of modern packaging and refrigeration reduced its primary role and necessity.

Salt in processed meats

Salt is basic to all meat curing mixtures and is the primary ingredient necessary for curing. It acts by dehydration and alters the osmotic pressure, inhibiting bacterial growth and subsequent spoilage (Pearson and Tauber, 1984). Processed meat products comprise one of the major sources of sodium in the diet in the form of sodium chloride (salt) (Desmond, 2006). Fresh meat is low in sodium but processed meats contain 2% added salt, a value that may increase to 6% in dried products. Processed meats contribute 20–30% to the daily salt (NaCl) intake in industrialized countries, amounting to between 9 and 12 g/day, a much larger value than the recommended value of < 5 g/day (Jiménez-Colmenero *et al*., 2001; WHO, 2003). Intake of dietary sodium has been linked to hypertension in about

20% of the population and consequently increased risk of cardiovascular disease (CVD). The estimated cost of CVD to both the EU and US economies is €169 billion and $403 billion, respectively (Desmond, 2006). The clear association between consumption of processed meats and the incidence of hypertension (Paik *et al*., 2005) confirms the importance of meat technology in relation to salt intake. Apart from the recommendation to 'limit consumption of salty foods and foods processed with salt (sodium)' (WCRF, 2007), a possible association of the processed meat–colorectal cancer relationship with the salt problem' should not be discarded. In Ireland and the UK the daily sodium adult intake is approximately three times the recommended daily allowance and therefore public health and regulatory authorities are recommending reducing dietary intake of sodium to 2.4 g (6 g salt) per day (Desmond, 2006).

Nitrate and nitrite in processed meats

The addition of nitrate-based compounds to cured meats is thought to have arisen from the salting of meats contaminated with saltpetre (KNO_3). Saltpetre is a commonly occurring impurity in salt, which enhances its preserving action and produces a red colour in the product (Honikel, 2008). It was subsequently adopted for the colour and flavour properties it contributed. Nowadays, the usual process for the accelerated manufacture of cured meats is to incorporate salt, nitrite, a reducing agent (such as ascorbate) and other ingredients such as seasonings (Kramlich *et al.*, 1973). Meat products that may contain nitrites include: bacon, bologna, corned beef, frankfurters, luncheon meats, ham, fermented sausages, shelf-stable canned cured meats, perishable canned cured meat (e.g., ham) and a variety of fish and poultry products (Pennington, 1998). Nitrate used to be used as the primary source for nitrite, supplied as either sodium nitrate or potassium nitrate, but nitrite is now preferred (Sebranek and Bacus, 2007). Nitrate is converted to nitrite by microbiological processes, therefore acts more slowly than nitrite and is generally not extensively used today in the accelerated manufacture of cured meats, however, it is still used in slower curing processes such as that utilized in Wiltshire ham manufacture and may be used in modern cured meat manufacture as a reservoir source for nitrite replacement in meats as it decomposes or becomes more depleted within the cured product. Nitrate and nitrite have strong antibacterial effects, particularly in relation to the growth of *Clostridium botulinum*. Nitrite is strongly inhibitory to anaerobic bacteria, like that of *Clostridium botulinum*, and contributes to the limited control of other microorganisms such as *Listeria monocytogenes* (Sebranek and Bacus, 2007).

Human health impact of nitrate and nitrite

Both nitrate and nitrite can be hazardous to humans if ingested in large amounts. Since the 1970s, there has been concern about a possible link between nitrite consumption and cancer. Nitrite can cause the formation of carcinogenic N-nitrosamines in cured products due to its reaction with secondary amines and amino acids in muscle proteins. Furthermore, residual nitrite in cured meats may form nitrosamines in the gastrointestinal tract (Shahidi and Pegg, 1991). There is

no conclusive evidence that nitrite is directly carcinogenic (Cantor, 1997); however, in high doses it has been implicated as a co-carcinogen (Schweinsberg and Burkle, 1985). The lethal oral doses for human beings are established as 80–800 mg nitrate/kg body weight and 33–250 mg nitrite/kg body weight (Schuddeboom, 1993). This may increase the incidence of colorectal cancer, and it is recommended that we Limit intake of red meat and avoid processed meat' as one of the ten universal guidelines for healthy nutrition (WCRF, 2007). The consumption of red meat and, in particular, processed meats, has been related to the incidence of colorectal cancer in several epidemiological studies since 1975, mainly in the USA and the UK (Demeyer *et al*., 2008). In cured meats, nitrosamines occur only in small amounts and they are easily avoidable through proper frying, grilling and pizza baking (Honikel, 2008). Although negative reports and scientific studies have demonstrated health risks associated with the consumption of processed foods, results from these reports and studies are variable and not wholly conclusive. The WCRF report, among others, published in peer-reviewed journals, is subject to some criticism because the large variability in composition and nature of meat and meat products is not sufficiently taken into account, nor are processed meats defined with sufficient precision (Demeyer *et al*., 2008). The WCRF report itself states that there is no generally agreed definition of processed meat. The term is used inconsistently in epidemiological studies. Judgments and recommendations are therefore less clear than they could be' (Demeyer *et al*., 2008).

In most countries, the use of potassium or sodium salts is limited. Either the ingoing or the residual amounts are regulated by law (Honikel, 2008). The curing process has been regulated in the USA, by the US Department of Agriculture (USDA), since the early 1900s. Sodium nitrite is allowed to be added at a maximum of 156 ppm (Cassens, 1997a). In Europe, the curing process is regulated by directive 2006/52/EC. In this directive the use of nitrates is limited to non-heated meat products with 150 mg sodium nitrite/kg and nitrite up to 100 mg, and 150 mg nitrite/kg meat in all meat products (Directive, 2006; Honikel, 2008).

The meat industry continues to search for alternative methods to produce nitrite-free meats that maintain the colour characteristics of nitrite-cured meat products (Zhang *et al*., 2007) and acceptable alternatives for the use of nitrate and nitrite exist in relation to colour development, flavour and microbiological safety (Demeyer *et al*., 2008). Zhang *et al*. (2007) showed that nitrosylmyoglobin could be generated in Harbin red sausage when *L. fermentum* (AS1.1880) was inoculated into the meat batter, and the formation of a characteristic pink colour with an intensity comparable to that in nitrite-cured sausage could be achieved by its use. This treatment did not seem to have a negative impact on the product texture and flavour, although the oxidative stability and microbial shelf life require further investigation to determine limits. Sindelar *et al*. (2007) compared uncured, no-nitrate/nitrite-added hams, frankfurters and bacons against nitrite-cured products considered to be industry standards in their respective product category. Consumer sensory differences existed between all the brands; nonetheless, the hams tested were considered acceptable by a majority of consumers. A greater amount of variation was identified between frankfurters than hams and consumer

sensory results for the bacon products revealed that a majority of the non-nitrate products had similar sensory scores to the nitrite-added control. These studies have proved promising, but greater work is required before such products become widely adopted, primarily because of the greater effectiveness of nitrite as an antimicrobial agent and its inhibitory effects on Clostridium and Listeria species and because of its positive effects on sensory flavour and colour qualities.

Natural and organic foods are not permitted to use chemical preservatives, therefore the traditional curing agents used for cured meats, nitrate and/or nitrite, cannot be added to natural and organic processed meat products. However, alternative processes that utilize ingredients with high nitrate content, such as vegetable-based ingredients, and a nitrate-reducing starter culture can produce processed meats with very typical cured meat properties (Sebranek and Bacus, 2007). However, when manufacturing natural and organic meat products using natural ingredients, the inherent variability of natural ingredients must be considered (Sebranek and Bacus, 2007).

Packaging technology and the reduction of preservative ingredients

Modern packaging technologies can be employed to improve the food safety and shelf life of processed meat products and allow the subsequent reduction of the preservative ingredients such as salt and nitrates in these processed foods. By incorporation of the preservative effects directly into packaging, preservation may be maintained which will compensate for the lesser preservative effect of the optimized processed meats. Active packaging systems can include oxygen scavengers, carbon dioxide scavengers and emitters, moisture control agents and antimicrobial packaging technologies (Kerry *et al*., 2006). Active packaging has the advantage of maintaining the preservative effects of various compounds (antimicrobial, antifungal or antioxidant), but without being in direct contact with the muscle food product (O'Sullivan and Kerry, 2012). Chemical preservatives can be employed in antimicrobial-releasing film systems, including organic acids and their salts (sorbates, benzoates and propionates), parabens, sulphites, nitrites, chlorides, phosphates, epoxides, alcohols, ozone, hydrogen peroxide, diethyl pyrocarbonate, antibiotics and bacteriocins (Ozdemir and Floros, 2004). The antimicrobial agent is incorporated into the packaging material by either spraying, coating, physical mixing, or chemical binding (Berry, 2000). This is an important development, considering the consumer drive towards clean labelling of food products and the desire to limit the use of food additives (O'Sullivan and Kerry, 2009). Looking to consumer demand for chemical preservative-free foods, food manufacturers are now using naturally occurring antimicrobials to sterilize and/or extend the shelf life of foods (Han, 2005). The preservative effect of active packaging can substitute for the reduced preservative effects of salt or nitrate. By reducing the growth and spread of spoilage and pathogenic microorganisms in meat foodstuffs, antimicrobial packaging materials can inhibit or kill the microorganisms and thus extend the shelf life of perishable products and enhance the safety of packaged products (Han, 2005). Similarly, packaging films that release organic acids offer potential for reducing the effect of the growth of slime-forming bacteria on meat (Rooney and Han, 2005).

3.3 Colour development in fresh and processed meats

The following sections describe the different issues relating to colour, important from the consumer's point of view, that are involved in the packaging of fresh and processed meat products.

3.3.1 Colour development in fresh meats

Muscle colour, at the point of purchase, is an indicator of freshness and anticipated palatability for the consumer (Brewer *et al*., 2002). In red meats, consumers relate the bright red colour to freshness, while discriminating against meat that has turned brown in colour (Hood and Riordan, 1973; Morrissey *et al*., 1994). However, Carpenter *et al*. (2001) showed that consumer preference for beef colour was sufficient to influence their likelihood to purchase, but was not enough to bias taste scores. It is likely that once a decision to purchase beef is made in the market, whether the beef is the red of fresh bloomed beef, the brown of discounted beef, or the purple of vacuum-packaged beef, consumer eating satisfaction at home will depend only on the beef quality attributes of tenderness, juiciness and flavour (Carpenter *et al*., 2001).

Myoglobin is the principle protein responsible for meat colour, although other heme proteins such as haemoglobin and cytochrome C may also play a role in beef, lamb, pork and poultry colour (Mancini and Hunt, 2005). Oxymyoglobin, which is the red colour of fresh meat, is oxidized to the grey-brown pigment of metmyoglobin during retail display conditions (Fig. 3.4). Oxymyoglobin is a heme protein in which iron exists in the ferrous form (Fe^{+2}), while metmyoglobin possesses the ferric form (Fe^{+3}). The conversion of the ferrous to the ferric form is a result of oxidation (Liu *et al*., 1995). The formation of metmyoglobin from oxymyoglobin is positively correlated to lipid oxidation and appears to be dependent on antioxidant status (Yin *et al*., 1993). Metmyoglobin formation also depends on numerous factors, including oxygen partial pressure, temperature, pH, meat's reducing activity and, in some cases, microbial growth (Mancini and Hunt, 2005).

After animal slaughter, the iron in muscle tissue is released from high molecular weight sources (e.g., haemoglobin, myoglobin, ferritin, haemosiderin) and

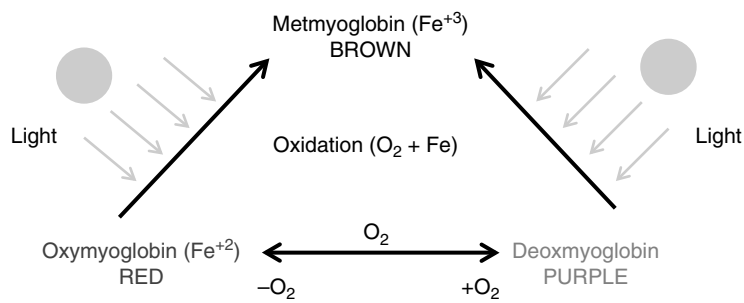

Fig. 3.4 Oxidation of oxymyoglobin and deoxymyoglobin to metmyoglobin.

made available to low molecular weight compounds such as amino acids, nucleotides and phosphates with which it is believed to form chelates (Decker and Crum, 1993; Morrissey *et al*., 1998). Free iron and copper accelerate the auto-oxidation of oxymyoglobin (Snyder and Skrdlant, 1966) and the photo-oxidation of oxymyoglobin (Assef *et al*., 1971). Therefore, free iron, either directly or indirectly, promotes the discolouration of meat by the oxidation of oxymyoglobin to metmyoglobin. However, oxymyoglobin may be maintained in meat by delaying oxidation to metmyoglobin (Lynch *et al*., 1999).

Antioxidants can increase the oxidative stability of muscle foods. These compounds may be incorporated in to meat through dietary means such as the case of dietary α-tocopherol, which is present in grass. Grass-fed beef may not be as prone to lipid oxidation than grain-fed beef because of the increased levels of vitamins A, C and E, carotenoids and flavonoids found in forages (Wood and Enser, 1997). O'Sullivan *et al*. (2003) found reduced susceptibility to lipid oxidation of meat from high herbage-fed cattle, which was due to higher vitamin E levels in grass. Grass-derived dietary α-tocopherol is incorporated in to the phospholipid membranes of the mitochondria within cells and is then bioavailable to retard oxidation in the subsequently produced meat.

Packaging fresh meat in a high O_2 atmosphere can help to maintain the muscle pigment myoglobin in its oxygenated form, oxymyoglobin, thus reducing fresh meat discolouration. For this reason, beef steaks are commonly displayed under high O_2 concentrations in MAP in order to promote colour stability (Okayama *et al*., 1995; Zakrys *et al*., 2008; Zakrys-Waliwander *et al*., 2009, 2010). The colour of lamb and pork may also be extended by storage under MAP conditions (Kerry *et al*., 2000; Lanari *et al*., 1995). As mentioned earlier, fresh red meats are typically stored in MAP containing 80% O_2:20% CO_2 (Georgala and Davidson, 1970).

Meat discolouration can be prevented also by the inclusion of low levels of carbon monoxide (CO) in the gas mixture (Luno *et al*., 2000). CO is a colourless, odourless and tasteless gas and can be used for prolonging meat colour integrity. Carboxymyoglobin (COMb), which is more resistant to oxidation than oxymyoglobin, forms due to the stronger binding of CO to the iron-porphyrin site on the myoglobin molecule (Wolfe, 1980). However, one of the concerns with using CO is that the consumer may perceive that product colour quality can be maintained within packs, even if the product has been exposed to temperature abuse (Wilkinson *et al*., 2006). Hunt *et al*. (2004) has demonstrated that the use of 0.4% CO during storage in MAP improved beef colour without masking spoilage. From a legislative perspective, the FDA have approved the use of CO in MAP applications and defended its decision on the basis that, while meat colour did not degrade in a package containing CO, offensive odours could still be produced normally by microbial growth in the product in the presence of CO, thereby warning the consumer that the product has spoiled (FDA, 2004).

As mentioned earlier, vacuum-packaged fresh meat is unsuitable for retail display because depletion of O_2 coupled with low O_2 permeability of the packaging film causes a change of meat colour from red to purple due to the conversion

of oxymyoglobin to deoxymyoglobin. These are not acceptable meat colours to the consumer (Allen *et al*., 1996; Parry, 1993). American consumers have demonstrated a bias against the purchase of vacuum-packaged beef which displays the purple colour of deoxymyoglobin (Meischen *et al*., 1987).

3.3.2 Colour development in processed meats

The characteristic addition of nitrate or nitrite to cured meats, as well as having a bacteriocidal effect, contributes specific colour and flavour sensory attributes to the resulting meat product. The chemical reactions leading to cured meat pigmentation are a complex series of processes, involving microbially, enzymatically and/or chemically catalyzed steps, which depend on pH, pigment concentration, redox potential, curing agent distribution, temperature and relative humidity (Chasco *et al*., 1996). Nitrate does not directly take part in the colour-developing reactions and must be reduced to the active agent nitrite. Therefore, nitrate-reducing Staphylococcus or Kocuria (formerly Micrococcus) species are required if nitrate is used instead of nitrite (Lücke, 1998). Nitrate reductase activity is strongly enhanced by anaerobic conditions, but activity is still present under aerobic conditions (Neubauer and GÖtz, 1996). After it was discovered that nitrite, rather than nitrate, was the genuine curing agent, it took only a few years until nitrite was introduced into general meat product manufacturing. Nitrite (NO_2^-) breaks down in the meat to nitric oxide (NO), which then binds to an iron atom in the centre of myoglobin's heme group, reducing oxidation and causing a reddish-brown colour (nitrosomyoglobin) when raw, and the characteristic pink colour (nitrosohemochrome) when cooked (Fig. 3.5). Curing agents are employed in combination with reducing agents (e.g., ascorbate/ascorbic acid) that accelerate the reduction of nitrite into NO (Gøtterup *et al*., 2008).

3.4 Flavour of fresh and processed meat products

The following sections consider the factors affecting flavour in various meat products.

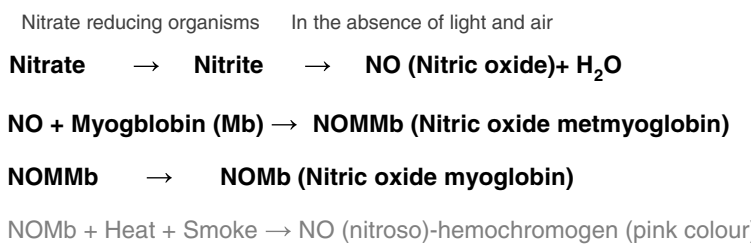

Fig. 3.5 Colour development in cured meats.

3.4.1 Catalysis of lipid oxidation

Meat quality is a complex term consisting of attributes such as colour, flavour and texture (Bredahl *et al*., 1998). These parameters are affected not only by pre-slaughtering management, but also by processes of proteolysis and lipid oxidation occurring during post-mortem storage (Sierra *et al*., 2006). The oxidation of PUFA in meat causes the rapid development of meat rancidity; it also affects colour, nutritional quality and texture of beef and other meats (Kanner, 1994). Lipid oxidation in muscle systems is initiated at cellular membrane level, specifically in the phospholipid fractions, as a free-radical autocatalytic chain mechanism (Labuza, 1971) in which prooxidants interact with unsaturated fatty acids resulting in the generation of free radicals and propagation of the oxidative chain (Asghar *et al*., 1988). Oxidation of unsaturated fatty acids is generally thought to occur in three stages: (1) initiation: the formation of free radicals; (2) propagation: the free-radical chain reactions; (3) termination: the formation of non-radical products (Tappel, 1962).

The ability of heme pigments and non-heme iron to accelerate the propagation step of the free-radical chain mechanism can explain the rapid rate of oxidation in cooked meats (Pearson *et al*., 1977). Transition metals, notably iron, are believed to be pivotal in the generation of species capable of abstracting a proton from an unsaturated fatty acid (Gutteridge and Halliwell, 1990; Kanner, 1994). Haemoglobin accounts for two-thirds of body iron, with smaller amounts in myoglobin and very small amounts in various iron-containing enzymes and in the transport protein transferrin. Iron can be released from ferritin and utilized by mitochondria for the synthesis of hemoproteins – for example, myoglobin. Ferritin is the main storage protein for iron in cells (Aisen and Listowsky, 1980). Seman *et al*. (1991) suggested that ferritin may be responsible for catalysing lipid peroxidation in muscle foods. O_2 releases iron from ferritin and is the primary reductant in ascorbate-mediated ferritin iron release (Boyer and McCleary, 1987).

3.4.2 Flavour of fresh and cooked meat

Meat flavour is thermally derived, since uncooked meat has little or no aroma and only a blood-like taste. Only after cooking and a series of thermally induced complex reactions that occur between the many different non-volatile compounds of the lean and fatty tissues does meat become flavoursome (Calkins and Hodgen, 2007; Mottram, 1998). Hundreds of compounds contribute to the flavour and aroma of meat and are very complex attributes of meat palatability. Many of these compounds are altered through storage and cooking, making meat flavour an incredibly complex topic (Calkins and Hodgen, 2007). The main reactions during cooking, which result in aromatic volatile production, are the Maillard reactions between amino acids and reducing sugars, and the thermal degradation of lipid (Mottram, 1998). In general, amino compounds condense with the carbonyl group of a reducing sugar in the presence of heat. This produces gylcosylamine, which is rearranged and dehydrated to form furfural, furanone derivatives, hydroxyketones and dicarbonyl compounds (Calkins and Hodgen, 2007). The broad array of flavour

compounds found in meat includes hydrocarbons, aldehydes, ketones, alcohols, furans, thriphenes, pyrrols, pyridines, pyrazines, oxazoles, thiazoles, sulphurous compounds and many others (MacLeod, 1994). Sulphurous compounds occur at low concentrations, but their very low odour thresholds make them potent aroma compounds and important contributors to the aromas of cooked meat (Mottram, 1998). Many of these sulphur compounds contribute sulphurous, onion-like and, sometimes, meaty aromas (Fors, 1983). Roast flavours in foods are usually associated with the presence of heterocyclic compounds such as pyrazines, thiazoles and oxazoles (Mottram, 1998).

A higher proportion of unsaturated fatty acids in the triglycerides of pork and chicken, compared with beef or lamb, produce more unsaturated volatile aldehydes in these meats and these compounds may be important in determining the specific aromas of meat species (Mottram, 1991). Additionally, the effects of dietary ingredients on the sensory attributes of meat are dependent on the type of diet being offered to the meat-producing species in question and also, to a large extent, on the meat species itself (Rødbotten *et al*., 2004).

In fresh meat the products of fatty acid oxidation produce off-flavours and odours which are usually described as rancid (Gray and Pearson, 1994). The oxidation of fatty acids occurs due to the exposure to O_2 and is accelerated in the presence of light and catalysts, such as free iron, and similar to the mechanism described previously for pigment and lipid oxidation. The relationship between rancidity and flavour is unclear. As rancid flavours develop, a subsequent loss in desirable flavour notes occurs (Campo *et al*., 2006). Greene and Cumuze (1981) reported that oxidized flavour in beef was detected over a broad range of TBARS from 0.6 to 2.0 mg MDA/kg meat, indicating a big variation in the threshold of the panellists. It is difficult to determine the limiting point at which beef can be rejected due to lipid oxidation, based on sensory perceptions (Campo *et al*., 2006). The general population of meat consumers would not detect oxidation flavours until oxidation products reached levels of at least 2.0 mg/kg tissue (Greene and Cumuze, 1981).

High O2 MAP increases lipid oxidation in meat – Kerry *et al*. (2000) in lamb; Lund *et al*. (2007b) in pork; Jakobsen and Bertelsen (2000), Zakrys *et al*. (2008) and Zakrys-Waliwander *et al*. (2009, 2010) in beef. The main drawback with using high O_2 MAP is the potential for off-flavour development in packs due to lipid oxidation (Jayasingh *et al*., 2002). Zakrys *et al*. (2008) found that the sensory quality of MAP beef steaks was best promoted by packaging under atmospheres containing 50% O_2, followed by 80% O_2 samples, with TBARS levels below 2 mg MDA/kg. Campo *et al*. (2006) also indicated that beef would be rejected due to a strong sensory perception of lipid oxidation with TBARS values of 2 or over. The removal of O_2 is particularly important with cooked MAP muscle foods. Cooking promotes lipid oxidation, partially due to the release of iron, which acts as a pro-oxidant. The presence of small amounts of O_2 accelerates oxidation of cooked MAP meat even further (Smiddy *et al*., 2002a). The odour of oxidized cooked meats is also referred to as 'warmed over flavour' (WOF) (Pearson *et al*., 1977). Raw meat is generally considered less susceptible to WOF

than heated meat. However, after grinding and exposure to air during processing, odours develop that are similar to those found in oxidized cooked meats (Sato and Hegarty, 1971).

3.4.3 Flavour of cured meat

Salt

Salt as a component of the cure will contribute directly to the taste of the resulting product. Use of salt alone gives a harsh, dry, salty product that is not very palatable (Pearson and Tauber, 1984). Additionally cure or brine sugars like dextrose, fructose, corn syrup, etc. will reduce the harshness of the saltiness of cured meats by contributing a sweeter taste. Recently, emphasis has been placed on reducing levels of salt in meat products in view of its relationship to hypertension in about 20% of the population. Salt has an essential function in meat products in terms of flavour, texture and shelf life (Desmond, 2006), but levels can potentially be reduced without compromising consumer satisfaction or quality. Tobin *et al*. (2011) showed that the most acceptable beef patty formulation in a salt and fat reduction study was 40% fat and 0.5% salt. This is a 20% decrease in fat and a 50% decrease in salt levels of average commercial patties. Since sodium is the element in salt that causes hypertension, other chloride-containing salts are being considered as alternatives to sodium chloride (Pearson and Tauber, 1984). Bacon has been manufactured using salt replacers which consist either of a salt such as potassium chloride alone, or a combination of potassium chloride with other salts such as potassium sulphate and potassium glutamate (Varnam and Sutherland, 1995). Salt reduction may be difficult to achieve, however, because of supermarket concerns about associated reductions in shelf life, as well as processing concerns pertaining to traditional product labels (Honikel, 2008).

Cure flavour

Varnam and Sutherland (1995) state that nitrite itself is not thought to contribute any specific flavour compounds, although it does have a beneficial effect on flavour by retarding the formation of off-flavours by inhibiting lipid oxidation. Additionally, according to Shahidi (1991), as curing with nitrite inhibits the formation of oxidation products, it may be assumed that the flavour of nitrite-cured meat of a given species is actually the natural flavour of meat without the overtones caused by the oxidation of lipid components. Conversely, other authors consider that nitrite contributes the characteristic cured meat flavour of cured meats and thus is the most important ingredient in terms of flavour development (Noel *et al*., 1990; Reineccius, 1994). Sebranek and Bacus (2007) state that cured flavour is an important quality attribute of cured meats that is derived from addition of nitrite. Tichivangana *et al*. (1984) found that even though ascorbate and phosphate were effective in reducing off-flavour development in bacon during storage, they failed to produce a typical cured meat aroma comparable to nitrite-cured samples. An amount of residual nitrite is considered by some to be essential to

maintaining typical cured meat properties during extended product storage, and 5–15 ppm residual nitrite has been reported for commercial cured meats in the USA (Cassens, 1997b). When nitrite is fully depleted from cured meat, changes in colour and flavour fading occur.

Cured meat products are resistant to lipid oxidation due to the reducing conditions of ascorbic acid combined with the synergism of phosphates. Additionally, as mentioned above, the nitrites used in the curing process have been associated with the inhibition of the lipid oxidation process. The development of warmed-over flavour is also very slow in cooked cured meats compared with uncured meats due to the inhibitory affect of a number of nitrite-derived compounds on lipid oxidation (Varnam and Sutherland, 1995). The actual mechanism by which this antioxidant capacity is facilitated is not fully understood; however, several mechanisms have been suggested (Igene *et al*., 1985; Morrissey and Tichivangana, 1985). Proposed mechanisms include: the formation of a stable complex between nitrite and heme pigments which prevents the release of non-heme iron (which itself could catalyse the lipid oxidation reaction); stabilization of unsaturated lipids within membranes; and possible direct interaction with liberated non-heme iron (Fe^{2+}) from the denatured heme pigments. S-nitrosocysteine, which possesses antioxidant properties, can also be formed by nitrite (Kanner and Juven, 1980). However, the most important and widely accepted mechanism is that of nitrite stabilization of the porphyrin ring during cooking, thereby preventing the release of Fe^{2+} (Gray and Pearson, 1987). Morrissey and Tichivangana (1985) reported that 50 ppm of nitrite reduced TBA values by 50–64% for beef, pork and chicken, and by about 35% for fish. While nitrite is effective as an antioxidant at 50 ppm, it is more effective at greater concentrations up to 200 ppm (Sebranek and Bacus, 2007).

3.5 Texture of fresh and processed meat

Meat quality characteristics play a major role for consumers in determining meat purchases, and several traits are considered fundamental, namely tenderness, juiciness, flavour, appearance and safety (Aktas and Kaya, 2001; Savell and Shackelford, 1992). Tenderness is considered by many authors as the most important qualitative characteristic of meat (Destefanis *et al*., 2008; Savell *et al*., 1987, 1989; Smith *et al*., 1987). Miller *et al*. (2001) found that the consumer would be willing to pay a higher price in the marketplace for beef as long as it is guaranteed tender.

Lipid and protein oxidation are closely associated deteriorative processes occurring in meat, although relatively little is known about the repercussions of the latter on the quality of meat products (Estevez and Cava, 2004; Rhee and Ziprin, 1987). Proteins are damaged by the action of free radicals, thereby resulting in a loss of protein functionality (Descalzo and Sancho, 2008). The oxidation of proteins can lead to physical and chemical changes in meat, such as amino acid destruction, decreases in protein solubility due to protein polymerization,

loss of enzyme activity and formation of amino acid derivatives, including carbonyls (Meucci *et al*., 1991; Stadtman and Oliver, 1991; Starke-Reed and Oliver, 1989; Uchida *et al*., 1992). These changes may lead to decreased eating quality such as reduced tenderness and juiciness, flavour deterioration and discolouration (Xiong, 2000). Decker *et al*. (1993) also reported that protein oxidation can affect the quality of meat and meat products. In particular, the loss of enzymatic activity and solubility and formation of protein complexes and non-enzymatic browning products associated with protein oxidation (Mercier *et al*., 2004) could be linked to meat tenderness.

Tørngren (2003) noted that beef steaks packaged in high O_2 MAP had decreased tenderness and flavour, as well as increased off-flavour, compared with steaks packaged in low O_2 MAP. Seyfert *et al*. (2005) found that steaks stored in low O_2 MAP increased flavour intensity, decreased off-flavours, and increased tenderness scores and suggested that the increased tenderness in low O_2 MAP was due to reduced protein oxidation. Lund *et al*. (2007a) reported an effect of packaging atmosphere on protein oxidation in beef patties showing significant increases of carbonyl content under high O_2 atmosphere after six days of shelf life in comparison to packaging with 100% N_2. Zakrys *et al*. (2008) observed a directional increase in protein oxidation of samples of beef *M. longissimus dorsi* muscle packed in increasing O_2 atmospheres over 15 days of refrigerated retail display. Warner-Bratzler shear force values had a positive correlation to O_2 levels in MA-packed beef samples, displaying that all samples appeared to become less tender with increasing O_2 level during the storage, although no significant differences were observed between experimental treatments (Zakrys *et al*., 2008). Additionally, these authors found that beef samples packed with 50% and 80% O_2 were tougher than low O_2-treated samples as determined by a trained sensory panel (Zakrys *et al*., 2008) and by 134 consumers (Zakrys *et al*., 2009). Lund *et al*. (2007a) investigated the effect of MAP (70% O_2/30% CO_2) and skin packaging (no oxygen) on protein oxidation and texture of pork *M. longissimus dorsi* muscle during storage for 14 days at 4°C and found that the high oxygen atmosphere resulted in reduced tenderness and juiciness of samples. Additionally, their SDS–PAGE data revealed cross-linking of myosin heavy chain through disulphide bonding, and the content of protein thiols was reduced indicating protein oxidation. Zakrys-Waliwander *et al*. (2010) found that tenderness of LD beef steaks stored under high oxygen atmospheres was reduced in comparison to steaks packaged under vacuum atmosphere. These results could support the explanation of decreased tenderness based on protein cross-linking, as a decreased content of free thiols was found in meat stored under high oxygen atmospheres demonstrating the oxidation of free thiol groups. Myosin was found to form intermolecular cross-links due to packaging under high oxygen atmosphere (Zakrys-Waliwander *et al*., 2011). The requirements for colour stability of fresh meat achieved through the use of high O_2 MAP must be balanced against the deteriorative action of lipid oxidation (Tørngren, 2003; Zakrys *et al*., 2008, 2009; Zakrys-Waliwander *et al*., 2010) and any reduction in meat tenderness.

3.6 Future trends

The food that we consume today is scrutinized more than it has ever been in the past in a whole variety of ways, such as product composition, labelling concerns, clean labels, health claims, product 'naturalness', safety concerns, issues pertaining to the environment and sustainability (Kerry and Troy, 2010). Also, the consumer has become very much more discerning with respect to the origins of the food they consume. One very important aspect of such food safety management systems, particularly where control has been lost at the point of manufacture, is through the effective use and operation of traceability systems (Kerry and Troy, 2010). Poor labelling by the supermarkets has resulted in a swing back towards the local butcher, where meat traceability is transparent and promoted as a selling point, in addition to green issues relating to product movement to markets (air miles) and support for local product producers. The impact of such developing trends on the pre-pack sales of meat at supermarket level remains to be seen (O'Sullivan and Kerry, 2008).

For many years, it was practice to promote the eating quality of continental cross-bred cattle due to the added benefit of reduced production costs involved for specific breeds such as Limousin, Charolais and Belgian Blue. However, the current trend seems to be for the promotion of specific breeds to the consumer – like Hereford and Angus – in an effort to add a premium to the meat and exploit niche markets, which are differentiated from the more generic mass supply that occurs in the supermarket.

The method by which meat is packaged in modified atmospheres may also be updated in the future. Packaging beef striploin in lower O_2 atmospheres (50% O_2) appears to improve the sensory quality of the meat in comparison to packaging in high O_2 atmospheres (80% O_2) due to the improvement in sensory score and increase in tenderness, both observed from a sensory perspective (Zakrys *et al*., 2008). Additionally, smart packaging technologies, which provide tray materials with the capacity to absorb drip loss from fresh meat, are being developed. These contain a layer of multi-absorbent material that resides in the base of the tray but above which lays a false perforated floor allowing the drip to migrate from the product to the base of the tray (Kerry and Troy, 2010).

In the case of cured meats, there are developments that may effect the future consumption of such products. The widely recognized nutritional and gastronomic value of meat and meat products may be damaged by several recent media reports relating, in particular, to processed meat consumption and the incidence of 'civilization' diseases (Demeyer *et al*., 2008). Perhaps the future of cured meat products lies in the optimization of non-nitrate-containing products as mentioned above or by formulating new products with ingredients that demonstrate a protective effect against colorectal cancer, as reviewed briefly by Demeyer *et al*. (2008). Such ingredients could include fibre (Münch and Honikel, 2007), amylose-enriched starch (Toden *et al*., 2007), pre- and pro-biotics (Geier *et al*., 2006), omega-3 polyunsaturated fatty acids (Hall *et al*., 2007) or antioxidants (Yilmaz, 2006).

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3.8 References

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4

Sensory properties of packaged fresh and processed poultry meat

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Abstract: Sensory and quality issues are among the most important factors that influence consumer purchasing decisions of meat products. This chapter discusses the most important sensory and quality issues, which include appearance, texture, flavor/taste, functional properties and nutritional values associated with a both packaged fresh and processed poultry product. The extent to which the mode of packaging is a factor is also addressed.

Key words: sensory and quality issues, consumer preference, oxidation-reduction potential.

4.1 Introduction

Consumer preference among meat products has dramatically shifted from red meat (beef) to white meat (poultry) consumption over the last few decades for numerous reasons, including healthier product image, relatively low price, increased diversity of further-processed products and the absence of cultural and religious preferences (Magdelaine *et al*., 2008). At the same time, consumers' concern over the quality of poultry products has also increased during the same period. Therefore, sensory and quality issues are of paramount importance to consumers and failure to concentrate attention can cause major consumer rejection of such products, resulting in tremendous economic losses to the poultry industry. The most important factors influencing consumer attitudes when purchasing various food types are quality, taste and healthfulness (Lennernas *et al*., 1997). The notably important quality attributes of poultry meat products, include appearance (especially color at point of sale), texture, flavor/taste, functional properties such as water-holding

capacity (WHC) and emulsifying capacity (EC), microbial quality and nutritional values. In this chapter, current sensory and quality issues associated with packaged poultry products of both a fresh and processed nature are discussed.

4.2 Color changes in packaged fresh and processed poultry meat

Color is generally recognized as the most important quality parameter that influences consumers' purchasing decision for poultry products, as well as assessment for product quality (Fletcher, 2002). Color of fresh and processed poultry products can be affected by age, sex, genotype, nutrition, pre- and post-slaughter handling and associated conditions (especially time and temperature) of live animals, as well as packaging and processing variables associated with such products, such as ingredient selection and usage, processing technologies used and meat condition prior to processing (Kropf, 2008). Color causes immediate positive or negative psychological responses in consumers to the products being observed at the point of purchasing and, subsequently, influences their evaluations on the preference to and the wholesomeness/freshness of these products (Kropf, 2008). Therefore, the discoloration of fresh and processed poultry products can cause remarkable economic loss to the poultry industry. The discoloration problems are associated with the concentration and chemical status of myoglobin, a primary muscle pigment, physicochemical conditions affecting chemical status of myoglobin, and light reflectance and transmission properties of the poultry products. Pale, soft, exudative (PSE) conditions and pinking have long been considered to be the major discoloration problems in fresh and processed poultry products, respectively. Breast-related products are especially affected by discoloration because subtle changes in color can be more easily noticed in light-colored breast meat.

4.2.1 PSE incidents in poultry breast meat

PSE meat is generally characterized as meat that possesses a pale color, soft texture and exudative surface due to poor WHC (Smith and Northcutt, 2009). Increased consumer demand for product diversity has resulted in an intensive market shift from whole birds to smaller cuts and further-processed products. This trend has led to increased attention to meat quality properties such as meat color and WHC because they are highly associated with consumer acceptance. PSE meat is a significant problem in the modern poultry industry, with an estimated incidence of between 5% and 47% in chicken and turkey breast meat produced from commercial processing plants in Europe and North America (Table 4.1; Petracci *et al*., 2009). PSE conditions are highly associated with a low ultimate pH of muscle: when animals undergo acute ante mortem stresses, postmortem glycolysis in their muscle is accelerated, resulting in rapid pH decline to an abnormally low pH (< 5.8) level while muscle temperature is still high (Owens *et al*., 2009). The combination of high muscle temperature and low pH causes the denaturation of muscle

Table 4.1 Estimated incidences of Pale, Soft, and Exudative (PSE)-like chicken and turkey breast meat produced from commercial processing plants in Europe and North America based on their lightness

Study	Species	Country	Observations	L*[1] range	L* cutoff	PSE-like Incidence (%)
Barbut, 1998	Chicken	Canada	700	41–56	> 49/50	10
Wilkins *et al*., 2000	Chicken	United Kingdom	7538	45–67		
Woelfel *et al*., 2002	Chicken	United States	3554	42–71	> 54	47
Petracci *et al*., 2004	Chicken	Italy	6997	41–66	> 56	10
Lesiów *et al*., 2007	Chicken	Poland	250	43–56	> 53	5
Barbut, 1998	Turkey	Canada	4000	38–57	> 50/51	12
Owens *et al*., 2000b	Turkey	United States	2995	41–63	> 53	40
Fraqueza *et al*., 2006	Turkey	Portugal	977	35–55	> 50	8

Source: Adapted from Petracci *et al*. (2009).
[1]L*, lightness.

proteins which then become responsible for a decrease in WHC and an increase in the autoxidation rate of oxymyoglobin (King and Whyte, 2006). These changes are responsible for the color fading observed in PSE meat. Excessive extracellular water generated by lower WHC increases light reflectance and decreases light transmittance on the surface of meat, resulting in the characteristic pale color of PSE meat (Swatland, 2008).

Several studies have investigated the characteristics of PSE poultry breast meat by comparing physicochemical and functional properties of poultry breast meats. Bianchi *et al*. (2005) reported that pale breast fillets showed significantly lower ultimate pH (5.67 vs. 5.94) and WHC than normal-colored meat, indicating a close relationship between meat color and ultimate pH and WHC. In addition, the WHC of pale poultry fillets was partially restored after pH adjustment to 5.9 (Fraqueza *et al*., 2006; Van Laack *et al*., 2000; Zhang and Barbut, 2005a). PSE poultry breast meat showed significantly lower functional qualities when compared to normal breast meat: decreased solubility of water-soluble and salt-soluble proteins, lower protein functionality, lower marinade pick-up, higher drip and cooking losses (Barbut *et al*., 2005; Pietrzak *et al*., 1997). The low ultimate pH of pale poultry breast meat is the primary determinant of its paleness, low WHC and low functional quality. Practices that limit pH decline or increase the pH of PSE to that of the normal meat during processing may be effective in reducing the incidence of PSE and improving functionality of the PSE poultry breast. Because breast meat color is closely related to ultimate muscle pH and low functional quality, breast meat color can be used as an indicator for PSE meat.

However, there are controversial issues pertaining to several quality indicators and biochemical characteristics of PSE poultry breast. The effect of postmortem glycolytic rate on the incidence of PSE condition in poultry breast has also shown controversial results. Fernandez *et al*. (2001) reported that turkey breast with fast postmortem glycolysis and assessed by muscle pH (5.90 vs. 6.24 for the slow), 20 min after slaughter showed typical PSE conditions – that is, lower ultimate pH, paler color and lower functional qualities. In a similar study, Pietrzak *et al*. (1997) also found higher lactate levels in muscles deemed to have a faster glycolytic rate, but the ultimate muscle pH of the meats with fast and slow glycolytic rates proved not to be different. In their study, Fraqueza *et al*. (2006) could not find any relationship between glycolysis rate and typical quality characteristics of PSE, such as ultimate pH, lightness, drip or cooking losses in turkey breast. Van Laack *et al*. (2000) also reported no relationship between ultimate pH and lactate concentration and glycolysis rate in chicken breast. Allen *et al*. (1997) and Fraqueza *et al*. (2008) reported that the shelf life of PSE poultry breast meat assessed for microbial growth and development of lipid oxidation under aerobic and modified atmosphere packaging was significantly longer than that of the darker breast meat, but Allen *et al*. (1998) found no significant differences in microbial growth or in oxidative qualities between the PSE and normal poultry breast meat. Therefore, further research is required to clarify the mechanisms of pH decrease in PSE poultry breast meat and the relationship between low pH and the incidence of PSE conditions in the same meat.

According to numerous studies reported over the past few decades, incidences of PSE conditions in poultry is associated with many genetic and environmental factors, including genetic make-up, strain, heat stress, gender, season, geographical region, pre- and post-slaughter handling and processing practices. Factors influencing the development of PSE conditions in pork have been well investigated for over 50 years and a few genetic markers such as a single-point mutation in the sarcoplasmic reticulum calcium-release channel (called the ryanodine receptor, RYR) and halothane screening test for PSE-susceptible swine have been identified and utilized to eliminate swine prone to developing PSE conditions (Barbut *et al*., 2008). However, there are no reliable genetic markers or screening tests related to the development of PSE conditions in poultry and this is primarily due to a lack of extensive investigation conducted to date to identify the relationships between the genetic variation in turkey RYR and the incidence of PSE conditions (Strasburg and Chiang, 2009) and availability of the halothane screening test for poultry (Cavitt *et al*., 2004; Owens *et al*., 2000a). It has been claimed that fast growth rates and large breast muscle sizes in modern poultry strains by the intensive selection to satisfy the growing demands of consumers has induced histological and biochemical modification to the muscle tissues, resulting in the increased potential for developing PSE conditions in poultry (Alvarado and Sams, 2004; Owens *et al*., 2009). However, other studies (Duclos *et al*., 2007; Werner *et al*., 2008) have indicated no correlation between muscle growth rate and muscle size to the incidence of PSE conditions in poultry. In addition, various environmental stressors during pre- and post-slaughter handling and postmortem muscle

temperature can be associated with the incidence of PSE in poultry meat. Among environmental stressors, heat stress is considered as a primary stressor during the end of growing phase and pre-slaughter handling of birds, because it can be associated with other physical stressors such as crowding during catching, transportation and holding (Owens *et al*., 2009). Holding time and temperature before slaughter have been demonstrated to be closely correlated with the incidence of PSE in poultry meat (Bianchi *et al*., 2006; Petracci *et al*., 2001). Seasonal effect on the incidence of PSE poultry meat has also been established in several studies (Bianchi *et al*., 2007; McCurdy *et al*., 1996), suggesting that excess heat stress during summer can induce PSE problems in poultry. Maintaining muscles at high postmortem temperature due to inadequate chilling has been demonstrated to contribute to the incidence of the PSE problem in poultry (Alvarado and Sams, 2004; Feng *et al*., 2008; Molette *et al*., 2003).

PSE poultry meat causes approximately 200 million dollars a year in losses for the US broiler industry alone (Lubritz, 2007). However, available strategies to prevent PSE are limited because the genetic markers are not available for the selection of commercial poultry strains. Barbut *et al*. (2008) suggested that the application of several improved pre- and post-slaughter handling strategies (e.g., a rest period after transportation, the employment of gentle catching methods, lower stress unloading methods, lower stress stunning methods, efficient carcass chilling methods, etc.) to reduce stress on poultry can be useful to minimize developing PSE meat. Fletcher (1999) found significant color variations within broiler breasts packed using multiple-fillet packaging formats which would have been rejected by consumers if presented to them. Barbut (2009) and Owens *et al*. (2009) suggested a sorting system of poultry breasts at the plant based on their color because of the strong relationship between color and quality properties of PSE poultry breast. PSE meat can be used in poultry products where there is no requirement to add water or to marinate, or in further-processed products which contain non-meat ingredients, such as regular and modified starch (Zhang and Barbut, 2005b), soy protein, collagen, carrageenan (Daigle *et al*., 2005) and other functional ingredients which can compensate WHC and other functional properties lost in PSE meat.

4.2.2 Pink discoloration in poultry breast meat

Pink discoloration has been one of major quality defects in cooked, uncured poultry products, especially breast-related products. The pink discoloration is a condition where cooked, uncured poultry breast products still exhibit a pink color even after the meat has been fully cooked to an internal temperature (73.9°C) recommended by the Food Safety and Inspection Service of the USDA (FSIS USDA, 2007). This phenomenon has been considered a quality defect because consumers may take it as an indicator of undercooked products and consider it unsafe to eat, resulting in significant product rejection.

Myoglobin is the primary pigment responsible for meat color. Myoglobin consists of globin protein and its prosthetic group heme, a porphyrin ring containing

an iron atom at its center. The heme ring absorbs the light at the end of the visible spectrum, resulting in its red color. Five coordination points of the iron atom are linked to the four nitrogen atoms of the heme ring and histidine residue of the globin, and its sixth coordination point can be complexed with other molecules, usually oxygen and water. Many other molecules such as CO, NO, ammonia, nicotinamide, pyridine, etc. can also be attached to myoglobin, leading to color changes in meat (Holownia *et al*., 2003). Therefore, meat color is determined by this sixth ligand, but the ability of the iron atom to bind the ligands is dependent upon the oxidation status of the heme iron: only ferrous ion (reduced form; Fe(II)) can bind to them.

Three major forms of myoglobin are found in fresh meat, resulting in the different colors of meat: oxymyoglobin (cherry-red, Fe(II)), deoxymyoglobin (purple-red, Fe(II)) and metmyoglobin (brown, Fe(III)). When meat is cooked, oxy- and deoxymyoglobins are denatured to ferrohemochrome (red, Fe(II)) and metmyoglobin to ferrihemichrome (brown, (Fe(III)) (King and Whyte, 2006). The former can bind to other ligands due to its possession of the ferrous ion, but the latter cannot. Therefore, the pigment classes associated with pink discoloration in uncured, cooked poultry breast products are likely to be due to undenatured myoglobin and ferrohemochrome which can be linked to the ligands, resulting in the development of a reddish color. In addition, cytochrome c, a heme-containing protein found in mitochondria, is another possible factor for producing pink discoloration in poultry because of its higher thermostability and reactivity compared to myoglobin (Ahn and Maurer, 1990b; Maga, 1994).

Endogenous conditions such as pH and oxidation reduction potential in fresh and cooked meat can influence the thermal denaturation, chemical status and reactivity of pigments, resulting in color variation in cooked meat. The thermal denaturation of myoglobin during cooking is significantly affected by meat pH. A higher pH in muscle significantly reduces the thermal denaturation of myoglobin at a given temperature (Hunt *et al*., 1999; Trout, 1989) and increases pigments containing ferrous ions in their heme ring (Ahn and Maurer, 1990a). Hunt *et al*. (1999) indicated that deoxymyoglobin is more heat-stable than metmyoglobin in the range of pH 5.4~6.6. Therefore, high pH may cause incomplete thermodenaturation and greater reactivity of the pigments with ligands, leading to pink discoloration. Oxidation-reduction potential (ORP) in meat is another important factor for pink discoloration. The ORP determines the chemical status of the iron in the heme ring and subsequently, the pigments present – that is, oxy- and deoxymyoglobin versus metmyoglobin or ferrohemochrome versus ferrihemichrome, and is, therefore, a determining factor in facilitating pigments to react with ligands, resulting in an increase in a reddish color intensity (Ahn and Maurer, 1989a, 1990b; Holownia *et al*., 2004). The ORP in meat is closely associated with pH and the presence of reducing agents in meat. The ORP seems to decrease to negative (reducing condition) as the pH increases (Ahn and Maurer, 1989a). Reducing agents can also decrease the ORP and convert ferric ions in the pigments to ferrous ions, thereby increasing their reactivity with ligands. Various reducing compounds, including ascorbic acid, NAD(P)H and thiol compounds such as

glutathione (GSH) are present in muscle tissues. Min *et al*. (2008) observed that the ferric ion reducing capacity in raw chicken breast was significantly higher than that in raw beef loin and pork loin, and was reduced, but still retained a significant presence after cooking.

Factors that can affect the endogenous conditions in meat such as the concentration of pigments, pH, ORP (particularly, the concentration of reducing agents) and the availability of ligands can be associated with pink discoloration: nitrate/nitrite contamination, pre-slaughter and slaughter conditions, oven/environmental gases, processing conditions such as end point of cooking temperature, ingredients such as salt, phosphate, etc. and irradiation of precooked products (Ahn and Maurer, 1989a; Holownia *et al*., 2003; Maga, 1994). Among all of these factors, nitrate/nitrite contamination is the most probable factor responsible for pink discoloration in uncured, cooked poultry breast product (Table 4.2; Ahn and Maurer, 1989b; Heaton *et al*., 2000; Holownia *et al*., 2004; Maga, 1994). Nitrite and nitrate are commonly used to form nitrosoheme pigments for the typical pink color of cured meat products. Ahn and Maurer (1987) found that the level of nitrite and nitrate in raw turkey breast was in the range of 0.0–0.7 ppm and 3.8–21.0 ppm, respectively, and that 100 ppm added nitrate could be converted to 5.1 ppm nitrite by the presence of naturally occurring microorganisms during a 40 h storage period at 4°C. They also showed that addition of 1 ppm of nitrite could cause pink discoloration in turkey breast (Ahn and Maurer, 1989b). Heaton *et al*. (2000) confirmed that the minimum nitrite ion concentrations for panel detection of pink discoloration were 1.3 and 0.7 ppm for cooked turkey and chicken breast, respectively. Feed and water for drinking or carcass washing/chilling can serve as sources of nitrate/nitrite for the development of the pink discoloration in poultry breast muscle (Maga, 1994). Therefore, it is suggested that the combined conditions (an uncommonly high level of nitrite/nitrate in feed or water supplies, a high microbial load and long-time storage under refrigeration) are likely to cause pink discoloration in poultry breast meat (Ahn and Maurer, 1987). Additionally, Heaton *et al*. (2000) found high levels of nitrate (> 250 ppm) and nitrite (> 42 ppm) in soy protein isolate. Scriven *et al*. (1987) observed the migration of nitrate

Table 4.2 Effect of nitrite level on pink discoloration in cooked chicken breast roll

Nitrite (ppm)	Panel color score[2]	NO-hemochrome (ppm)	Redness[3]
0	1.1[a]	2.7[a]	3.0[a]
1	1.3[b]	4.4[a]	4.3[b]
2	2.1[c]	9.2[b]	6.0[c]
3	2.4[d]	10.9[b]	6.7[d]
4	2.2[c]	9.6[b]	6.5[d]

Source: Adapted from Heaton *et al*. (2000).
Notes: [1] Means with different letters (a–d) within the same row are significantly different ($P < 0.05$).
[2] Panel color score 1 = not pink, 2 = slightly pink, 3 = moderately pink, 4 = very pink, and 5 = extremely pink.
[3] Redness measured as Hunter color a.

from paper materials for packaging to chicken breast meat. These indicate that ingredients and packaging materials can be sources of nitrate/nitrite for fresh and further-processed products. In addition, certain amino acids, nicotinamide (Ahn and Maurer, 1990b, 1990c), ammonia (Shaw *et al*., 1992) and nitric dioxide produced in gas ovens (Cornforth *et al*., 1998) have been suggested as possible ligands in the development of pink discoloration.

Because the mechanisms of pink discoloration are not fully understood yet, the poultry industry has mainly focused on applying and following good manufacturing practices to reduce or eliminate the external contamination of the possible ligand sources such as nitrite/nitrate during processing. Schwarz *et al*. (1997) hypothesized that non-pink generating ligands such as metal chelators can competitively occupy the sixth coordinate point of the iron heme ring to prevent the formation of pink color-generating ligand complexes such as nitrosoheme pigments. They reported the effectiveness of several non-pink-generating metal chelators such as diethylenetriamine pentaacetic acid (DTPA), ethylenedinitrilotetraacetic acid (EDTA), trans 1,2-diaminocyclohexane-N,N,N′,N′ tetraacetic acid (CDTA) and non-fat dried milk to prevent the development of pink discoloration in poultry. Several efforts have been made to prevent pink discoloration in uncured, cooked poultry breast products by using non-meat ingredients: citric acid, sodium citrate, non-fat dried milk, whey protein concentrate, calcium chloride, tricalcium phosphate and sodium tripolyphosphate (Sammel and Claus, 2006, 2007; Sammel *et al*., 2007). However, the effectiveness and availability of these ingredients has been controversial. Some of these are not approved for the use in foods, some show inconsistent results in different studies and some adversely affect other meat functional properties such as WHC, cooking yield and possibly oxidative stability.

4.2.3 Discoloration of irradiated poultry meat

Irradiation is a promising technology to improve the safety of poultry meat and meat products by reducing or eliminating food-borne pathogens. However, it increases storage-stable redness, which is similar to pink discoloration in fresh poultry breast meat, and this redness can persist after cooking (Du *et al*., 2002). In a series of studies on pink color development in turkey breast by irradiation, Nam and Ahn (2002a, 2002b) suggested that irradiation generates reducing conditions and carbon monoxide (CO), resulting in the formation of CO-heme pigments which are primarily responsible for the pink color in irradiated turkey breast. The intensity of redness, ORP and CO production in irradiated poultry breast has been shown to vary, depending upon irradiation dose and packaging conditions (Table 4.3; Ahn *et al*., 1998; Nam and Ahn, 2002a). An increase in irradiation dosage was found to increase poultry redness values and CO production, but decreased ORP. Vacuum-packaged, irradiated raw turkey breast also had significantly higher redness values and CO production levels and lower ORP when compared to aerobically packaged equivalents (Nam and Ahn, 2002a). Interestingly, Du *et al*. (2002) indicated that the redness induced by irradiation in raw chicken breast remained after cooking, but only when

Table 4.3 Redness (CIE a value), production of CO, and oxidation-reduction potential (ORP) in raw turkey breast with different packaging, irradiation, and storage conditions[1]

Parameter	Irradiation dose (kGy)					
	Aerobic packaging			Vacuum packaging		
	0	2.5	5.0	0	2.5	5.0
Redness	3.02[c]	4.69[b]	6.45[a]	2.86[c]	5.72[b]	6.93[a]
ORP (mV)	−15.7[a]	−174.7[b]	−91.2[b]	−74.0[a]	−193.2[b]	−279.0[c]
CO (ppm)	0[c]	328[b]	593[a]	0[c]	445[b]	999[a]

Source: Adapted from Nam and Ahn (2002a).
Note: [1]Means with different letters (a–c) within the same row with same packaging are significantly different ($P < 0.05$).

raw meat was stored under vacuum. The authors suggested that this was most likely due to the increased ORP and the increased competition between CO and O_2. This implies that short-term aerobic display (~ 3 days) of raw poultry breast after irradiation can prevent the development of pink discoloration by irradiation. However, long-term aerobic display accelerates oxidative quality deterioration of irradiated meat products. Nam and Ahn (2003a) suggested a 'double-packaging' concept to prevent pink discoloration: (1) several meat or product pieces individually packaged in oxygen-permeable plastic bag are vacuum-packaged together in one big oxygen-impermeable plastic bag, irradiated and stored; (2) the inner bags can be removed from the outer oxygen-impermeable bag for aerobic exposure for a few days before marketing or consumption. Irradiation can generate considerable amounts of free radicals such as hydroxyl radicals, which not only promote lipid oxidation, but also induce the breakdown of biomolecules, thereby leading to the production of CO. The addition of antioxidants can be one of preventive strategies used against quality deterioration of meat by irradiation such as lipid oxidation, off-odor production and pink discoloration. The application of one method, however, may not be sufficient to prevent the quality deterioration of irradiated meat. Nam and Ahn (2003b) demonstrated that the combined use of 'double-packaging' and antioxidants can be an efficient strategy to control pink discoloration as well as lipid oxidation and off-odor production in irradiated raw and cooked turkey breast. This strategy can be applied to prevent pink discoloration in all poultry breast meat products.

4.2.4 Modified atmosphere packaging to reduce discoloration of poultry meat

The color of poultry products is significantly affected by packaging because packaging can control endogenous and exogenous factors affecting the quality of products. Nowadays, modified atmosphere packaging (MAP), including vacuum packaging (VP), has been widely used in the meat industry because it can maintain or extend the shelf life and quality of the products. Common gases used in modified atmosphere packaging are carbon dioxide (CO_2), oxygen (O_2) and nitrogen

(N_2), in different concentrations (Rao and Sachindra, 2002). Carbon dioxide is used primarily for controlling microbial growth. Oxygen is used to extend, in particular, red meat color, but its proportion should be strictly controlled because it can promote oxidation of lipid and pigments, resulting in the discoloration of such products. Zakrys *et al*. (2008) indicated that lipid oxidation of beef loin steaks packed in various levels of O_2 atmospheres (0–80%) during a 15-day refrigerated storage is dependent upon O_2 concentration and seems to drive changes in color and oxymyoglobin content. Nitrogen gas serves as a filler gas and purge reducer. Keokamnerd *et al*. (2007) reported that high N_2 atmospheres (90%) maintained the surface color of ground chicken breast meat better than high O_2 atmospheres (90%). Seydim *et al*. (2006) and Mastromatteo *et al*. (2009) demonstrated that the discoloration of ground poultry patties packed in high oxygen atmosphere (O_2 80% and CO_2 20%) during a refrigerated storage was significantly greater than those held in high nitrogen (N_2 80% and CO_2 20%), vacuum and low oxygen (O_2 20% and N_2 80%; O_2 20%, N_2 65% and CO_2 30%) atmospheres. Additionally, nitrosoheme pigments in cured poultry products were sensitive to even low concentration of O_2, resulting in a dull greyness on the surface (Pexara *et al*., 2002). Therefore, the incorporation of O_2 in modified atmosphere for poultry products should be restricted to prevent the development of discoloration during storage. In addition, carbon monoxide has been used to stabilize and extend the color shelf life of beef (Belcher, 2006); however, as alluded to previously, CO should not be used for poultry breast products because it may cause pink discoloration. Because the discoloration in poultry products during storage is mainly caused by pigment oxidation through free radical attack, such as reactive oxygen species (ROS), the addition of antioxidants to products can be another preventive strategy for development of discoloration. As observed in an attempt to prevent pinking problems in irradiated poultry breast (Nam and Ahn, 2003b), addition of antioxidants have proved to be one of promising strategies employed to prevent discoloration problems in poultry products. Previous research into other meat systems has demonstrated the positive effects that antioxidants have bestowed on numerous meat quality attributes (Ahn *et al*., 2007; Carpenter *et al*., 2007; Han and Rhee 2005; Min *et al*., 2009; Rojas and Brewer 2007), as well as the synergistic effects that have resulted when antioxidant usage and packaging systems have been combined for similar applications (Camo *et al*., 2008; Lund *et al*., 2007; O'Grady *et al*., 1998, 2000; Sánchez-Escalante *et al*., 2001; Vissa and Cornforth, 2006).

4.3 Lipid oxidation in packaged fresh and processed poultry meat

Lipid oxidation is one of the major causes of quality deterioration and shortened shelf life of fresh and processed poultry products. Lipid oxidation causes detrimental effects on sensory, functional and nutrition quality aspects of such products (Kanner, 1994). Free radical chain reaction is the primary mechanism responsible for causing lipid oxidation in meat, and consists of three primary steps: initiation,

propagation, and termination (Min and Ahn, 2005). In the initiation step, highly reactive free radicals, such as the hydroxyl radical (•OH), abstracts a hydrogen atom from bisallylic carbon contained within a lipid molecule. Free ionic iron is the most important catalyst to produce •OH via $O_2^{\bullet-}$ and the ascorbic acid-assisted Haber-Weiss reaction because it is the most abundant transit metal ion in muscle (Halliwell and Gutteridge, 1999). The oxidation status of iron most likely determines the reactivity of lipid oxidation as it serves as a catalyst for lipid oxidation: Fe(II) is much more reactive than Fe(III) (Ahn and Kim, 1998). Myoglobin is a central compound source for the primary meat catalyst (i.e., free ionic iron) and/or initiators (ferrylmyoglobin and hematin). The formed lipid radical undergoes molecular rearrangement to form conjugated dienes which, subsequently, turn into lipid peroxyl radicals (LOO•) in the presence of O_2. In the propagation step, the formed lipid peroxyl radical abstracts a hydrogen atom from an adjacent lipid molecule to form a lipid hydroperoxide (LOOH). This reaction produces another lipid radical that can attack other lipid molecules, and the reaction continues. In the termination step, LOO• undergoes self-destruction to form a non-radical. Relatively stable LOOH is also destructed to form the lipid oxidation secondary products under high temperatures or exposure to transit metal ions such as Fe(II) (Halliwell and Gutteridge, 1999). Hexanal, a product from n-6 fatty acid, is the most predominant volatile, so that it has been used as an indicator of meat flavor deterioration (Shahidi and Pegg, 1994). Aldehydes such as malondialdehyde are capable of reacting with proteins to form protein adducts, leading to the degradation of protein solubility and functionality (Lynch *et al*., 2001). They can also stimulate oxymyoglobin autoxidation and prooxidant activity of metmyoglobin, resulting in the deterioration of meat color and flavor (Lynch and Faustman, 2000). Some aldehydes, such as 4-hydroxy-2-nonenal, are carcinogenic (Halliwell and Gutteridge, 1999). Thus, concentrations of hexanal, malondialdehyde and total volatiles, abundant lipid oxidation secondary products in meat, have been widely used as indicators for its lipid oxidation development and are well correlated to each other as shown in Fig. 4.1.

4.3.1 Factors affecting lipid oxidation in packaged fresh and processed poultry meat

The postmortem biochemical changes that take place during the conversion of muscle to meat causes imbalances between prooxidant and antioxidant factors, resulting in initiation of lipid oxidation. Many endogenous and exogenous factors affect lipid oxidation rate of fresh and further-processed meat products, such as animal species, total fat content, fatty acid composition, iron availability, level of antioxidant molecules and enzymes, muscle damage during pre- and post-slaughtering events, pH, carcass temperature, mechanical processes such as grinding, cooking, additives, oxygen availability, packaging, storage conditions, etc. (Kanner, 1994; Min and Ahn, 2005).

The susceptibility of muscle lipids to oxidation depends on the degree of unsaturation in fatty acids (Song and Miyazawa, 2001). Phospholipids in muscle cell

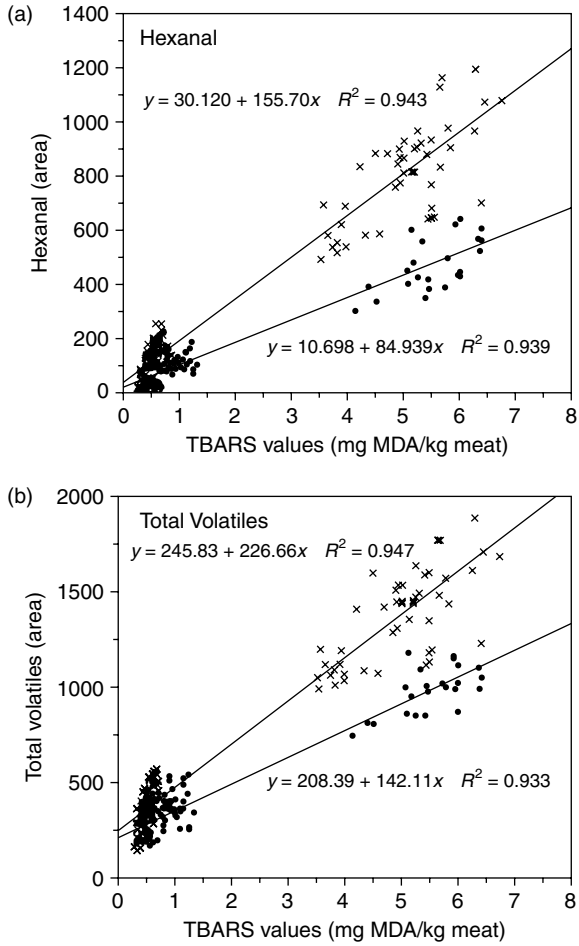

Fig. 4.1 Relationships between TBARS, hexanal and total volatiles of cooked-meat patties from three different pork muscles (●, *L. dorsi* (LD) muscle; **x**, *L. psoas* (PS) and *R. femoris* (RF) muscles). (Source: Adapted from Ahn *et al*. (1998).)

membranes are major substrates for lipid oxidation in the initial stages of oxidation because of the highly unsaturated nature of their fatty acids and accessibility to prooxidants in the sarcoplasm. After the disruption of cell membranes, PUFA of triglycerides (TG) stored in adipose tissues plays an important role in the development of lipid oxidation.

Oxidative stability varies among meats from different animal species, muscle type and anatomical location. Several studies have compared oxidative stability among poultry (chicken and turkey) breast and thigh, pork loin and beef loin (Min and Ahn, 2009; Min *et al*., 2008, 2010; Rhee *et al*., 1996). They demonstrated that poultry breast is the most oxidatively stable in both raw and cooked forms, and

beef (in both forms) was the most susceptible to lipid oxidation. In addition, poultry breast meat had a significantly higher oxidative stability than poultry thigh meat (Min *et al*., 2008). Min *et al*. (2008) reported that lipid oxidation did not increase in ground chicken breast patties during 10 days of refrigerated storage, although lipid oxidation in cooked chicken breast patties significantly increased during the storage. Yet, its increase was remarkably smaller than that in chicken thigh and beef loin. Differences in endogenous pro- and antioxidant factors are responsible for the difference in the oxidative stability of meat from different animal species: the amounts of heme pigment and free ionic iron, total antioxidant capacity, reducing compounds, catalase, total fat and fatty acid composition (Chan and Decker, 1994; Min and Ahn, 2009; Rhee *et al*., 1996).

Min *et al*. (2008) found that PUFA composition in phospholipids of chicken breast was similar to that of beef loin but that in TG and total fat of the former was significantly higher than that of the latter, suggesting that differences in amounts of prooxidants in the sarcoplasm, rather than the amount of total fat and PUFA, may be responsible for the lipid oxidation rate of the intact or unprocessed raw meat, as most lipids are stored in adipose tissues where the amounts of prooxidants are low (Min *et al*., 2008). However, any processes causing disruption of membranes such as deboning, size reducing processes (grinding, etc.), cooking and addition of salt result in the increased accessibility of prooxidants to the PUFA in adipose tissues. Reducing compounds such as ascorbic acid can serve as either an antioxidant or a prooxidant, depending on its relative concentration to iron present (Decker and Hultin, 1992). Min *et al*. (2008) and Min and Ahn (2009) reported that ferric ion reducing capacity and non-heme iron content in raw meats from different animal species vary significantly. The ferric ion reducing capacity in raw chicken breast was the highest, but decreased significantly upon storage and cooking; that in beef loin, however, did not change upon storage and was even higher in the cooked form. Non-heme iron content in raw and cooked beef was significantly higher and increased significantly during storage, compared to chicken breast. Therefore, ferric ion reducing capacity of meat can serve as a prooxidant when meat is salted, cooked, held under prolonged storage etc., which can increase the level of free ionic iron.

Cooking and addition of sodium chloride, the most adopted processes for processed meat products, cause the acceleration of lipid oxidation. High temperature usage causes reductions in the activation energies for oxidation and decomposes preformed hydroperoxides to free radicals (Min and Ahn, 2005). Cooking destroys muscle cell structure, inactivates antioxidant enzymes, destructs antioxidants and releases free ionic iron, hematin and/or oxygen from heme pigments, resulting in an increased lipid oxidation rate (Min and Ahn, 2005). Cooking rate and final temperature also affect the release of free ionic iron from heme pigments, leading to the promotion of lipid oxidation; slow cooking increases iron release (Chen *et al*., 1984). Sodium chloride (NaCl) is the most important ingredient for successful processed meat product manufacture as it enhances preservation, flavor, tenderness, juiciness, WHC and binding ability (Rhee, 1999). However, addition of NaCl accelerates lipid oxidation depending on its concentration by (1) disrupting muscle

cell membrane; (2) releasing free ionic iron from heme pigments; and (3) inactivating antioxidant enzymes (Min and Ahn, 2005; Min *et al*., 2010; Rhee, 1999).

4.3.2 Preventing lipid oxidation in packaged fresh and processed poultry meat

Oxygen availability is probably the most important factor for lipid oxidation in raw and cooked meat because the primary lipid oxidation products, lipid hydroperoxides, are formed from the reaction of oxygen with polyunsaturated fatty acids. Subsequently, they are broken down into secondary products, leading to the deterioration of sensory and nutritional product qualities. The development of lipid oxidation in raw and cooked meat is dependent upon the concentration of oxygen present in the packaging headspace (O'Grady *et al*., 2000; Smiddy *et al*., 2002a, 2002b). Cholesterol oxidation is also greatly affected by oxygen availability, resulting in the production of cholesterol oxidation products, which are associated with atherosclerosis and coronary heart disease (Nam *et al*., 2001). In fact, oxygen availability is more important for cooked meat because it possesses more oxidation-favorable conditions such as a disintegrated structure with an increased availability of prooxidants, compared to raw meat. The utilization of vacuum and modified atmosphere packaging without incorporating oxygen improves the oxidative stability of cooked poultry products (Patsias *et al*., 2006; Seydim *et al*., 2006). Ahn *et al*. (1992, 1993a) reported that turkey breast and thigh patties vacuum packaged immediately after cooking ('hot-packaging') developed significantly lower levels of lipid oxidation during refrigerated storage than those vacuum packaged after chilling for 3 h (Table 4.4). This suggested that the 3-h exposure to oxygen provided enough time to promote lipid oxidation in cooked meat. They also reported that the addition of prooxidants such as free ionic iron, hemoglobin and NaCl had little effect on the oxidation of 'hot-vacuum packaged' cooked turkey patties during storage. Brunton *et al*. (2002) confirmed the findings of Ahn *et al*. (1992) as shown in Fig. 4.2. They found that the 'hot packaged' turkey breast meat held in modified atmosphere packaging (100% N_2) was more oxidatively stable than that held under vacuum packaging conditions, possibly due to incomplete removal of all traces of air during vacuum packaging. In addition, Brunton *et al*. (2002) proposed that the likely reasons for the enhancement of oxidative stability by 'hot packaging' was that, during cooking, the hydrostatic pressure of the water vapor from the meat surface caused the oxygen-excluding effect to protect meat from oxidation, but during cooling, the rapid decrease in the hydrostatic pressure accelerated the surge of air from the surrounding atmosphere into meat matrix, resulting in the rapid buildup of oxidation-favorable conditions in the cooked meat. In addition, the irregular, pore-rich surface of the cooked meat caused by an irregular orientation of myofibrillar proteins may have facilitated the increased ingress of oxygen. Therefore, 'hot packaging' immediately after cooking prevented this surge of oxygen from the atmosphere into cooked meat matrix, leading to minimization of the development of lipid oxidation. The 'hot packaging' concept is one strategy which effectively controls lipid oxidation in cooked meat.

Table 4.4 Effect of prooxidant treatment[1] on the TBARS values (mg MA/kg meat) of hot- and cold-packaged[2] cooked turkey patties from breast, leg and meat mixture[3] during refrigerated storage

	Storage (weeks)							
	0		1		2		3	
Treatment	Hot pkg	Cold pkg	Hot pkg	Cold pkg	Hot pkg	Cold pkg	Hot pkg	Cold pkg
Breast meat								
Control	0.79 ± 0.1a	1.70 ± 0.3a	0.94 ± 0.1a	1.97 ± 0.1a	1.05 ± 0.1b	1.89 ± 0.1a	1.02 ± 0.1a	1.60 ± 0.1a
Myoglobin	0.57 ± 0.0a	1.91 ± 0.3a	0.87 ± 0.1a	2.09 ± 0.2a	0.94 ± 0.1a	1.94 ± 0.1a	0.86 ± 0.1a	1.67 ± 0.1a
$FeCl_2$	1.57 ± 0.2c	2.77 ± 0.2b	1.87 ± 0.2b	2.85 ± 0.3b	2.18 ± 0.1d	3.07 ± 0.2b	2.10 ± 0.2b	2.77 ± 0.1b
Mb + $FeCl_2$	1.24 ± 0.2b	2.76 ± 0.2b	2.26 ± 0.2c	3.26 ± 0.4c	1.99 ± 0.0c	3.21 ± 0.9b	1.88 ± 0.5b	3.01 ± 0.1c
Leg meat								
Control	0.76 ± 0.2a	1.40 ± 0.3a	0.61 ± 0.0a	1.75 ± 0.1a	0.89 ± 0.1a	1.83 ± 0.1a	0.83 ± 0.1a	1.57 ± 0.2a
Mb	0.59 ± 0.1a	1.31 ± 0.1a	0.66 ± 0.1a	1.76 ± 0.1a	0.72 ± 0.1a	1.79 ± 0.1b	0.71 ± 0.0a	1.47 ± 0.1a
$FeCl_2$	1.53 ± 0.4b	2.16 ± 0.2c	1.99 ± 0.3b	2.67 ± 0.3b	2.14 ± 0.2c	3.15 ± 0.1c	2.01 ± 0.1b	2.86 ± 0.1b
Mb + $FeCl_2$	0.95 ± 0.4a	1.78 ± 0.3b	1.62 ± 0.5b	2.70 ± 0.4b	1.41 ± 0.2b	2.74 ± 0.1a	1.93 ± 0.3b	3.26 ± 0.6b
Meat mixture								
Control	0.45 ± 0.0a	0.70 ± 0.1a	0.60 ± 0.0b	1.36 ± 0.1b	0.58 ± 0.0b	1.47 ± 0.2b	0.60 ± 0.1b	1.39 ± 0.2b
Mb	0.41 ± 0.0a	0.61 ± 0.1a	0.45 ± 0.0a	1.03 ± 0.2a	0.47 ± 0.1a	1.08 ± 0.1a	0.51 ± 0.0a	1.13 ± 0.1a
$FeCl_2$	0.77 ± 0.0b	1.16 ± 0.1b	0.84 ± 0.0c	1.38 ± 0.1b	0.87 ± 0.1c	1.81 ± 0.2c	0.91 ± 0.1c	1.66 ± 0.1c
Mb + $FeCl_2$	0.79 ± 0.1b	1.24 ± 0.1b	0.82 ± 0.1c	1.50 ± 0.2b	0.84 ± 0.0c	1.32 ± 0.1b	0.94 ± 0.1c	1.41 ± 0.1b

Source: Adapted from Ahn *et al*. (1992).

Notes: Mb, myoglobin; TBARS, 2-thiobarbituric acid reactive substances; MA, malondialdehyde.

[1] Treatments: myoglobin: 0.4 mg Mb/g meat; free iron: 12.7 ppm as free Fe^{++} ion.

[2] In 'hot-packaging', patties were immediately vacuum-packaged after cooking whereas in 'cold packaging', patties were cooled without packaging. Means with different letters (a–b) within the same row are significantly different ($P < 0.05$).

[3] Meat mixture: 25% breast, 25% leg and 50% mechanically deboned turkey meat.

[a–d] Different letters within a column of same meat type are significantly different ($p < 0.05$).

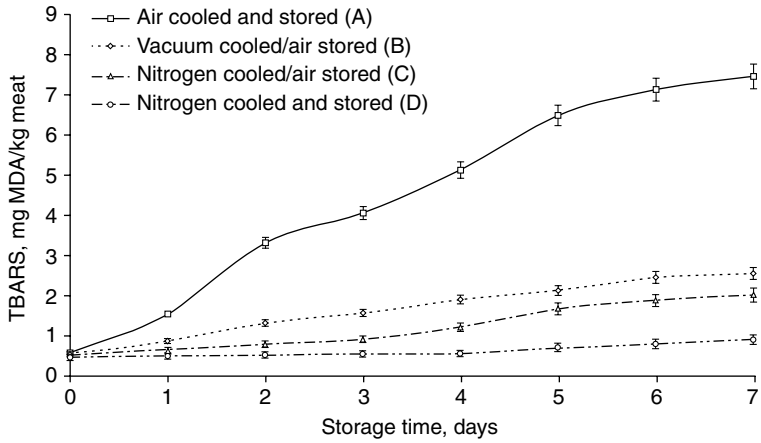

Fig. 4.2 Effect of 'hot-packaging' under vacuum and modified atmosphere (100% N_2) on lipid oxidation in cooked turkey breast meat during 7-day storage at 4°C. Immediately after cooking, samples were either air-cooled (A), cooled in vacuum packaging (B), or in 100% N_2 (C) to 4°C in a refrigerator for ~ 6 h. Then, (B) and (C) was unsealed and stored for 7 days at 4°C with (A) For (D), samples were cooled and stored in 100% N_2 for the duration of storage. Data are presented as TBARS values (mg MDA/kg meat). (Source: Adapted from Brunton *et al*. (2002).)

Both vacuum and modified atmosphere packaging remove oxygen from the packaging atmosphere and employ oxygen-impermeable barriers to protect the products from the exposure to oxygen. Packaging materials with oxygen permeability of 100 $cm^3/m^2/atm/day$ at 25°C are generally used for vacuum and modified atmosphere packaging (Rao and Sachindra, 2002). Although the meat products are appropriately packaged using vacuum and modified atmosphere packaging, the oxygen-free atmosphere in the packaging is not always guaranteed and permeation of oxygen through packaging barriers occurs (Kerry *et al*., 2006). Many factors may affect the residual oxygen concentration in modified atmosphere and vacuum packaging: the ability of food to trap air, oxygen permeability of the packaging materials, integrity of the packaging to prevent leaking and ineffective gas flushing (Smith *et al*., 1986). Smiddy *et al*. (2002c) surveyed the oxygen content in commercial modified atmosphere packs of processed cooked meats using four categories of oxygen levels (0.0, 0.01–0.5, 0.51–1.2 as ideal, desirable, unacceptable and rejectable) outlined by manufacturers; 29.4% and 20.4% of all surveyed packs were placed in unacceptable and rejectable categories, respectively, at Day 1 and 5.3% and 79.3%, respectively, after 21 days of refrigerated storage. They suggested that the combination of entrapped oxygen and oxygen permeability of barrier materials may be the primary factors responsible for increased levels of oxygen detected in packs. Randell *et al*. (1995) found that the oxygen level increased and the sensory shelf life of modified atmosphere-packaged marinated chicken breast meat (60% N_2 and 40% CO_2) decreased as

product packs leaked. Smiddy *et al*. (2002b) found that the residual oxygen level in modified atmosphere (70% N_2 and 30% CO_2) packs of cooked chicken patties (0.9–1.1 %) were significantly higher than that of vacuum-packaged (0.11–0.15 %) equivalents and the lipid oxidation level of the former was significantly higher than the latter during refrigerated storage. However, Smiddy *et al*. (2002c), reported that around 50% of modified atmosphere-packed products surveyed had less than 0.5% of residual oxygen level. This indicated that the effectiveness of gas flushing was an important factor in the residual oxygen levels determined in these modified atmosphere packs.

Because the residual oxygen in packaging is important, the application of oxygen-scavenging technologies can be another good strategy to ensure the oxidative stability of vacuum- and modified atmosphere-packaged raw and, especially, cooked poultry products (Vermeiren *et al*., 1999). The oxygen-scavenging technologies available today, include iron powder oxidation, ascorbic acid oxidation, photosensitive dye oxidation, enzymatic oxidation, unsaturated fatty acid rice extract, or immobilized yeast on a solid substrate (Floros *et al*., 1997). Most commercial O_2 absorbers currently available on the market are based on iron powder oxidation and are provided in sachet form (Coma, 2008). Martínez *et al*. (2006) reported that the combined use of commercial O_2 absorber and modified atmosphere packaging (20% CO_2 and 80% N_2) successfully prevented lipid oxidation and extended shelf life of fresh pork sausages. In addition, the oxygen-scavenging plastic film, within which O_2 absorbers are incorporated, is another alternative to sachets, particularly for vacuum-packaged products where the meat makes direct contact with the packaging film and can minimize negative responses to the sachets, avoid the risk of product contamination through accidental rupture of the oxygen absorber and prevent accidental consumption of the sachet by the consumer (Kerry *et al*., 2006). The utilization of O_2 absorbers is a good example of an 'active packaging' concept where specific additives are incorporated into packaging systems to preserve the quality of meat products and maintain or extend their shelf life (Kerry *et al*., 2006).

One of the efficient strategies to prevent lipid oxidation in raw and cooked meat is the incorporation of antioxidants in raw and cooked meat products. Synthetic antioxidants such as butylated hydroxyl-anisole (BHA) have been legally approved and widely used in the meat industry. However, consumers' concerns about the use of artificial preservatives in meat products have been increased because of their possible toxicity to human health. Consequently, attention has focused on the use of natural antioxidants, such as vitamin E, herbal extracts (rosemary, sage, oregano, etc.), green tea (tea catechins), grape seed extracts and others to replace synthetic antioxidants. These natural antioxidants show not only strong preventive effects on lipid oxidation, but may also function as nutraceuticals and protect consumers against developing chronic diseases. Antioxidants such as those listed above have efficiently prevented the development of lipid oxidation in a range of further-processed poultry products (Antony *et al*., 2006; Brannan, 2008; Mielnik *et al*., 2002; Mitsumoto *et al*., 2005; Rababah *et al*., 2006). Dietary supplementation of poultry diets with vitamin E and other natural antioxidants, primarily phytochemicals, have been shown to enhance the oxidative stability of poultry meat, but the effects

vary between the different antioxidants used (Bou *et al*., 2006; Jang *et al*., 2008; Smet *et al*., 2008). Both vacuum and modified atmosphere packaging can also serve to replace synthetic antioxidants through prevention of lipid oxidation in meat products (Ahn *et al*., 1992; Brunton *et al*., 2002; Rao and Sachindra, 2002). Therefore, the simultaneous application of both natural antioxidant and vacuum or modified atmosphere packaging not only meets consumer demands for replacement of synthetic preservatives, but also provides stronger protective effects on lipid oxidation in poultry products. Ahn *et al*. (1993b) reported that the combination of 'hot vacuum' packaging and antioxidants provided cooked turkey meat patties with better protection from lipid oxidation than either treatment alone. Smiddy *et al*. (2002b) observed that modified atmosphere-packaged chicken patties manufactured from chicken fed a vitamin E-supplemented diet showed higher oxidative stability than patty equivalents fed a control diet (not supplemented with vitamin E). These studies suggest that poultry meat supplemented with antioxidants is protected against the rapid development of lipid oxidation, caused by a small amount of oxygen available during brief product exposure to air or to residual oxygen levels in modified atmosphere packaging formats.

4.4 Tenderness and packaged fresh and processed poultry meat

Meat tenderness (texture) is one of the most important organoleptic properties influencing acceptability and eating satisfaction of meat products for consumers, as well as impacting on the future decision to repeat purchase (Miller *et al*., 2001). Tenderness is the consequence of postmortem physicochemical and biochemical changes in muscle, acting primarily on myofibrillar structure. After slaughter, muscle is still extensible and elastic until the onset of rigor mortis, when energy for muscle relaxation is depleted. The duration of pre-rigor phase is dependant on animal species: less than 0.5–1.0 h for chicken and turkey and 4–6 h for beef (Aberle *et al*., 2001). After the onset of rigor, muscle becomes gradually stiff and its tension reaches maximum on the completion of rigor. Rigor mortis development is due to the formation of an irreversible actomyosin complex in muscle, leading to the shortening of sarcomere length. The sarcomere shortening during rigor mortis development causes muscle toughening at the beginning of the postmortem process (Koohmaraie *et al*., 1996). The amount of time that rigor mortis persists varies among animal species: more than 24 h for cattle and sheep and 6 h for chicken breast (Lee *et al*., 2008a).

A tenderization process begins soon after the completion of rigor, resulting in the gradual reduction of muscle toughness. The length of time taken for the tenderization process to reach maximum tenderness also differs greatly between animal species. Dransfield (1994) reported that the time taken to reach 80% of maximum tenderness was 0.3 days for chicken, 4.2 days for pork, 7.7 days for lamb and 10 days for beef. There has been general consensus that proteolytic enzyme systems are responsible for progressing the tenderization process in the

post-rigor phase (Kemp *et al*., 2010; Koohmaraie *et al*., 2002). The proteolytic degradation of myofibrillar and cytoskeletal proteins, such as; desmin, vinculin, nebulin, titin, dystrophin, and troponin-T, causes the loss of structural integrity of myofibrils, thereby, enhancing meat tenderization (Koohmaraie *et al*., 2002). Three proteolytic enzyme systems have been extensively investigated for their involvement in post-rigor proteolytic degradation and meat tenderization; calpain/calpastatin (calcium-dependent), proteosomal (multicatalytic protease complex) and lysosomal (cathepsin) systems (Kemp *et al*., 2010; Koohmaraie *et al*., 2002). The calpain/calpastatin system generally consists of two isoforms of calpain (μ- and m-calpain requiring micromolar and millimolar concentrations of calcium ion for activation, respectively) and calpastatin, their inhibitor (Kemp *et al*., 2010). Among these systems, the calpain/calpastatin system is the most researched protease system regarding its involvement in the tenderization process and this is probably because of: (1) its ability to degrade key myofibrillar proteins such as nebulin, titin, troponin-T and desmin; (2) *in vitro* reproduction produces similar myofibril degradation patterns to those observed in postmortem samples; and (3) the association of overexpressed calpastatin with a large reduction of postmortem proteolytic degradation of myofibrillar proteins (Huff-Lonergan *et al*., 1996; Kemp *et al*., 2010; Koohmaraie and Geesink, 2006). Both μ- and m-calpains are activated by calcium-associated partial autolysis and are, subsequently, inactivated by prolonged autolysis (Li *et al*., 2004). It is suggested that only μ-calpain is likely to be involved in postmortem proteolytic degradation due to its reduced requirement for calcium concentration, unlike m-calpain which remains intact for several days after slaughter (Koohmaraie and Geesink, 2006). The calpain/calpastatin system is ubiquitously present in mammalian and avian tissues but their relative proportions may be variable between different species (Ishiura *et al*., 1982; Wolfe *et al*., 1989). Northcutt *et al*. (1998) reported that the activities of μ- and m-calpains and calpastatin varied with age and muscle type; breast muscle from younger turkey showed higher calpains and calpastatin activities than that from older birds; the calpastatin activity in thigh muscle increased with age; the activities of m-calpain and calpastatin in thigh muscle were significantly higher than in breast muscle, irrespective of age. In addition to the two ubiquitous calpains, a unique isoform of calpain, so-called μ/m-calpain, was found in chicken skeletal muscle and requires an intermediate concentration of calcium ions between those for μ- and m-calpain (Lee *et al*., 2007). Lee *et al*. (2007, 2008a) suggested that the ratio of μ-calpain to m-calpain is different among different skeletal muscles and the calpains in poultry are more calcium-sensitive than mammalian equivalents. In addition, the potential associations of lysosomal cathepsin and proteosomal systems with poultry meat tenderization have been demonstrated in several studies (Etherington *et al*., 1990; Thomas *et al*., 2004). However, no direct evidence to support the involvement of these enzymatic systems in myofibrillar protein degradation in postmortem muscle has been reported to date (Koohmaraie *et al*., 2002). The application of new approaches using innovative analytical instruments such as proteomics, transcriptomics and metabolomics advanced in the past decade may be useful for understanding their relationship to meat tenderness (Remignon *et al*., 2006).

The amount and nature of connective tissues is another important determinant of meat tenderness. The background toughness, 'the resistance to shearing of the unshortened muscle' defined by Marsh and Leet (1966), exists in the pre-rigor muscle and is hardly affected during postmortem storage (Purslow, 2005). The connective tissues contribute to the background toughness of meat. Three connective tissue sheaths surrounding muscles and their components are epimysium, perimysium and endomysium. Epimysium surrounding a muscle can easily be separated from cuts of meat and is generally not considered to be a factor for background toughness (McCormick, 1999). The intramuscular connective tissue (IMCT), including perimysium and endomysium, is closely related to the background toughness of meat (Purslow, 2005). In particular, perimysium most likely plays a major role in background variations in meat toughness because it is a dominant component (~ 90%) of IMCT and is highly variable among individual muscles (McCormick, 1999). Collagen is a major structural protein of muscle connective tissues (Aberle *et al*., 2001). Although five types of collagen fibers from type I to V, depending on their structure, are observed in the skeletal muscle connective tissues, type I and III collagens of filamentous structure are major types of collagens in the connective tissue sheaths of muscles (McCormick, 1999). The number of collagens varies among muscles, depending on their physical activity (Aberle *et al*., 2001). Chicken breast meat has lower collagen content (3.3–4.4 mg/g meat) than chicken thigh meat (8.7–10.2 mg/g meat) (Ding *et al*., 1999; Wattanachant *et al*., 2004). The collagen content is positively correlated to collagens that undergo intermolecular cross-linking processes, and the amount of the collagen cross-linkage in muscle increases with age (Aberle *et al*., 2001). The cross-linkages increase the insolubility and tensile strength of collagens. It is suggested that the background toughness is attributed to the additive effect of both collagen concentration and amount and types of cross-linkages among collagens (Liu *et al*., 1996; McCormick, 1999; Purslow, 2005).

4.4.1 Genetic diversity and tenderness

Rigor mortis development, myofibrillar protein degradation, and amount and nature of connective tissues account for most variations in meat tenderness after postmortem processes. Genetic diversity is one of the most important causes for variations in meat quality, especially tenderness (Lonergan *et al*., 2003). In particular, the effect of recent breeding trends to select fast-growing strains with larger breast muscle over slow-growing strains of poultry on meat quality, especially meat tenderness, has been investigated intensively, but the consequences are obscure (Werner *et al*., 2008). It is hypothesized that high growth rate may increase muscle fiber cross-sectional area and proportion of glycolytic (white) muscle fiber and decrease proteolytic potentials (Dransfield and Sosnicki, 1999). As in sheep callipyge and chicken fed with β-adrenergic agonist, muscle hypertrophy, due to the reduction of protein catabolism, increases meat toughness (Koohmaraie *et al*., 2002). Schreurs *et al*. (1995) reported lower concentrations of μ-calpain and cathepsins in the breast muscle from a fast-growing strain of

chicken than those from a slow-growing strain, suggesting that the larger muscle in the fast-growing strain was a result of reduced protein degradation. A type IIB muscle fiber (white, fast twitch, glycolytic and large diameter) is generally a predominant muscle type in poultry muscle, and its proportion increases in fast-growing chicken, which leads to rapid pH decline and increased potential for PSE development and toughness (Dransfield and Sosnicki, 1999; Santé *et al*., 1995). Fanatico *et al*. (2007) reported that chicken breast muscles from slow-growing strains were more tender than those from fast-growing birds. In contrast, other studies found that breast muscles from fast-growing chicken strains showed higher or similar tenderness values compared to slow-growing chicken strains (Fanatico *et al*., 2005; Wattanachant *et al*., 2004; Werner *et al*., 2008), probably due to more collagen cross-linkages in slow-growing chickens, which require more time to reach market size (Fletcher, 2002) and higher intramuscular fat content in breast muscle from fast-growing birds (Le Bihan-Duval, 2003). Fast-growing strains have higher ultimate pH in breast meat than slow-growing birds (Fanatico *et al*., 2007; Wattanachant *et al*., 2004). Debut *et al*. (2005) demonstrated that slow-growing chicken strains showed more intense struggling on the shackle line and higher lactate concentration in their breast muscles than fast-growing birds, leading to a more rapid postmortem pH fall.

4.4.2 Processing factors and tenderness

Processing factors immediately before and during slaughter (e.g., catching, transportation, unloading, holding, shackling, stunning and scalding) can be stressors on poultry if practiced improperly, resulting in degrading yield and meat quality, including tenderness and regardless of genotype (Debut *et al*., 2005; Sams, 1999a). Heat exposure is one of the primary stressors during pre-slaughter handling that reduces yield and accelerates rigor mortis development and rapid pH decline (Bianchi *et al*., 2006; Lee *et al*., 1976; Petracci *et al*., 2001). Because most pre-slaughter handling of poultry is practiced in non-controlled temperature conditions and often outdoors, heat stress during summer can be a factor which increases the incidence of meat quality deterioration (Bianchi *et al*., 2007; McCurdy *et al*., 1996). However, the effect of heat stress before slaughter on meat tenderness has shown itself to be inconsistent in many studies (Lee *et al*., 1976; Petracci *et al*., 2001). Transportation induces significant stress responses from birds as measured by plasma corticosterone levels (Kannan *et al*., 1997). Zang *et al*. (2009) suggested that transportation increases glycolysis and the level of plasma corticosterone and glycopenia that affect the contractive status of muscle fibers by changing their area and density. Kannan *et al*. (1997) found that a 4-h rest after transportation lowered plasma corticosterone levels. Ehinger (1977) reported that a 2-h transportation reduced tenderness, but Kannan *et al*. (1997) showed transportation for up to 4 h had an overall effect on meat quality characteristics and tenderness. Inconsistent effects of ante mortem stressors on meat quality could be due to different experimental conditions such as use of different genotypes of birds, weather and bird number per crates during transportation,

holding time, etc. For instance, Debut *et al*. (2005) showed that fast-growing chicken strains are less sensitive to shackling stress than slow-growing birds.

Stunning is the process that immobilizes birds without stopping heart action prior to slaughter and bleeding (Aberle *et al*., 2001). Electrical stunning is currently the most common stunning system used in the USA and Europe, because it is inexpensive, convenient and safe (Bilgili, 1999; Göksoy *et al*., 1999). The effectiveness of electrical stunning depends on the electrical variables, such as current, voltage, waveform, frequency, duration and by the electrical impedance presented by the birds (Bilgili, 1999). Electrical stunning is another ante mortem stressor that significantly affects carcass and meat quality. The primary defects of electrical stunning with high voltage or current are bruising, hemorrhage, discoloration, poor plucking and broken or dislocated bones, resulting in carcass downgrading (Bilgili, 1999). However, many studies (Alvarado and Sams, 2000; Lee *et al*., 1979; Papinaho *et al*., 1995) have found that breast muscles from electrically stunned birds show higher pH, adenosine triphosphate (ATP) and creatine phosphate and lower lactate level at the early stage of the postmortem process. In addition, Alvarado and Sams (2000) reported that electrical stunning delayed rigor mortis development up to 2 h, compared to the control. Ma and Addis (1973) also showed that the time for rigor mortis completion in breasts from electrically stunned turkey was significantly longer (314 min) than that from unstunned birds (143 min). These authors showed that electrical stunning significantly delayed rigor mortis development in poultry muscle, probably due to a reduction in the degree of struggling during slaughter (Papinaho *et al*., 1995; Sams, 1999a).

Despite the positive effect of electrical stunning on chicken breast meat tenderness after 24-h postmortem (Lee *et al*., 1979), several other studies (Alvarado and Sams, 2000; McNeal and Fletcher, 2003) have suggested that electrical stunning does not improve final meat tenderness and other quality traits compared to unstunned birds. Because of the carcass damage induced by electrical stunning, gas is used as an alternative stunning method. Gas stunning can be achieved by the exposure of birds to the gas atmosphere such as: (1) increased level of carbon dioxide to induce hypercapnic hypoxia, (2) depletion of oxygen by substituting with another inert gas such as argon to induce anoxia or (3) a combination of the two to induce hypercapnic anoxia (Hoen and Lankhaar, 1999). Gas stunning reduces carcass damage compared to electrical stunning (Kang and Sams, 1999a, 1999b; Raj *et al*., 1997). Raj *et al*. (1997) reported that gas stunning of chicken and turkey with 30% CO_2 in argon accelerated rigor mortis development and improved meat tenderness compared to electrical stunning, suggesting that it reduced aging time prior to deboning without any adverse quality effects. However, Poole and Fletcher (1998) found that its effect was only superior to high-current electrical stunning, but not to low-current (voltage) electrical stunning, suggesting that gas stunning cannot provide the opportunity for early deboning of chicken breast meat compared to low-voltage electrical stunning. High-current electrical stunning is required in Europe, whereas low-voltage electrical stunning is conventional in the USA (Sams, 1999a). Other studies (Kang and Sams, 1999a, 1999b) showed that CO_2 gas and air mixture did not affect, or even slow rigor mortis

development, and did not improve meat quality attributes, including tenderness, when compared to electrical stunning or where no stunning method was used at all, possibly due to the anesthetic effect of CO_2 (Bilgili, 1999; Sams, 1999a). Battula *et al*. (2008) compared the effects of vacuum stunning and electrical stunning on chicken breast meat quality, but they did not observe any differences in meat quality between the two.

According to the US regulations (the Code of Federal Regulations, 2009), poultry carcasses should be chilled to 4.4°C or to a lower internal temperature within 4–8 h after slaughter, depending on carcass weight. Rapid chilling of poultry carcasses primarily aims to minimize microbial growth for food safety, but also to reduce the potential for the incidence of PSE in poultry meat (Alvarado and Sams, 2004; Feng *et al*., 2008; Molette *et al*., 2003). Ice-water immersion (IC) and air chilling (AC) methods are the two most commonly used methods in the USA and Europe, respectively (Huezo *et al*., 2007a). Both methods have similar effects on final tenderness and other sensory characteristics of chicken breast meat (Huezo *et al*., 2007a, 2007b; Zhuang *et al*., 2009). IC is economical and requires less time to reach the recommended temperature compared to AC (Huezo *et al*., 2007b), but causes moisture uptake in the muscle, (around 10%), excessive drip loss, higher thaw loss, has a higher transportation cost and cooking loss compared to AC (Huezo *et al*., 2007a, 2007b). Microbial quality of air-chilled products is better than that of immersion-chilled products, probably due to cross-contamination by bird-to-bird contact in IC (Carroll and Alvarado, 2008). In addition, wastewater discharge restriction and changes in the US federal regulations on carcass moisture retention in 2001 are unfavorable for the continued use of IC (Huezo *et al*., 2007a).

The market share of processed poultry products including cut-up/parts and further-processed products has been increased dramatically, and now only around 10% of broilers produced in the USA are retailed as whole-carcass (National Chicken Council, 2010). Boneless, skinless broiler breast meat products are the most favored poultry products to consumers, probably due to inherent nutritional values and easy preparation time associated with such products over whole-carcass products (Seabra *et al*., 2001). Although many ante mortem stressors affect meat tenderness, sufficient postmortem aging time between slaughter and deboning allows for rigor mortis completion and tenderization processes to take place and is the most critical factor pertaining to meat tenderness (Sams, 1999a). Insufficient aging (i.e., early deboning before rigor mortis development) results in objectionable toughness in breast meat (Dawson *et al*., 1987; Stewart *et al*., 1984). Four to six hours of aging time are typically required to ensure that the tenderness of broiler breast meat is acceptable for consumption (Huezo *et al*., 2007a; Lee *et al*., 2008b; Zhuang *et al*., 2009). Aging of intact carcasses or breast halves is an expensive process that requires additional storage space, equipment, labor, logistics and energy costs for refrigeration to store carcasses for an additional 2.5–4.5 h after chilling and reduces meat yield (Huezo *et al*., 2007a; Sams, 1999b). Therefore, numerous technologies to minimize postmortem aging time by accelerating rigor mortis development and tenderization processes have been investigated: (1) portmortem electrical stimulation (Sams, 1999a, 1999b), (2)

wing restraints or muscle tensioning (Lyon *et al*., 1992a; Seabra *et al*., 2001), (3) post-chilling flattening or extended holding time of pre-rigor deboned breast (Lyon *et al*., 1992b; McKee *et al*., 1997), (4) clamping of pre-rigor deboned breast during chilling (Cason *et al*., 2002), (5) breast muscle opposition during rigor mortis development (Cason *et al*., 1997), (6) application of hydrodynamic shockwave to early deboned breast (Meek *et al*., 2000), (7) calcium chloride and/or sodium chloride marination to accelerate the tenderization process by activating proteolytic enzymes such as the calpain enzyme system (Seabra *et al*., 2001; Young and Lyon, 1997a) and (8) their combinations, such as electrical stimulation with electrical stunning (Craig *et al*., 1999), wing restraints (Birkhold *et al*., 1992), or marination (Young and Lyon, 1997b).

Among these employed technologies, postmortem electrical stimulation has been extensively evaluated for improved tenderness of early deboned poultry breast meat (Li *et al*., 1993; Sams, 1999a, 1999b). Electrical stimulation facilitates rigor mortis development by accelerating pH decline and energy depletion, as well as inducing physical disintegration of muscle fibers, resulting in the improvement of tenderness in early deboned breast meat (Sams, 1999a, 1999b). Although electrical stimulation has been well established and widely used for red meats, it has not been broadly applied to the poultry breast production because the effect by electrical stimulation is not sufficient to consistently achieve the tenderness levels that consumers and processors desire (Craig *et al*., 1999; Li *et al*., 1993; Meek *et al*., 2000; Sams, 1999b). In addition, most of the other technologies have not been fully assessed or verified for commercial application. Therefore, conventional aging using refrigerated temperatures is still the primary method employed by commercial poultry plants to improve the tenderness of breast muscle.

In addition, oxidation of proteins such as proteolytic enzymes and myofibrillar proteins can result in the loss of enzymatic activity, formation of hydroperoxides and carbonyls, inter- and intramolecular cross-linking through the formation of disulfide bonds and dityrosine, and decreased protein solubility, leading to an adverse effect on meat tenderness (Decker *et al*., 1993; Mercier *et al*., 2004; Rowe *et al*., 2004; Xiong, 2000) although little information is available for the direct relationship between protein oxidation and meat tenderness. Studies (Lund *et al*., 2007; Zakrys *et al*., 2008) showed that protein oxidation in beef packed under high O_2 atmosphere was significantly higher than that under low O_2 or 100% N_2 atmosphere. In addition, Zakrys *et al*. (2008) reported that Warner-Bratzler shear force values of beef loin steak packed in modified atmosphere packaging with a series of O_2 concentrations (0–80%) were positively correlated to the O_2 concentration after 15-day storage, suggesting that the samples seems to become less tender as O_2 concentration increases. Therefore, packaging technologies to reduce O_2 availability such as vacuum, modified atmosphere and active packaging with O_2 absorber, as well as incorporation antioxidants, can preserve meat tenderness during storage as they can prevent lipid oxidation.

Cooking strongly influences appearance (color, visible texture properties and dimension), juiciness, cooking loss and product texture (Aberle *et al*., 2001). Poultry products should be cooked to an internal temperature of > 73.9°C (FSIS

USDA, 2007) in order to ensure food safety. Cooking causes changes in physicochemical properties of intramuscular connective tissues and myofibrillar proteins, resulting in changes in meat tenderness. Heat induces protein coagulation and structural changes within muscle by thermal denaturation of myofibrillar proteins, resulting in an increase in toughness and a decrease in WHC. On the other hand, as meat temperature increases, collagen shrinkage takes place (until the temperature reaches to 61–62°C) and becomes further hydrated on extended heating (Aberle *et al*., 2001). Thus, cooking to the recommended internal temperature of 73.9°C increases the solubility of collagen and the hydration of collagen, leading to the softening of connective tissue, thus increasing tenderness. In general, cooked meat tenderness is closely related to raw meat tenderness (Bouton *et al*., 1981). Although meat toughness increases with temperature during cooking, two distinctive increases in two phases have been observed: around 40–55°C and 60–80°C as shown in Fig. 4.3 (Bouton *et al*., 1981; Christensen *et al*., 2000; Purslow, 2005). Several studies (Bouton *et al*., 1981; Christensen *et al*., 2000; Lewis and Purslow, 1989) using beef muscles suggested that the intramuscular connective tissues primarily contribute to the increase in toughness at the lower temperature phase due to collagen shrinkage, while the myofibrillar component mainly contributes to the toughness at the higher temperature phase due to denaturation of actin and other sarcoplasmic proteins. Wattanachant *et al*. (2005) also showed increases in the toughness of intact chicken breast muscle in the two phases (40–60°C and 70–80°C), but they were reported as lacking distinction. Murphy and Marks (2000) observed that the hardness of ground chicken breast patties dramatically increased (150%) from 40°C to 60°C meat temperature, but decreased (14%) from 60°C to 80°C. This study indicated that the tenderness of cooked ground chicken breast with a relatively low amount of collagen (Ding *et al*., 1999; Wattanachant

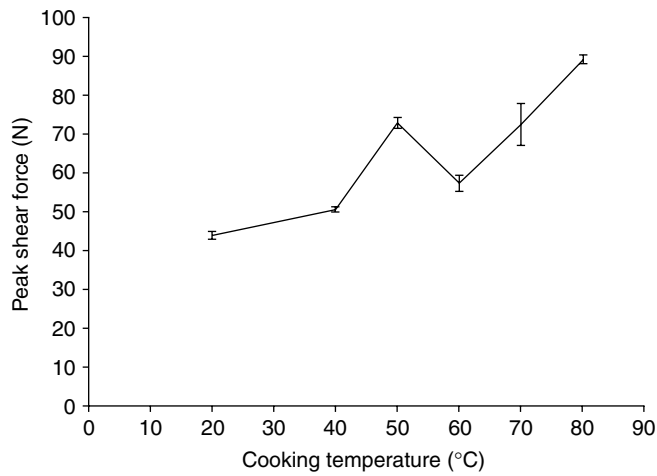

Fig. 4.3 Changes in whole meat toughness from beef semi-tendinosus with cooking temperature, measured using Warner-Bratzler peak shear force. (Source: Adapted from Christensen *et al*. (2000).)

et al., 2004) compared to beef (Bertola *et al*., 1994) is still significantly affected by changes in structural and chemical properties of collagen types present in intramuscular connective tissues by heating. The effect of meat temperature on meat tenderness may be different, depending on animal species, muscle type, structure (e.g., intact or ground) and composition (amount and nature of collagen types) in intramuscular tissues. In addition, Bouton *et al*. (1981) and Wattanachant *et al*. (2005) found that meat toughness is not likely to increase above 80°C.

4.5 Other sensory and quality issues associated with packaged fresh and processed poultry meat

In addition to the appearance/color and tenderness, flavor (taste) is another important sensory attribute which determines the eating satisfaction experienced by consumers for poultry products. During cooking, a broad range of flavor and aroma compounds are generated from many muscle constituents, including connective and adipose tissues by various reactions, such as Maillard reactions between sugar and amino acids, thermal oxidation, and degradation of proteins, lipids, and nucleotides: free amino acids, peptides, nucleic acids, inosine monophosphate (IMP), hypoxanthine, fatty acids, sulfur- and nitrogen-containing compounds, hydrocarbons, aldehydes, ketones, alcohols, furans, acids and many others (Aberle *et al*., 2001; Calkins and Hodgen, 2007; Northcutt, 2009; Sasaki *et al*., 2007). In general, the most flavor-active compounds responsible for 'meaty' flavor are water-soluble (Aberle *et al*., 2001). Among these, umami-related compounds such as free amino acids, peptides and IMP significantly contribute to 'meaty' flavors in beef, chicken and pork (Fuke and Konosu, 1991). Fujimura *et al*. (1996) identified that glutamate and IMP are major contributors to the chicken meat flavor. Nishimura *et al*. (1988) reported that an increase in free amino acids during postmortem aging was closely associated with the development of umami and 'meaty' tastes in beef, chicken and pork muscles. IMP is the intermediate product of ATP and other nucleotide metabolism, and thus its concentration in muscle depends on the activities of ATP hydrolase and IMP degrading enzymes during postmortem aging. Shu *et al*. (2008) suggested that purH gene, producing 5-amino-4-imidazolecarboxamide ribonucleotide transformylase/IMP cyclohydrolase for the last two steps of the *de novo* purine biosynthesis pathway, is a candidate locus or linked to a major gene affecting IMP concentration in muscle. The concentration of IMP in chicken depends on genotype, age, sex and muscle location within the carcass (Shu *et al*., 2008; Song *et al*., 2002).

However, specific flavor and aroma associated with chicken is mainly attributed to differences in fatty acid composition compared to other species (Aberle *et al*., 2001; Northcutt, 2009). Factors affecting the flavor and aroma of poultry products include age, genotype, diet, growing conditions, scalding temperature, chilling, cooking, product packaging and storage (Aberle *et al*., 2001; Calkins and Hodgen, 2007; Northcutt, 2009). Recently, polyunsaturated fatty acids, especially n-3 fatty acids, have been intensively incorporated into poultry diets to enhance the nutritional composition of poultry meat, but can cause off-flavor developments

in the final poultry products, as well as producing rapid lipid oxidation development (Sárraga *et al*., 2008). Zhang *et al*. (2008) reported that dietary inosinic acid supplementation induced the deposition of IMP in broiler breast and thigh, and improved growth and product tenderness.

Packaging materials can interact with flavor constituents in poultry products, and thus can cause loss of desirable flavor and/or migration of undesirable substances from the packaging materials (so-called 'flavor scalping') to meat, resulting in detrimental quality effects and raising concerns as to the safety of the products as shown in Fig. 4.4 (Sajilata *et al*., 2007). Polyethylenes (PE) and polypropylenes (PP) are commonly used as contact components with poultry products because of their chemical resistance and inertness, good humidity barrier property and thermal sealability (Min and Ahn, 2007). They can absorb non-polar flavor compounds due to their lipophilic characteristics and their chemical constituents such as their monomers and other starting substances, residual solvents, plasticizers, inhibitors and mold-release agents can migrate to the products, thereby causing safety problems (Sajilata *et al*., 2007). Factors affecting the absorption of flavor compounds by packaging materials, include the nature of flavor compounds (concentration, molecular weight and polarity), the properties of plastic polymers (surface area, polarity and glass transition temperature) and environmental conditions (pH, food composition, relative humidity and storage conditions such as temperature and duration) (Sajilata *et al*., 2007). Because important flavor constituents for poultry-specific flavors are lipophilic, the flavor absorption into the packaging material may cause significant flavor loss during storage. The migration of packaging materials into the products is influenced by the volatility,

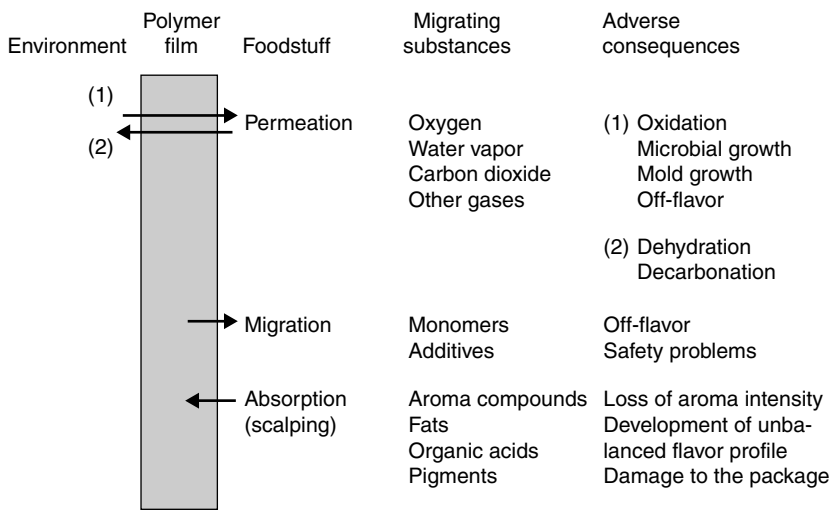

Fig. 4.4 Possible interactions between foodstuff, polymer film and the environment, and their adverse consequences. (Source: Sajilata *et al*. (2007).)

polarity and aroma strength of the migrants (Sajilata *et al*., 2007). Silva *et al*. (2007) indicated that the migration of a model migrant from low-density PE packaging film to the mixture of chicken breast with different levels of pork fat during storage at different temperatures increased as fat content and storage temperature increased. Therefore, careful selection of packaging materials should be made based on the properties possessed by individual poultry products. Approaches to reduce the 'flavor scalping' problem such as the application of scavengers for possible migrants into packaging materials (Del Nobile *et al*., 2002) and the use of bio-based packaging materials (Ikada and Tsuji, 2000) have been proposed.

Microbial quality significantly affects the quality and safety of meat products; it is one of the most important determinants for the shelf life of poultry products (Aberle *et al*., 2001). The nutrient-rich environment of meat is suitable for the growth of spoilage microorganisms and common food-borne pathogens, which can cause quality deterioration (off-flavor, off-odor, discoloration and slime production) and meat safety problems. In particular, chicken and other poultry are more perishable than red meat (Thomas *et al*., 1984), probably due to poultry having a relatively higher pH. The lag phase time of microorganisms is reduced at the higher pH, resulting in faster growth rate (Allen *et al*., 1997; Newton and Gill, 1981). Dark poultry meats such as dark, firm, dry (DFD) breast meat, as well as leg meat, show relatively higher pH compared to lighter-colored breast meat, leading to higher susceptibility to microbial spoilage (Allen *et al*., 1997; Newton and Gill, 1981). Synder (1998) proposed that poultry contains higher counts of spoilage microorganisms and food-borne pathogens than almost any other food. The normal shelf life under aerobic, chilled or refrigerated conditions after slaughter ranges from 4 to 10 days for fresh chicken carcass and breast meat (Chouliara *et al*., 2007; Economou *et al*., 2009; Jiménez *et al*., 1997; Mielnik *et al*., 1999). Predominant bacteria on the surface of poultry meat under cold conditions are psychrophilies such as *Pseudomonas*, *Psychrobactor*, *Moraxella* and *Acinetobacter*, which are spoilage microorganisms (Aberle *et al*., 2001). These psychrophilies are responsible for the production of chicken spoilage odors (putrid and ammonia-like odors) due to degradation of amino acids in muscle when microbial counts reach 10^6–10^8 cells per cm^2 of sample (Mielnik *et al*., 1999; Pooni and Mead, 1984). In addition, their growth probably results in increased meat value due to the accumulation of amines and ammonia produced by organisms (Quio *et al*., 2002). Among these, *Pseudomonas* is a predominant psychrophilie; producing sulfurous off-odors and slime (Mielnik *et al*., 1999; Russell *et al*., 1995).

Modified atmosphere packaging and vacuum packaging have long been used to preserve product quality and extend product shelf life. Vacuum packaging achieves its inhibitory effects on the growth of spoilage microorganisms by creating oxygen-deficient atmospheres, whereas, modified atmosphere packaging employs the antimicrobial effects of a CO_2-enriched microenvironment within the pack and in addition to the removal of oxygen removal. CO_2 has a bacteriostatic effect on Gram-negative bacteria, such as *Pseudomonas*, by increasing the lag phase and generation time (Church and Parsons, 1995). A 20–60% CO_2 concentration within the pack is required for the effective inhibition of spoilage microorganisms through

the alteration of certain enzyme systems and modification of metabolic activities (McMillin, 2008; Rao and Sachindra, 2002). CO_2 is highly soluble in muscle and fat tissues and dissolved CO_2 appears to inhibit microbial growth more than CO_2 levels present in the packaging headspace (Jakobsen and Bertelsen, 2004). The shelf life of poultry products can be increased by 50–400% depending on type of meat, initial microbial load at the time of packaging, temperature and the permeability of packaging films (Rao and Sachindra, 2002). While both forms of CO_2 are effective in inhibiting aerobic spoilage microorganisms, they allow the survival and growth of facultative anaerobic or microaerophilic spoilage microorganisms such as lactic acid bacteria, *Brochothrix thermosphacta* and *Campylobacter jejuni* which are predominant microorganisms in poultry meat products stored in modified atmosphere and vacuum packaging (Kakouri and Nychas, 1994; Rao and Sachindra, 2002). Lactic acid bacteria produce lactic acid and acetic acid, causing so-called 'souring' and slime formation (Rodríguez-Pérez *et al*., 2002). The extended shelf life of poultry meat products in modified atmosphere and vacuum packaging is probably due to very slow growth rate of these microorganisms in these packaging systems and the less offensive spoilage effects produced when compared to *Pseudomonas spp*. (Rao and Sachindra, 2002).

Furthermore, facultatively anaerobic, psychrophilic pathogens such as *Listeria monocytogenes*, *Aeromonas hydrophila* and *Yersinia enterolitica* can grow in poultry products held in both systems during refrigerated storage (Coma, 2008). Therefore, extended storage of these products has a potential for the incidence of food safety problems. Food safety is globally a top priority issue for regulatory agencies, processors and consumers. Although the hazard analysis and critical control point (HACCP) program has been widely established in slaughtering and processing facilities, as well as in further processing plants, to ensure the safety of poultry products, numerous outbreaks of food-borne illnesses associated with consumption of poultry meat still take place, and this negatively impacts on consumer confidence in such meat products, as well as resulting in huge financial losses for the processors. In addition, consumer demand for high quality, natural, minimally processed, convenient, safe poultry products is greater than ever. In order to meet these expectations, many innovative decontamination technologies have been introduced to ensure post-packaging quality and the safety of poultry products: biopreservatives and natural antimicrobial agents, irradiation, high-pressure processing, active packaging, high-frequency heating, ohmic heating, steam pasteurization, etc. (Aymerich *et al*., 2008). Unfortunately, every technology has its limitations and the technology required to ensure food safety generally can affect other quality attributes of the products: for example, irradiation effectively destroys microorganisms in poultry products, but causes quality problems such as pink discoloration, off-odor and increased lipid oxidation. In addition, high variation in demands on the quality of different types of products – for example, fresh versus further-processed products – may limit their application. Therefore, combinations of various antimicrobial technologies may reduce the intensity of each technology required to achieve food safety and produce synergistic effects, and preserve other product quality attributes as suggested in the concept and application of hurdle technology (Leistner, 2000).

4.6 Future trends

The consumption of poultry products will grow continuously because of their perceived high nutritional status and affordability compared to red meat. There are several significant quality issues that should be addressed by the poultry industry: PSE conditions, pink discoloration, tenderness of early deboned breast meat, extension of product shelf life and food safety. These issues have caused significant economic losses in the past and the significance of these issues will increase as the market grows. Genetic markers for PSE-prone birds and other quality traits such as tenderness have not been identified yet. Environmental factors such as diet and ante and postmortem processes also greatly affect sensory and functional qualities of poultry products and should be controlled. In addition, the terms 'natural', 'organic' and 'environment-friendly' typify current consumer food demands. Therefore, research on practical approaches to improve the quality of individual products affected by these issues should be continued, especially with respect to the modification and utilization of poultry diets on fresh meat composition and quality, and additive or ingredient usage in further-processed products. A number of natural bioactive components with multi-functional properties such as antioxidant, antimicrobial and nutraceutical potentials are available as potential natural quality enhancers to improve sensory and nutritional qualities and safety issues in the poultry products, as well as satisfying consumers' demands.

In addition, active packaging has brought much attention as a novel preservation technology. Active packaging is an innovative packaging system where specific substances such as antioxidants and antimicrobial agents are incorporated into packaging systems and slowly migrate from packaging materials to the products during storage and distribution, resulting in improved post-packaging microbial and sensory qualities of poultry products (Aymerich *et al*., 2008). Furthermore, the incorporation of natural preservatives into conventional packaging systems and utilization of eco-friendly biodegradable packaging materials such as biopolymers and edible films (and combinations of these) are promising areas which meet the all demands for high-quality, natural, safe, eco-friendly poultry products. In addition, their combination with other innovative microbial intervention technologies using hurdle concepts (Leistner, 2000) is likely to be the path chosen to further ensure the ultimate quality of packaged poultry products.

4.7 References

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5

Sensory and quality properties of packaged seafood

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Abstract: This chapter will outline the most important deterioration processes affecting fish sensory quality, with special focus on lipid oxidation. Varying types of fish, with muscle tissue bearing differing qualities, are considered. Factors affecting various forms of degradation in the fish are also studied. The relationship between packaging and storage methods and the sensory quality of fish will be discussed.

Key words: sensory quality, storage condition, lipid oxidation, pelagic fish, demersal fish, autolytic process, freezer burn.

5.1 Introduction

The total world production of seafood, including shellfish (which constitutes approximately 25% of total production), has increased since 1950 and is about 144 million tonnes at time of writing, with 110 million tonnes used for human consumption and the rest used for the production of non-food materials such as fish meal and oil (Fig. 5.1). The production of aquacultured fish has grown extensively, whereas the activities of the wild fisheries sector has levelled off, as indicated by the decreased consumption of wild fish and the increased consumption of farmed fish (FAO, 2010; Anon, 2010).

The fishing industry is very diverse, dealing with many species and producing a wide variety of products including fish meal and oil, raw fish products such as sushi and highly processed combined products. Because of the great variety in raw material and the short shelf life associated with fish flesh, it is a huge challenge for the fish industry to develop uniform, high-quality and stable fish and fish-based products. Fish freshness is rapidly lost at a temperature above 2°C for

chilled products (Huss, 1995). Consequently, fish products require accurate time/temperature control and excellent chilling and freezing facilities in the production and distribution chain from catch to consumer. This chain is illustrated in Fig. 5.1.

Quality is a concept that must be carefully defined before it can be measured (Bremner, 2000). Definitions pertaining to fish quality have been proposed and debate around appropriate terminology is ongoing. Many definitions address the idea of fitness for use, which implies that it is the user who evaluates the necessary quality. One firm standpoint is that the quality must always be correlated to the sensory (appearance, flavour and texture) properties associated with fish products and their acceptability. For as long as fish has been traded there have been special rules guaranteeing that only fish of a high sensory quality are sold to the consumer. Almost 100 years ago protection against the supply of poor-quality fish became part of general food legislation. Laws proposed and directed were initially based on sensory assessment of the fish. In Europe today, the most com-

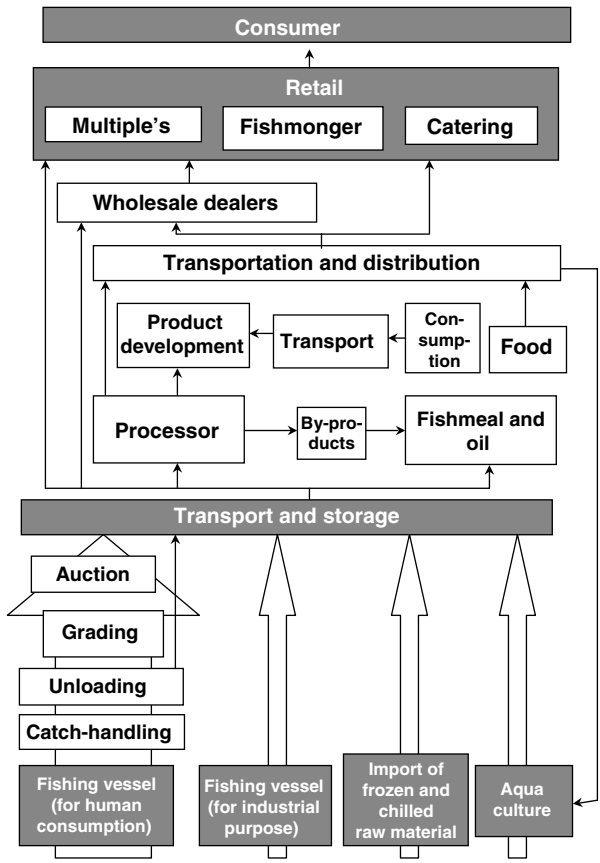

Fig. 5.1 Flow diagram of the fish chain.

monly used method for quality assessment by inspection services and the fishing industry is sensory analysis (Anon., 1996).

This chapter will outline the most important deterioration processes affecting fish sensory quality, with special focus on lipid oxidation. The relationship between the packaging and storage methods and the sensory quality of fish will be discussed. The microbiological aspects will be discussed elsewhere (see Chapters 2 and 9).

5.1.1 Fish handling

The fish distribution chain (Fig. 5.1) is diverse and challenging due to the high perishability of fish but also because the supply of raw material is dependent on weather, time of the year and the capability of the fishermen. The supply of raw material can come from small day boats fishing a short distance from the shore to big ocean trawlers landing fresh fish after 14 days at sea or frozen fish after 1–2 months at sea. Aquacultured fish come from either land-based or sea-based farms. Fish is often sold via auctions but can also be landed directly to the industry for production of food and non-food (fish meal and oil) products.

The handling procedures of fish from capture to processing can have a large effect on the quality of the final packaged fish and fish-based products (Olsen, 1991). The on-board handling operation often involves the use of mechanical devices such as fish pumps, large nets, hooks or lifts of up to 100 tons of fish, depending on the fishing method utilized (Huss, 1995). When the catch is landed on deck, the handling time is, of course, dependent of the size of the catch. A holding time of 2 h is not unusual before bleeding and gutting of the larger white fish, for example cod, and then the fish have to be sorted and iced in returnable tough deep freeze-grade HDPE plastic boxes holding between 20 and 50 kg of fish. For 100% traceability, the boxes can be fitted with radio-frequency identification and tracking system.

Small pelagic fish species are normally stored in the fish hold, ungutted and mixed with ice or held in tanks of cooled seawater. The reason for this is partly that a large number of small fatty fish are caught at the same time and partly because of the potential to accelerate product deterioration through processing, primarily due to problems associated with discolouration and oxidation of exposed muscle surfaces. Three methods are commonly used to store chilled fresh fish on board:

- ice mixed with fish in boxes
- chilling seawater (CSW) with ice in a tank/container on board the vessel
- refrigerated seawater (RSW) using mechanical cooling normally installed as an independent motor unit.

When the fishing vessel comes to harbour the portion of the catch intended for consumption is unloaded and distributed either via a fish auction or directly to a processor. If the catch is stored in tanks – for example, in the case of small fatty fish such as herring and mackerel – the next step can be icing in boxes or transport

directly to the processing factory in containers. Size sorting of fish normally takes place at the quayside or in the factory. This sorting process is often automatic and based on the thickness of the fish. After unloading, box-iced fish such as cod and similar species are normally de-iced and graded according to species, size and quality class. If this sorting has taken place on board the fishing vessel, the boxes are transported to an auction or directly to the processing factory.

Transportation of fresh fish has always been a challenge. If no cooling agent is available during transportation and, consequently, haulage temperature is high, the deterioration of the fish is fast – the shelf life is reduced by 50% every time the temperature increases by 5°C. Fish is traded globally and modern transportation uses all means of transport from lorries to air cargo This kind of transportation requires great flexibility and can only exist with the help of a sophisticated logistic systems, standardization of boxes, pallets, lorries, lifting gear and reliable transport companies.

The last link in the fresh fish chain, before the consumer buys the product, is the fish retailer. There are great differences in approach to the global retailing of fresh fish and, consequently, it is difficult to define an industry standard. However, an interesting trend is that sale of fresh fish at supermarket level is growing. The trend to retail fresh fish from chill cabinets in locations such as supermarkets demands the development of a wide variety of packaged fish and fish products, semi-prepared products and ready-to-cook meals; there is increasing demand for these types of fish and seafood. Time and temperature control is mandatory and the consumer will, in the future, require that the display of information about catch and handling in the chain is mandatory.

5.2 Fish composition

Fish are the largest group of vertebrates, with over 30 000 different species (Thurman and Webber, 1984). They are characterized by absorbing oxygen from the water through gills, and by being equipped with fins for locomotion. The cartilage fish (e.g., sharks, skates, etc.) and bony or teleost fish (e.g., cod, herring, plaice, etc.) are commercially important. Bony fish can again be divided into pelagic fish (those dwelling in the upper free water column – e.g., herring, mackerel, etc.) and demersal fish (mid-water to bottom dwellers – e.g., cod, haddock, turbot, halibut, etc.). In fish, the flesh is constructed of adjacent muscle blocks and the muscle mass on each side of the fish constitutes the fillet, of which the upper part is termed the ‘dorsal muscle’ and the lower part termed the ‘ventral muscle’. This anatomy and the content of the muscle will have an important influence on the quality changes that may occur during storage. Fish muscle differs from muscle in mammals and birds as follows (Love 1970, 1988):

- it is not attached to the skeleton with tendons
- it is divided into muscle blocks (myomers) separated by connective tissue (myocommata)

- red muscle (type I fibers) and white muscle (type II fibers) are distinctly divided
- it has a very low content of connective tissue
- it contains less glycogen, which means that *postmortem* pH drops to only 6.0–6.7.

Most of the muscle contained in fish is white, but, depending on the species, many fish will have some dark tissue of a brown or reddish colour. The dark muscle is located along the side of the body close to the skin and fins. The proportion of dark muscle varies with the activity of the fish species. Pelagic fish swimming more or less continuously may consist of up to nearly 50% of dark muscle as it is required for prolonged aerobic muscle activity, whereas demersal fish tend to have a small amounts of dark muscle as they drift in the water using the muscles only for short bursts of low-activity swimming. There are profound differences in the chemical composition of the two muscle types; higher levels of lipids and myoglobin are present in the dark muscle.

The chemical composition of fish is different from that of mammals and birds (Huss, 1995) as evidenced by the following:

- fish contain a higher percentage of monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA), including n-3 fatty acids, therefore they are more prone to oxidative rancidity
- fish contain specific N-containing non-protein low molecular weight substances that can be extracted in water and, therefore, fish have a high content of ammonia, free amino acids such as taurine and in the case of the cartilage-containing fish, a high content of urea
- marine fish and some freshwater species contain trimethylamine-N-oxide (TMAO).

These gross compositional differences have a major impact on the chemical degradation reactions that fish undergo during storage and processing.

The composition of each fish species can vary tremendously with the seasons (Connell, 1990). The variation in fish composition with respect to protein and fat are dependent on food uptake, migration and spawning season. Fasting periods for the fish may be natural (e.g., spawning period or migrations), or it may be due to external circumstances such as lack of food (hunger). The energy depots for the fasting periods consist of fat. Species that migrate long distances before they reach spawning grounds may also use protein as an energy source.

5.3 Initial biochemical and microbiological deterioration of fish

After the fish is caught and slaughtered a chain of important biochemical reactions commence during phase one and two shown in Fig. 5.2. The speed and extent of these reactions depend on the fish species in question, its condition and temperature, but also on fish handling before and after death. In the first phase

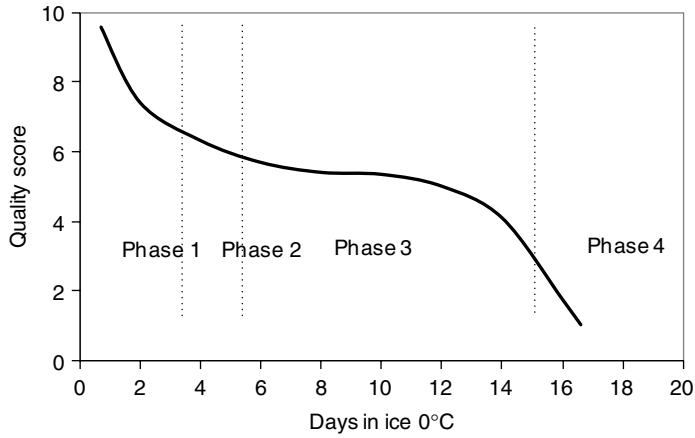

Fig. 5.2 Change in the sensory quality of iced fish. The deterioration is divided into four phases.

(Fig. 5.2) muscle glycogen is degraded, followed by an increase in lactate causing a fall in pH. Glycogen concentration in fish muscle is low and pH only falls to 6.2–6.5 (Nielsen and Nielsen, 2006). The adenosine triphosphate (ATP) level in muscle decreases, *rigor mortis* sets in and the muscle becomes stiff. After a couple of days in ice, the fish will again soften as *rigor* resolves. However, at this stage, the texture is also affected by the autolytic (degradation of fish by endogenous fish enzymes) processes that start immediately after the fish is dead and continuous through phases one and two (Fig. 5.2). A major part of these processes is the action of the proteolytic enzymes in the fish muscle cleaving certain proteins in the muscle structure (Nielsen and Nielsen, 2006) including proteins constituting the attachment between muscle fibres (Taylor *et al*., 2002), proteins that bind the myofibrils to the connective tissue (Bremner, 1999; Ofstad *et al*., 2006; Taylor *et al*., 2002) and collagen fibres surrounding the muscle fibres (Sato *et al*., 2002). Another important biochemical reaction is the degradation of nucleotides ATP, adenosine diphosphate (ADP), adenosine monophosphate (AMP) and integral membrane protein (IMP) which leads to formation of hypoxanthine, ionosine and xanthine, all of which contribute to unpleasant taste and indicating spoilage (Nielsen and Nielsen, 2006). In live fish, there is a balance between fish lipids and pro- and antioxidants. This stability is changed when the fish dies and lipid hydrolysis and oxidation are initiated, causing rancidity as cell membranes degrade autolytically or are physically harmed by slaughter and processing.

Bacterial deterioration of chilled fish also starts slowly just after death, but the effect on spoilage through the biochemical changes induced by the bacterial growth is not pronounced until the specific spoilage organisms have increased to a certain level, which does not take place before phases three and four (Fig. 5.2). In addition, as microbial counts increase, enzymes secreted by microorganisms may also cause additional softening of the fish. For more details see Chapter 2 concerning microbiology.

5.4 Lipid oxidation

One of the major deteriorative processes that occurs during the storage of fish and which has a major influence on quality is lipid oxidation. The lipolysis process leads to formation of free fatty acids, diacylglycerides and monoacylglycerides, glycerol and nitrogen bases (Belitz and Grosch, 1987). The sensory properties of fish are particularly affected by the level of free fatty acids (Refsgaard *et al*., 2000). The rapid development of rancidity in stored muscle from fish such as mackerel, herring, capelin and bluefish is often attributed to the fact that these species contain high levels of lipid. However, recent research suggests that the lipid content is not the only factor determining the susceptibility of fish muscle to lipid oxidation. Studies by Richards and Hultin (2001) indicate that blood-mediated oxidation of washed cod (*Gadus morhua*) lipids required ≤ 0.1% phospholipid to cause rancidity. This finding has been supported by other studies and it can be concluded that the type and level of pro-oxidants in fish appear to be of greater importance than the lipid content (Jacobsen *et al*., 2008).

The highly unsaturated nature of the lipids present in fish makes them very susceptible to lipid oxidation. Thus, the two important n-3 PUFA, eicosapentaenoic acid (EPA; C20:5, *n*-3) and docosahexaenoic acid (DHA; C22:6, *n*-3) contain numerous 1,4-cis-pentadiene systems that are easily attacked by radicals/initiators. Lipid oxidation is one of the most important quality deterioration processes in fish as it may affect both the odour and flavour of fish and, in severe cases, its nutritional value. Lipid oxidation in fish muscle can be caused by non-enzymatic processes such as autooxidation and photosensitized oxidation, as well as catalysed by enzymes such as lipoxygenase. The autoxidation and photosensitized oxidation reactions are the same as for other lipids. Briefly, lipid radicals are formed in the presence of initiators such as heat, light, trace metals or heme-bound iron. The lipid radicals quickly react with oxygen, whereby peroxyl radicals are formed. During the propagation phase the peroxyl radicals attack intact fatty acids forming odourless and tasteless primary oxidation products, lipid hydroperoxides (LOOH). Low molecular weight (LMW) and heme-bound transition metals quickly break down LOOH to an array of new radicals (hydroxyl radicals, peroxyl radicals and alkoxyl radicals), which can reinitiate oxidation reactions. Alkoxyl radicals can also be cleaved in a β-scission reaction, whereby various volatile oxidation products like aldehydes, ketones, acids and alcohols are formed.

Volatile oxidation products from n-3 long chain PUFA have extremely low odour thresholds. This makes oxidation a more severe sensory problem in seafood than in more saturated systems such as meat. Some secondary oxidation products such as aldehydes are very reactive and can, for example, react with free amino groups of proteins whereby tertiary products such as Schiff's bases are formed. These products can polymerize into yellow-brownish pigments (Pokorny *et al*., 1974).

As for the enzymatic lipid oxidation reaction, lipoxygenase activity has been detected in various tissues of fishes and shrimp in blood plasma, gill, skin, fish eggs, brain, muscle, erythrocytes and platelets (Pan and Kuo, 2000). Lipoxygenases are iron-containing enzymes, which are situated in the cell cytosol or microsomal

fraction (Harris and Tall, 1989). The enzyme catalyses the insertion of one molecule of $^3O_2$ into an unsaturated fatty acid containing a 1,4-cis-pentadiene group (Belitz and Grosch, 1987).

5.4.1 Sensory impact of lipid oxidation in seafood products

The most important flavour change during frozen storage of fatty fish such as salmon is the formation of train oil, bitterness and metallic tastes (Refsgaard *et al*., 1998). Likewise, Milo and Grosch (1995) observed the development of a train oil flavour during prolonged frozen storage in a lean fish species such as cod.

Milo and Grosch (1995) suggested that the increased concentration of 1-octen-3-one, (Z)-1,5-octadien-3-one, hexanal, (Z)-3-hexenal, (Z)-4-heptenal, (Z,Z)-2,6-nonadienal and (E,Z)-2,6-nonadienal observed during storage most likely were important contributors to the train oil odour in the stored cod sample. In contrast, Refsgaard *et al*. (1998) suggested that the formation of volatile oxidation products may not be the most important factor responsible for the pronounced sensory changes found during frozen storage of salmon. Rather, they proposed that compounds of low volatility contributed to the increased intensity of train oil taste, bitterness and metallic taste. Subsequently they hypothesized that the lipid hydrolysis, and thereby the release of free fatty acids that occurred parallel to lipid oxidation, contributed significantly to the sensory deterioration of salmon during frozen storage (Refsgaard *et al*., 2000). This hypothesis was corroborated by data showing that addition of each of the unsaturated fatty acids – palmitoleic acid (16:1, *n*-7), linoleic acid (C18:2, *n*-6), eicosapentaenoic acid (EPA; C20:5, *n*-3) and docosahexaenoic acid (DHA; C22:6, *n*-3) – to fresh minced salmon increased the intensity of train oil taste, bitterness and metal taste. The added level of each fatty acid (1 mg/g salmon meat) was equivalent to the concentration of the fatty acids determined in salmon stored as fillet at −10°C for 6 months. The effect of addition of the fatty acids on the intensity of train oil taste, bitterness and metallic taste was in the order: DHA > palmitoleic acid > linoleic acid > EPA. Taken together these findings suggest that the formation of unpleasant train oil, bitter and metallic off-flavours may not be solely ascribed to lipid oxidation, but may be a due to combination of lipid oxidation and lipid hydrolysis.

In a recent study on frozen stored rainbow trout, formation of volatile oxidation products and free fatty acids increased in parallel to the formation of rancid off-flavours (Baron *et al*., 2009). Moreover, an increased grainy, firm and fibrous texture was observed in the rainbow trout, which were most rancid. These changes in texture were most likely due to protein oxidation. The impact of protein oxidation on myofibrillar protein functionality and on muscle food quality has recently received more attention (Martinaud *et al*., 1997; Saeed *et al*., 1999), and protein oxidation has been shown to affect protein solubility, decrease gel elasticity and affect water distribution in muscle foods (Bertram *et al*., 2007; Ooizumi and Xiong, 2004; Rowe *et al*., 2004) and this may have severe impact on fish texture.

Apart from affecting odour and flavour, lipid oxidation may also affect colour. Thus, autoxidation of heme iron may decrease the red colour of fish muscle

(Sannaveerappa *et al*., 2007), whereas lipid oxidation of the lipids will lead to an increase in a more yellow hue.

5.5 Sensory quality changes in stored and packaged fish products

The following sections look at the ways by which various preservation methods affect the fish tissues, with consequent implications for flavour, texture, and other factors of interest to the retailer and consumer, among others.

5.5.1 Ice storage

In general fresh fish can be described by the following sensory attributes:

- for odour/flavour: sea/seaweed, sweet, cooked potato, warm milk, cucumber, fresh fish oil, mushroom
- for texture: juicy, oily and firm (Green-Petersen *et al*., 2006; Hyldig, 2009, 2010; Sveinsdóttir *et al*., 2010).

When fresh fish is stored in ice the flavour and odour compounds that characterize newly caught fish decrease and disappear over the first few days of storage, and the fish flesh becomes almost flavourless and odourless for a while. However, after this time period, an increase in foul-smelling sulphur and nitrogenous volatiles will result in rejection of the fish for human consumption.

5.5.2 Modified atmosphere packaging

In fish storage studies involving modified atmosphere packaging (MAP), the development of various sensory attributes over time are quite different from those that develop for the same fish species when stored in ice. Hong *et al*. (1996) found that the odour associated with Atlantic mackerel (*Scomber scombrus* L.) held under MAP conditions changed dramatically over storage time: day 0 (seaweed, fishy and rancid), day 7 (seaweed, cucumber-like, sour, fishy, painty and rancid), day 14 (seaweed, sour, fishy, rancid) and day 21 (seaweed, sour, fishy, metallic and rancid).

5.5.3 Freezing

Freezing is an effective means of preserving fish over long storage periods. Frozen fish stored for up to three months under ideal conditions (low non-fluctuating temperature) cannot be distinguished from fresh fish with regard to colour, taste and texture (Cappeln *et al*., 1999; Nielsen and Jessen, 2007). Packaging for frozen fish must have a number of specific properties, first, because frozen fish is exposed to large temperature variations: there is both cooling during freezing and

heating during thawing and during frozen storage and transport. Water barrier properties are also an important parameter of any packaging material used for the freezing of fish to prevent dehydration, which causes freezer burn. Freezer burn manifests as whitish or yellow brown, dry, tree-like areas on the fish flesh, and has thus a major impact on the appearance and the sensory quality of the product (Pham and Mason, 1997). High levels of water evaporation may also accelerate protein denaturation giving a tough texture and lipid oxidation causing off-flavour production (George, 1996; Sikorsky and Kolokowska, 1994). Glazing is often used to protect the surface of both lean and fatty fish from oxidation and dehydration. The frozen product is either sprayed with or dipped in water, thereby, forming an 'ice cap' around the product. When cold storage is prolonged, it might be necessary to renew or reapply the glazing layer. Trials have shown that drip loss can be reduced by dipping the fish in a salt solution before freezing, but this treatment has also been shown to accelerate the development of an rancid freeze house taste (Paine and Paine, 1992) due to the presence of cis-4-Heptenal formed by oxidation of n-3 fatty acids.

Whole fish and fish fillets are frozen in either vertical or horizontal plate freezers. The vertical plate freezer design is often used on board ship. The frozen blocks of fish are often glazed and afterwards lined with a robust plastic paper-lined bag packed before transportation and storage. Fish fillets are packed in a folded carton block liner inside a freezing frame. The block liner is designed to absorb a certain amount of water giving an oxygen barrier when the water freezes. The blocks can be shaped in various forms for further processing as fish fingers etc. The blocks are normally packed in master cartons before palleting. The range of packaging material for the final product is very wide and is dependent on the product type. A processed fish product, such as fish sticks or single frozen fillets, may be wrapped in a primary package of laminated plastic material, which is in direct contact with the frozen food, and then stored in an outer carton. There are no differences between the packaging materials used for frozen fish and meat products with the exception of molluscs or crustaceans, where plastic-based packaging and laminates are of thicker gauges to prevent cutting/tearing.

Other than packaging, frozen fish quality is affected by factors such as fish species, stress levels, handling before slaughter and *rigor* status. The most important factors determining the quality of frozen fish are, however, temperature management during freezing, storage, transportation and thawing. Freezing must be fast and the temperature must be low and constant throughout the process and fluctuations must be avoided during transport and storage (Kristoffersen *et al*., 2006; Sørensen *et al*., 1995).

5.6 Case studies of sensory quality changes in stored and packaged fish products

In the following, we look at the varying effects of differing approaches to different types of fish and the implications for the quality of such fish after storage.

5.6.1 Case study: ice storage of farmed salmon

Sensory profiling of ice-stored farmed salmon showed that sensory attributes characterizing the salmon on day 1 of storage were seaweed, cucumber, sourish odour, sweetish, sourish, fish oil and mushroom flavour, while after 22–24 days of storage the salmon were characterized by being described as rancid, sour, amine odour and rancid flavour (Sveinsdóttir *et al*., 2003). The authors grouped the sensory attributes as 'positive sensory parameters' or 'negative sensory parameters' in their storage experiment. Samples from every second storage day were analysed. The changes in the sensory attributes indicate that the salmon was approaching the end of acceptable flavour after 20–21 days, when the salmon was characterized by increasing intensity of sour, amine and rancid odour and flavour. All positive attributes had a high intensity and were very characteristic for the salmon at the beginning of the storage time, but after 21–22 days of storage they were hardly detectable. It was concluded from the result of the sensory profiling that shelf life, where the fish is no longer fit for human consumption, was 20 days in ice (Sveinsdóttir *et al*., 2003).

5.6.2 Case study: influence of temperature on frozen lean fish

Many studies have explored the relationship between quality changes in fish and frozen storage temperature, but most of these studies focus on the temperature range between −20°C and –30°C; all agree that –30°C is a more effective storage temperature than –20°C (Bøknæs *et al*., 2000, 2001; Sørensen *et al*., 1995). A limited number of studies have focused on temperatures below –30°C. These studies have primarily focused on the effects of very low freezing temperatures on tuna colour (Watabe and Hashimoto, 1986) as they are used worldwide for the transportation of tuna for sushi. Recently a study has investigated the relationship between quality-related changes in North Sea cod and storage temperatures in the temperature range from −10°C to −80°C (Burgaard and Jørgensen, 2011). The cod was vacuum packed and frozen in a blast freezer. The primary finding from these storage experiments showed that a storage temperature of −30°C was sufficiently low to maintain a high quality of post-*rigor* cod up to 12 months of frozen storage, while a storage temperature of −40°C was considered optimal when storage of cod was required to be longer than 12 months.

5.6.3 Case study: influence of temperature on frozen fatty fish

In a recent study in our laboratory, the effect of storage temperature on lipid and protein oxidation, as well as sensory changes in rainbow trout, was evaluated (Baron *et al*., 2007). Rainbow trout fillets were non-bled but slaughtered by electrocution, filleted as butterfly fillets using an industrial filleting machine, frozen at −30°C on an industrial steel belt freezer with a freezing time of 20 min and stored at −30°C for one week ($t = 0$) before they were transferred to storage temperatures of: −20°C, −30°C or −80°C. Since this study aimed at establishing links between protein and lipid oxidation and the consequences for both on sensory properties,

the fish samples were stored under conditions that would not protect the samples optimally against oxidation. Therefore, the fish were vacuum-packed individually in standard PA/PE bags and stored frozen for 13 months. Lipid oxidation was followed by measuring lipid hydroperoxides (PV), free fatty acid (FFA) as well as secondary oxidation products (volatiles) using dynamic headspace GC-MS. Protein oxidation was followed using the spectrophotometric determination of protein carbonyls and immuno-blotting. Significant oxidation was observed in samples stored at −20°C and, at this temperature, lipid and protein oxidation seemed to develop simultaneously. Thus, there was a significant increase in the level of lipid hydroperoxides and protein carbonyls after eight months of frozen storage for fish stored at −20°C, and the increase in PV and protein carbonyls was even more pronounced after 13 months. Likewise, the concentration of some of the volatile oxidation products also increased even after four to eight months in this sample. In contrast, fish stored at −30°C and −80°C did not show any significant increase in PV and protein carbonyls during the entire storage period but the more sensitive GC-MS method used for measurement of the volatiles showed that fish stored at −30°C oxidized faster than those stored at −80°C. Using this method, the ranking was found to be −20°C > −30°C > −80°C. Sensory analysis showed that the fish stored at −20°C developed more rancid off-flavours and a grainier, more fibrous structure than the other fish samples. Taken together, these data show –20°C is not a suitable storage temperature for rainbow trout if oxidative flavour and texture deterioration is to be avoided. Even at –30°C, lipid oxidation could be detected in fish samples, indicating that as low a temperature as –80°C might be necessary to completely avoid oxidative flavour deterioration.

5.6.4 Case study: sensory evaluation of flavour changes in frozen fish

In sniff tests it has also been shown that it is not always the sample that presents the highest odour intensity that determines what the final flavour of fish will be; sometimes the odour/flavour is first recognized when the muscle structure is broken down during product mastication. The most pronounced sensory changes determined for salmon (*Salmo salar*) held during frozen storage, for example, were first recognized by assessors when salmon samples were evaluated in the oral cavity. Significant time-temperature effects were determined and the intensities of train oil, metal and bitter taste increased during storage at −10°C and −20°C. The intensity of earthy and fish oil flavour decreased in salmon stored at the higher temperatures (Refsgaard *et al*., 1998). They also found that both cooked and raw samples showed significant colour changes during storage. The salmon colour intensity decreased during frozen storage and this change was not dependent on storage temperature.

5.6.5 Case study: Salmon and salmonids products

Green-Petersen *et al*. (2006) have provided an overview of both the sensory properties and differences between the most common salmonids products available

on the Danish market. Twelve salmon samples which differed in storage condition, packaging and species were used in the experiment. All the samples were obtained from local shops or companies and bought as consumer products. Of the samples, there were nine samples of *Salmo salar*, two were packed in MAP, four were stored in ice and three were frozen. The four samples of *Salmo salar* stored in ice had very similar sensory profile described by the following attributes: sea/seaweed odour, fresh fish oil flavour, sweet and mushroom and the texture juicy and oily (Hyldig 2009, 2010). Even though the ice-stored samples represented different fish farms, different batches and different storage times in ice (7 or 16 days), no clear sensory differences were observed between samples by these authors and this is consistent with studies carried out by Sveinsdóttir *et al*. (2003). After five days of chilled storage, MAP samples and ice-stored samples had relatively similar sensory profiles. However, following seven days of storage, MAP samples were determined to be rancid and sour. For the frozen samples, results showed that a longer freezing time produced a softer texture, increased discolouration and reduced desirable sea/seaweed odour. However, it was found that freezing generally had limited influence on flavour.

5.7 Shrimps

Bak *et al*. (1999) investigated the effect of packaging atmosphere, temperature fluctuation and light exposure on frost formation, lipid oxidation, discolouration and meat toughness of shell-on, cold-water shrimps (*Pandalus borealis*) during 12 months of frozen storage. They found that the single most important handling factor affecting shrimp quality was the exposure to oxygen during storage. The sensory score for rancid flavour was significantly higher for samples packed in atmospheric air compared to samples packed in modified air. The effect of light on the rancid flavour of atmospheric air-packed shrimps was significant. The sensory score for rancid flavour for modified air-packed samples did not increase significantly between nine and 12 months of frozen storage.

The sensory evaluation of shrimp meat toughness revealed a clear effect of the packaging method and storage conditions used. They found a significantly higher score for toughness for samples packed in atmospheric air compared to samples held in MAP. In addition, storage in light resulted in significantly tougher shrimp meat. The sensory score for toughness was almost constant for samples packed in MAP for the first nine months of storage, whereas a significant increase was observed between nine and 12 months of storage.

5.8 Future trends

It is a great challenge for the fish industry to develop uniform and high-quality fish and fishery products in order to improve the competitiveness of products towards other commodities like chicken, beef and pork. In many places quality

management systems have been introduced, but there is still a great need for good-quality monitoring methods in order to achieve the goal. It is expected that consumer demands for convenience seafood of high quality will continue to grow. Consumers also show an increasing interest in complete meals consisting of seafood together with other ingredients such as vegetables, rice and sauces of different types. Likewise, there will be an increased focus on using biodegradable packaging materials and active packaging. Together these trends will lead to number of challenges for fish producers with respect to development of new product, processing and packaging technologies that ensure the product has maintained its nutritional and sensory quality by the time it reaches the consumer. In order to develop such technologies, not only knowledge about interactions between the different ingredients and the effect of such interactions on (1) changes in lipids and proteins due to, for example, oxidation, (2) sensory properties due to, for example, masking effects, but also knowledge about the interactions between the ingredients and the packaging material will also be required. The generation of this knowledge and its successful implementation in the fish industry is dependent on collaboration between academia and industry.

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6

Advances in the packaging of fresh and processed meat products

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Abstract: Advances in traditional, vacuum (VP) and modified atmosphere (MAP) packaging of fresh and processed meat have been propelled by economic, social and technological forces. Overwrap packaging continues to be a dominant form of fresh meat retail packaging, whether accomplished in the store or as a master pack, while VP is common for processed meat unless specialized applications are better served with MAP. Consumer demands for quality, convenience and flexibility and processor needs for economy and processing efficiencies have spurred industrial and institutional research. Logistical and display requirements have increased presence of case-ready and integrated packaging systems. Active packaging systems that influence environment of packaged products, including oxygen scavengers, antimicrobial agents and bio-based materials, have more development and use than intelligent systems that utilize sensors to relay needed changes or communications. Cost and convenience considerations will continue to necessitate improvements in packaging materials, equipment, accessories and systems.

Key words: modified atmosphere packaging, vacuum packaging, packaging systems, polymer films, air permeable, moisture impermeable, antimicrobial agents, active packaging.

6.1 Introduction

Food packaging serves many purposes, including the containment of the product, protection of product against deteriorative effects, communication of information to consumers and providing ease of use and convenience (Yam *et al*., 2005). An overview of the development of meat packaging and current systems is provided and advances in packaging materials, equipment and systems are described. The effective applications of advanced packaging and packaging systems to improve

the quality of fresh and processed meat products are presented in a comparative format. The possible and likely short-term and long-range trends in packaging for fresh and processed meats are speculated upon in the last section.

Many advances have been made to provide foods in new packaging forms that are simultaneously protective and convenient (Brody *et al*., 2008). Packaging systems have been developed that integrate packaging materials having desired gas and water vapor barrier and mechanical properties to prevent product physicochemical or biological deterioration with logistical systems and retailing schemes to maintain overall product quality during storage and handling (Rhim and Ng, 2007). Safety, convenience, quality and shelf life demands have resulted in meat packaging improvements through technologies involving equipment, base materials and incorporation of active and intelligent approaches to packaging (Kerry *et al*., 2006).

6.2 Current technologies and use of packaging for fresh and processed meat

The following sections consider the developments that have led the meat packaging industry to the point at which we currently find it, looking at how technologies have progressed and how practice may evolve in the future.

6.2.1 Development of current systems of packaging for meat

The change from a butcher cutting and wrapping the meat in paper or waxed paper for direct sale to the purchaser to in-store cutting and packaging of meat for refrigerated self-service display cases required more advanced forms of meat packaging (Brody, 2002). The chemical industry provided plastics and other polymer forms of materials to satisfy the packaging requirements of shelf life, cost and attractiveness for air-permeable, moisture-impermeable polyvinyl chloride film for stretching around polystyrene trays as overwrap packaging of raw chilled meat packaging and barrier packaging for processed meats (Brody, 2002). Consumers began to associate the bright red bloomed oxymyoglobin color of overwrapped meat in air-permeable packaging with meat freshness because this was the meat color first seen on display in self-service meat cases (Jenkins and Harrington, 1991). Modified atmosphere packaging (MAP) can be in one of two forms: removal of air by evacuation before the package is sealed (vacuum packaging, VP) or with mixtures and combinations of gases other than atmospheric composition inserted before sealing. MAP usually refers to the latter unless otherwise specified. Combinations of overwrap and MAP may be used for fresh case-ready meat applications while processed meat products are generally in VP or MAP.

Fresh meat packaging is only minimally permeable to moisture, so surface desiccation is prevented (Faustman and Cassens, 1990), while gas permeability varies with the application. Meat color is used more than any other quality factor in making meat purchase decisions (Mancini and Hunt, 2005), with color preference

and consumer purchase intent highly related to discrimination against beef that is not red (Carpenter *et al*., 2001). The absence of O_2 binding to myoglobin to form red oxymyoglobin gives meat a purple color (deoxymyoglobin) while oxidation of the pigment heme group causes brown metmyoglobin color. Consumers discriminate against meat with 20% metmyoglobin (MacDougall, 1982) and metmyoglobin exceeding 40% causes downgrading or purchase rejection (Greene *et al*., 1971). Consumers rate appearance of beef steaks and patties with purple color below those with red color, but above those with brown color (Carpenter *et al*., 2001). A more detailed discussion of meat color changes and measurement is provided by Mancini and Hunt (2005).

VP began for primal cuts and cured meats due to advances in VP materials and equipment to meet the demands for economies of scale in large operations (Brody, 2002). Breaking of carcasses in processing plants and the shipping of primal cuts in VP in boxes to retail stores for fabrication rather than the cutting of carcasses, sides or quarters in retail stores created different demands on the meat industry. Similarly, VP became accepted, and possibly expected, by consumers for cured and/or cooked meat products because the color of these products was relatively fixed and consumers could easily view the contents. In many developed countries, case-ready or centralized packaging of meat has replaced the traditional system of packaging raw refrigerated meat in air-permeable overwrap packaging at individual stores. Case-ready, centralized, or retail-ready packaging (synonymous terms) is packaging of consumer-sized retail products in a centralized non-retail location for transport and subsequent display in retail stores with minimal or no package manipulation before display except for removal from the shipping carton (McMillin *et al.*, 1999). Retail-ready packaging allows shoppers to find specific products and place them into the grocery cart and decreases time for stores in reducing, rotating, stocking, opening, locating and identifying stock items (Reynolds, 2010). Case-ready packaging constitutes about 43% in European fresh markets (Belcher, 2006) and 66% of packages in US fresh meat self-service cases (Cryovac, 2010; Demetrakakes, 2010). Overviews compare the advantages and give descriptions of major current case-ready meat systems (Belcher, 2006; Brody, 2007). Growing numbers of retailers and consumers accept VP that results in purple meat color, but a majority of retailers still desire the bloomed red color of meat given by gas-flushed MAP or overwrap technologies (Dressler, 2010a). Economic considerations for packaging and packaging systems include costs of raw and finished materials and availability of trained labor to merchandise retail meat products.

MAP systems began with VP and expanded to include master pack concepts for placement of individual overwrapped packages into larger barrier bags or pouches, use of oxygen (O_2) and carbon dioxide (CO_2) higher than ambient levels for red meat color and inhibition of spoilage microorganism growth, and pillow pouches to allow separation of sliced or shingled meat products. The continued success of the many different retail MAP formats has been dependent upon product, package and system interactions; relationships of processors and retailers; and consumer acceptance of the merchandising format (Brody, 2002).

Plastic is highly suitable for food packaging by having desirable properties of machineability, functionality and cost (Jenkins and Harrington, 1991). Each type of packaging material has advantages, disadvantages, consumer and marketing issues, environmental considerations and cost factors (Marsh and Bugusu, 2007). Major properties of the different plastic resins used singly or in combinations in laminated or extruded films for meat packaging are summarized in Table 6.1 (additional details in McMillin, 2008) with polyolefins often used because of their desirable properties (Acosta *et al*., 2011). Manipulations of film barrier or diffusion properties, incorporation of active packaging technologies, nanotechnology use and coupling of packaging with other preservation methods have improved quality and shelf life of packaged meat products (Lee, 2010).

The primary packaging options for raw chilled meat are air-permeable overwrap, low O_2 VP, low O_2 MAP with anoxic gases, high O_2 MAP, overwrap packages in master bag and permutations of carbon monoxide (CO) incorporation (Belcher, 2006; Brody, 2007; Cole, 1986; Eilert, 2005; Gill and Gill, 2005; Jenkins and Harrington, 1991; McMillin *et al*., 1999; Renerre and Labadie, 1993). Anoxic MAP or VP result in long shelf life of meat with minimal oxidative deteriorative changes, but the meat has a purple deoxymyoglobin meat color. MAP with O_2 higher than ambient air results in red oxymyoglobin pigments, but deteriorative oxidative changes in heme pigments, myofibrillar proteins and lipids may occur before achieving the desired shelf life (Lund *et al*., 2007). CO_2 is often included in MAP for antimicrobial affects, but it is highly absorbed into meat at refrigerated temperatures (Jakobsen and Bertelsen, 2002). Nitrogen (N_2) is used as an inert gas. CO has received much attention because carboxymyoglobin pigments are red in color and have a high stability during extended storage. Low levels of CO (0.4%) are allowed to be used in the United States because although a red color of meat is produced and maintained throughout storage and display, spoilage due to offensive odors and flavors is not masked and growth of spoilage microorganisms is not inhibited. Hygiene and temperature control are essential regardless of packaging system to prevent spoilage (Eilert, 2005).

6.2.2 Technologies for packaging systems

The implementation of packaging systems to achieve the desired product characteristics for necessary distribution, retail storage, display and household use necessitated development of technologies to address increased consumer convenience and centralized packaging efficiencies. The demands for high-speed packaging of cuts with uniform size and shape resulted in development of automated overwrapping equipment that also weighs, prices and labels the finished package. MAP and VP can be accomplished on machines with single chambers or multiple chambers and in form-fill-seal or tray sealing configurations. Specific details of the different systems for air-permeable, VP and MAP systems are in review papers (Belcher, 2006; McMillin, 2008).

Consumers desire foods that will be cooked or reheated to be microwaveable with heating and serving in one package, attractive and informative graphics,

Table 6.1 Major resins for meat and poultry packaging[a]

Packaging resin	Abbreviation	Water vapor transmission rate, g/m²/24 h	O_2 transmission rate, cc/m²/24 h	Tensile strength, MPa	Tear strength, g/mL	Impact strength, J/m	Heat seal temperature range, °C	Notes	Common packaging system use, singly or in combination with other resins
Polyinyl chloride	PVC	1.5–5	8—25	9–45	400–700	180–290	135–170	Moisture impermeable; resistant to chemicals	Overwrap, master pack
Polyvinylidene chloride	PVdC	0.5–1	2–4	55–110	10–19	—	120–150	Vapor barrier; high hardness; abrasion resistant	Overwrap, VP
Polypropylene	PP	5–12	2000–4500	35.8	340	43	93–150	Clear, readily processed	Master pack, tray
High-density polyethylene	HDPE	7–10	1600–2000	38.2	200–350	373	135–155	Used for structure	VP, MAP, master pack, tray
Low-density polyethylene	LDPE	10–20	6500–8500	11.6	100–200	375	120–177	Lidding film use; high strength, low-cost sealant	VP, MAP, master pack
Linear low-density polyethylene	LLDPE	15.5–18.5	200	7–135	150–900	200	104–170	Superior hot tack; poor sealing through grease	VP, MAP, master pack

(*Continued*)

Table 6.1 Continued

Packaging resin	Abbreviation	Water vapor transmission rate, g/m^2/24 h	O_2 transmission rate, cc/m^2/24 h	Tensile strength, MPa	Tear strength, g/mL	Impact strength, J/m	Heat seal temperature range, °C	Notes	Common packaging system use, singly or in combination with other resins
Ionomer	—	25–35	6 000	24–35	20–40	150	107–150	Metallic salts of acid copolymers of PE; broad heat sealant range	VP, MAP, master pack
Ethylene vinyl acetate	EVA	40–60	12 500	14–21	40–200	45	66–177	4% improves heat sealability; 8% increases toughness and elasticity	VP, MAP, master pack
Ethylene vinyl alcohol	EVOH	1000	0.5	8–12	400–600	—	177–205	Vapor barrier	VP, MAP, master pack
Polyamide (nylon)	PA	300–400	50–75	81	15–30	50–60	120–177	High heat and abrasion resistance, clear, easily thermoformed; printable	VP, MAP, master pack

Polyethylene terephthalate	PET	15–20	100–150	159	20–100	100	135–177	Polyester from terephthalic acid reaction with ethylene glycol; abrasion and chemical resistant; structure use	MAP, master pack
Polystyrene	PS	70–150	4500–6000	45.1	39 493	59	121–177	High-impact PS (HIPS) for multilayer sheet extrusion; strong; structure use	Tray

Source: Adapted from McMillin (2008).
Note: [a]Based upon 1 mm film.

easy-open and self-venting packaging, chilled rather than frozen display and storage, and economical costs (Belcher, 2006). There are continuing improvements in case-ready packaging, value-added features in packaging and flexible packaging (Harrington, 2011b). A flexible microwaveable packaging with a form-fill-seal pouch with a patterned microwave susceptor between layers of polyethylene terephthalate (PET) and kraft paper provides for heating of protein foods such as meat requiring higher temperatures for texture development. Susceptors are desirable in microwaveable packaging to cause browning and crisping of products similar to those achieved in conventional oven heating (Zweep, 2010). Susceptors must be designed for the specific product. Fully metallized film produces one heat output and can cause overheating of food edges and undercooking of the center, while demetallized film reduces overcooking, but gives less browning. Printed susceptors may have a metallization layer that cracks at high temperatures to assist in heat modulation (Higgins, 2011b). Heating generates steam that enables temperatures inside packages to be higher than 100°C and assures heating of proteins to at least 74°C while the moisture from the steam keeps the cooked meat from drying. Steam valves can be tailored to specific cook-in-package applications that allow measured escape of steam from packages. Polystyrene trays are not usually used for heat-and-serve foods because of the low melting temperature and fumes released upon heating. It is possible to form trays of suitable polymers on line with a skin film that develops into a steam chamber when microwaved. This package type also allows post-packaging high-pressure pasteurization (Hancek, 2009).

6.3 Advances in overwrap, vacuum packaging (VP) and modified atmosphere packaging (MAP) for fresh and processed meat

Numerous different technologies, systems and equipment are currently used for processing and packaging case-ready fresh red meat. Many of the newer developments have been through company studies rather than institutional or government agency investigations so detailed information is not always readily available. Important current fresh meat technologies used commercially are master or mother packs and MAP. For master or mother packs, retail meat cuts are air-packaged in individual overwrapped packages placed together in a large bag that is evacuated and back flushed with the desired gas (Brody, 2007). The number of packages in the master bag is determined by the size of the individual packages, overall master pack bag dimensions, and the amount and type of gas necessary to provide the desired shelf life. These mother bags or individual MAP red meat packages for fresh meat often use mixtures of O_2 and CO_2 or O_2, CO_2 and N_2 (Brody, 2007), but development of lipid and pigment oxidation during storage with high (greater than 50%) O_2 led to the use of 0.4% CO with CO_2 and/or N_2 rather than O_2. This extended the shelf life of most fresh meat from less than 16 days to more than 30 days (McMillin, 2008). Carbon monoxide provides desirable red color stability, flavor acceptability with no oxidation, no bone darkening, no premature browning

during cooking, decreased growth of spoilage and pathogenic microorganisms and increased tenderness compared with aerobic overwrap packaging or high O_2 MAP (Cornforth and Hunt, 2008).

6.3.1 Advances in active components

Successful packaging systems require more than packaging materials, so bio-based plastics and products like labels, absorbent pads, netting, tape, tags and others are among packaging advances (Harrington, 2011b). Active and intelligent packaging have been confounded in many reports. Active packaging responds by sensing and changing some functional aspect. Intelligent packaging responds by sensing and signaling. Intelligent packaging includes temperature indicators, time-temperature integrators and sensors for temperature, moisture and gases. Gas sensors respond to respiratory gases while biosensors measure biological changes such as ripeness or spoilage that cause gas evolution or generation of volatile compounds. Time-temperature indicators can operate due to mechanical changes, internal enzyme activities or biochemical activities. Enzymatic sensors may sense hydrolysis of a substrate that changes pH, which is indicated by a color change. Diffusion of polymeric materials into a porous substrate are the sensing components of other intelligent packaging. Photosensitive crystals or changes in concentrations of polar compounds form the basis of some newer time-temperature sensors. Microbiological growth may be sensed by antigen contact with growing bacteria; these can be tailored so the antibody-antigen reactions are specific to certain spoilage or pathogenic microorganisms (Brody, 2010b).

Time-temperature indicators are common for food applications when temperature abuse may be suspected, although even more reliable and sophisticated systems are being developed (Ozdemir and Floros, 2004) that are low cost, small, reliable and usable in food packaging. Most time-temperature indicator types commercially available rely on diffusion where the measureable response is indicated by distance or change in light transmission, color change due to enzymatic activity, or temperature-dependent polymerization changes (Kerry *et al*., 2006). Intelligent packaging may provide direct or indirect product quality changes due to microbial growth or chemical changes (Kerry *et al*., 2006). Much of the sensor technology is still in developmental stages and not yet commercially available.

Active packaging has been incorporated into many systems to emit or absorb O_2, CO_2, odors and aromas, and ethylene; delay oxidation; control respiration rate; inhibit microbial growth; and mitigate moisture migration (Ozdemir and Floros, 2004). Control of purge and moisture with absorbent or soaker pads keeps products fresh, protects packaging systems from unsanitary meat juices and creates an aesthetically attractive package (Fernández *et al*., 2010). The pads may be paper or fiber inside of plastic film for placement in the bottom of trays or webs before the meat is inserted. Some trays have absorbent materials imbedded in the tray bottom that has pores to allow purge migration into the absorber, which eliminates the need for a separate soaker pad (Pelligrini, 2011). Spoilage

microflora were reduced by silver-based antibacterial hybrid materials adsorbed onto cellulose fibers used as pads (Fernández *et al*., 2010).

Micro- or macroperforations in film can improve gas passage into or out of packages. When overwrap packages are inserted into a master pack and gas is enclosed in the master pack environment, microperforated film for overwrapping improves the gas passage into the overwapped package. The shape, size and obstructions of film microperforations indicate the consistency and reproducibility of the process that made the microperforations, which also appear differently on the upper and lower surfaces of the films. Microperforations larger than 55 μm can lose diffusion if convection is present, so holes less than 55 μm in diameter should be used to achieve the required O_2 transmission rates (Allan-Wojtas *et al*., 2008). The permeability ratio between CO_2 and O_2 was 0.83 and gas exchange coefficients changed with decreasing temperature and pore diameter (Montanez *et al*., 2010). It had been previously suggested that the volume of gas that would flow through a hole of a defined size per unit time would be a more effective indicator of gas diffusion than hole size alone (Bix *et al*., 2005).

Small amounts of O_2, when combined with exposure to light, cause significant oxidative deterioration of products (Jakobsen *et al*., 2005). Many vacuum packagers or tray sealers do not remove air from packages with sufficient vacuum levels in the preferred amount of time for efficient packaging line speeds, so O_2 scavengers are used to create acceptably low-oxygen atmospheres in packages. Meat discoloration can be prevented by O_2 scavengers only if the residual O_2 is reduced below 10 ppm within an hour or so (Gill, 1996). The O_2 scavengers may be in various forms of sachets placed in the package or may be incorporated into labels, package materials or closures. Materials that absorb O_2 include ferrous iron, ascorbic acid, sulfites, catechol, some nylons, unsaturated hydrocarbons, photosensitive dyes and enzymes (Brody *et al*., 2008). Care must be taken to match O_2 absorbing capacity with system requirements to remove O_2 and that the scavenging material is not activated before it is desired that O_2 absorption begin. The rate of O_2 removal may not be sufficiently fast (less than 24 h) to prevent anoxic conditions with low levels of O_2 that promote metmyoglobin formation (Brandon *et al*., 2009). The O_2 concentration was the primary limiting factor for O_2 absorption in atmospheres with 500 or higher ppm O_2 because of diffusion while higher temperatures improved absorption rate (Tewari *et al*., 2002a). Relative humidity, the O_2 concentration and the gas composition inside the package are major factors that influence O_2 absorption kinetics, with an increase in rate of O_2 absorption causing a decrease in porosity and increase in surface area of iron powder corrosive products. Heat from the exothermic reaction of O_2 absorption results in a decrease in the amount of water adsorbed on the corrosion products (Polyakov and Miltz, 2010). An experimental sachet using iron powder and synthetic zeolite in a microperforated film had improved O_2 absorption over commercial sachets at 23°C, but not at 37°C (Braga *et al*., 2010). Benzyl acrylate, ascorbates, sulfur dioxide and other O_2-binding compounds imbedded into packaging materials are being tested (Brody, 2010a).

Thermoforming, as is often done in vacuum packaging and MAP operations on form-fill-seal equipment and for forming trays used in tray sealing equipment, changes

the material properties of the initial film or sheet. Oxygen transmission rate increased with increased drawing depth, but not in a linear manner. There was no correlation between package O_2 transmission rate and wall thickness, but drawing depth and relative wall thickness were highly related (Pettersen *et al*., 2004a). When material was thinned due to thermoforming, O_2 transmission rate increased, but not proportionally.

Carbon dioxide above 20% inhibits microorganism growth (McMillin, 2008), so CO_2 absorbers are usually not desirable because meat in MAP with CO_2 normally absorbs CO_2. CO_2 emitters would compensate for this absorption by producing CO_2 after sealing of the package. A CO_2 emitter prepared by adding sodium bicarbonate and citric acid to a liquid absorber based on weight developed too much CO_2 during storage and adding the bicarbonate and citric acid based on headspace gave too little CO_2 (Hansen *et al*., 2009). Carbon dioxide can be generated though moisture activation of biocarbonates in sachets or absorbent pads (Brody *et al*., 2008), with CO_2-generating chemicals being incorporated into absorbent pads for use with poultry trays (Brody, 2010a).

MAP packaging with a headspace can have beading of moisture on the inside surface of the film if the film becomes colder than the gas or meat product inside the MAP due to temperature changes during transport, storage or display. This moisture on the inside surface of the film can be controlled with reduction in the surface tension of the water by surfactant antifog agents that are dipcoated, sprayed, or blended to the polymer. Glycerol esters, polyglycerol esters, sorbitan esters and their ethoxylates, alcohol ethoxylates and nonyl phenol ethoxylates are common agents to prevent film fogging or moisture droplet accumulation (Osswald *et al*., 2006). However, many of these agents may cause meat to become brown if there is meat contact with the agent.

6.3.2 Advances in film components

Food safety is of particular concern with meat due to the pathogenic microorganisms associated with meat animals that cause foodborne illnesses. Control of microorganisms by antimicrobial agents can reduce surface contamination, but should not substitute for sanitation and hygiene practices (Cooksey, 2005). Antimicrobial substances can be used with packaging through inclusion into bioactive edible coatings directly applied to food surfaces, forming of polymers with antimicrobial properties into functional films, chemical modification of polymers to have antimicrobial properties, coating of packaging with a matrix carrying the agent, having the agent released from a sachet or other carrier in the package released during storage, and direct incorporation of the antimicrobial into the packaging film (Coma, 2008). It is more common for antimicrobials to be imbedded into packaging materials for release to the food surface or included as vapors during packaging (Brody *et al*., 2008). Research has been reported on silver ions, ethyl alcohol, chlorine dioxide, nisin, organic acids, allyl isothiocyanate, essential spice oils and metal oxides (Wilson, 2007).

Traditional food preservatives, including sodium benzoate, sodium nitrite, potassium sorbate and sodium lactate, incorporated into low-density polyethylene,

poly(maleic acid-co-olefin), polystyrene and polyethylene terephthalate as plaques and films showed variability in antimicrobial activities. Packaging with sodium nitrite was effective against *Aspergillus niger* and *Bacillus subtilis* while sodium lactate did not have antimicrobial activity and no samples inhibited *E. coli*. Strongest activities were observed when antimicrobial agents were added to polystyrene and polyethylene terephthalate (Vartiainen *et al*., 2003), but these packaging materials are not used for meat because of the brittle structure. No antimicrobial activity of 2.5–15% sodium benzoate and sodium nitrite in low-density polyethylene was found (Vartiainen *et al*., 2003), even though this packaging material is often included in coextruded or laminated film. Antimicrobial films have limitations and should be used as part of an overall strategy to produce safe foods (Joerger, 2007).

Several herb and spice extractives exhibit antimicrobial activity. Low-density polyethylene films containing linalool or methylchavicol from basil had slightly decreased transparency, water vapor and O_2 transmission rates, but no difference in crystallinity or melting temperature of the films (Suppakul *et al*., 2006). Films and coatings containing oregano essential oil incorporated in a thin layer had antimicrobial efficacy against *Salmonella*, *Staphylococcus*, *Pseudomonas* and mixed microbial population during 13 days of refrigerated chicken storage. Other essential oil spices (clove, rosemary, white thyme, tea tree, coriander, sage and laurel) known to have antimicrobial properties did have antimicrobial activity on the surface of the meat (Harrington, 2011a). Polypropylene packaging with a layer of 1–2% oregano extract improved oxidative stability of beef steaks in high O_2 MAP more than spraying the meat surface with the oregano extract through 28 days of storage at 1°C (Camo *et al*., 2011).

A coating of a styrene-acrylate copolymer containing triclosan inhibited growth of *E. faecalis* (Chung *et al*., 2003). Triclosan at 2000 and 4000 mg kg^{-1} incorporated by extrusion into polyethylene did not affect the mechanical film properties and had antimicrobial effect against *E. coli* and *S. aureus*, but not against *L. innocua*, *S. choleraesuis* and *P. aeruginosa* on cured ham (Camilloto *et al*., 2009). Antimicrobial activity was also observed when triclosan was incorporated into polyethylene- and cellulose-based films, but hydrophobicity of polyethylene films with triclosan was reduced and hydrophobicity of cellulose films with triclosan was increased (Camilloto *et al*., 2010). Controlled release of allyl isothiocyanate, an antimicrobial from the *Brassicaceae* plant family, in MAP (30% CO_2:70% N_2) reduced *Salmonella* and *Listeria* on chicken through 21 days at 4°C, but levels higher than 1.2 μg/h release affected the color of the chicken breasts (Shin *et al*., 2010).

Contamination of cooked products after heating, especially those that are ready-to-eat, must be stringently controlled to prevent the potential for foodborne illness. Several companies have proprietary films that are flexible enough to contain raw product and maintain integrity during thermal processing and chilling without need for repackaging the product post-processing. When packages must be opened by processors or consumers after heat treatment, bags and pouches that provide easy gripping and tearing to open the package and remove the contents

are available (Salvage, 2011). Post-pasteurization bags are available in side-seal, curved-seal and straight end-seal bag types for repackaging and high-speed sealing of heated and chilled products that can be exposed to temperatures up to 99°C for 10 min to give desired post-process lethality. Multilayer coextruded films with printing laminated between the layers allows information to be communicated without need for a label that might become contaminated or illegible and eliminates chemical contamination of products from printing inks.

Nanocomposites, where the filler has at least one dimension smaller than 100 nm, offer possibilities to improve the water vapor barrier properties of bio-based packaging materials. Nanocomposites are incorporated through solution, interlamellar polymerization, or melt processing with the layered clay materials interacting with polymers through nonintercalated, intercalated and exfoliated or delaminated arrangements. Starch, cellulose, polylactic acid and protein noncomposites are discussed in a review article (Arora and Padua, 2010). These materials allow improvements in strength, barrier properties, antimicrobial properties and stability. Montmorillonite clay at 1–5% has been used in many package polymers such as polyethylene, nylon, polyvinyl chloride and starch. Silver-montmorillonite nanoparticles imbedded into agar, zein and poly(ε-caprolactone) polymer matrices had antimicrobial properties when in agar, but not in zein and poly(ε-caprolactone). The antimicrobial effectiveness was attributed to the water content of the polymer matrices (Incoronato *et al*., 2010). Montmorillonite clay at 70% can be mixed with a variety of polymer materials to make a thin transparent film that could be layered through dipping or spraying onto existing films to provide strength and improved O_2 barrier properties (Anon., 2011). Silicate nanoparticles interspersed in polyamide films can block O_2, CO_2 and moisture from reaching fresh meats. Nanocrystals imbedded in packaging may minimize loss of CO_2 from packages and limit O_2 entry into packages (Brody *et al*., 2008). Biocomposites containing carrageenan with clay in the blend had less water permeability and uptake than those with zein prolamine, although the composites were transparent and exhibited the ability to block UV-visible radiation (Sanchez-Garcia *et al*., 2010). Corn-zein coatings on polypropylene improved water vapor and oxygen-barrier properties (Tihminlioglu *et al*., 2010). Response surface methodology showed that O_2 transmission rate, water vapor transmission rate, tensile strength and percentage elongation of polypropylene-based nanocomposite films could be optimized through the developed regression models to match desired food storage requirements (Manikantan and Varadharaju, 2011). Nanocomposites also allow creation of packages with printed electronic displays on a wide variety of packaging substrates at low cost and minimal power requirements to give dynamic and interactive displays rather than static images. The technology depends on electrochromism where materials change color when a charge is applied.

Deposition of gelatin-based bio-coating on oriented polypropylene, low-density polyethylene and polyethylene terephthalate decreased O_2 transmission rate by more than 40% and increased UV barrier characteristics by more than 12%, but resulted in lower transparency and haze optical properties. There were no differences in water vapor permeability, but the solubility of the coating in water

and other results suggested that the lipid protein coating gave negative aspects for use with plastic films. Seal strength of polypropylene films coated with a gelatin-based thin layer of monoglyceride acetic acid esters and glycerol was affected negatively by sealing bar pressure and positively by sealing temperature with no influence by dwell time (Farris *et al*., 2009).

Immobilization of antioxidant rosemary extracts in polypropylene or polyethylene lowered specific and overall migration rates by 20 times lower than established limits (Tovar *et al*., 2005). Polypropylene film containing a natural antioxidant improved the lipid and pigment stability of beef (Nerín *et al*., 2006) and polypropylene film with immobilized rosemary antioxidant agents reduced lipid oxidation without direct contact with the food (Nerín *et al*., 2008). Catechin or quercetin (natural flavonoids) extruded into ethylene vinyl alcohol did not modify water and O_2 permeabilities of the film and improved thermal resistance and were released into food, although the release extent and kinetics depended upon the food type (López-de-Dicastillo *et al*., 2010). L-ascorbic acid and L-tyrosine cast into cellulose acetate films decreased pore size and porosity of the films, indicating that antioxidant release can be controlled by varying the structural features of films with preparation conditions or different surfaces (Gemili *et al*., 2010).

Oxygen indicators that would visually indicate the integrity of low O_2 MAP systems would be useful. A simple O_2 indicator system causes a colorless reduced species of methylene blue to revert to the original blue color with O_2 contact. This system had slow reduction and fast oxidation by O_2 compared with a polyviologen (electrochrome, titanium dioxide and EDTA) indicator with more anodic reduction potential to detect O_2 up to 4% (Roberts *et al*., 2011).

Biodegradable, bio-based and degradable packaging reduce packaging waste, which has remained constant at about 31% of residential waste in the United States over the past 20 years (Marsh and Bugusu, 2007). Bio-based packaging sources include proteins, polysaccharides and lipids for films, plasticizers, functional additives and emulsifiers (Han and Gennadios, 2005) with many bio-based materials having positive influences on moisture loss, lipid oxidation, flavor, color and microbiological properties (Cutter, 2006). The renewable resource polylactic acid can be run on conventional packagers at lower temperatures than polyethylene materials (Eilert, 2005).

Soy protein isolate, glycerol and gellan gum or carrageenan were suitable for manufacture of soy-based biodegradable or edible trays to replace polystyrene trays (Mohareb and Mittal, 2007). A review of natural biopolymer-based films for foods included descriptions and properties of the various packaging materials (Rhim and Ng, 2007). There are many technical and physical parameters to be optimized for bio-based coatings or films in individual or composite configurations before commercialization for meat.

6.3.3 Advances in systems, packaging, processes and equipment

Traceability of products from source to use is an important concern that raises packaging considerations. Radio frequency identification (RFID) is a noncontact

identification technology that works well with plastic containers (Singh *et al.*, 2011). RFID devices are now applied to packages as tags and labels, but RFID antennas can be printed directly on packaging using conductive inks. Advances in plastic and metallic ink technologies promise to give ability of presses to actually print the circuitry for RFID tags directly on the package and these will compete with silicon-based RFID and EAS tags (Moore, 2009). Development, miniaturization and decreased costs of data collection technologies like RFID, machine vision and laser marking have improved traceability potentials, but no one technology will be useful by itself in the global food trade (Welt and McEntire, 2011). Additional discussion on traceability and RFID information can be found in Chapter 21 on traceability in the meat, poultry and seafood industries.

Consumers do not like the headspace in conventional MAP sealed trays, but meat contact with lidding film, with or without antifog agents, often causes it to become brown. Several package system developments are designed to minimize these difficulties. The Sealed Air Corporation's Darfresh Bloom™ package combines two case-ready technologies. The meat product is vacuum skin packed in permeable film and this is enclosed within a formed barrier tray and lidding film package containing the desired gas (Fig. 6.1). A smaller headspace is required and the permeable film prevents contact of the meat with the outer barrier film during transport and sales handling (Fig. 6.2).

The Cryovac Mirabella® package system consists of two coextruded shrink films, an inner permeable sealant film and an outer high-barrier film, that are used as lidding films for sealing rigid polypropylene or barrier foam trays on MAP tray sealers (Fig. 6.3). Prior to sealing on a modified tray and lid packager, the double film is separated on the roll to allow for the modified atmosphere gas mix

Fig. 6.1 Picture of meat in the Darfresh MAP system.

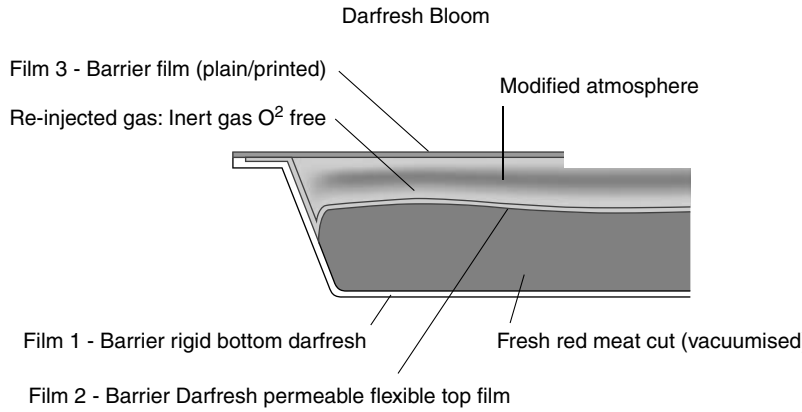

Fig. 6.2 Schematic diagram of the Darfresh MAP system.

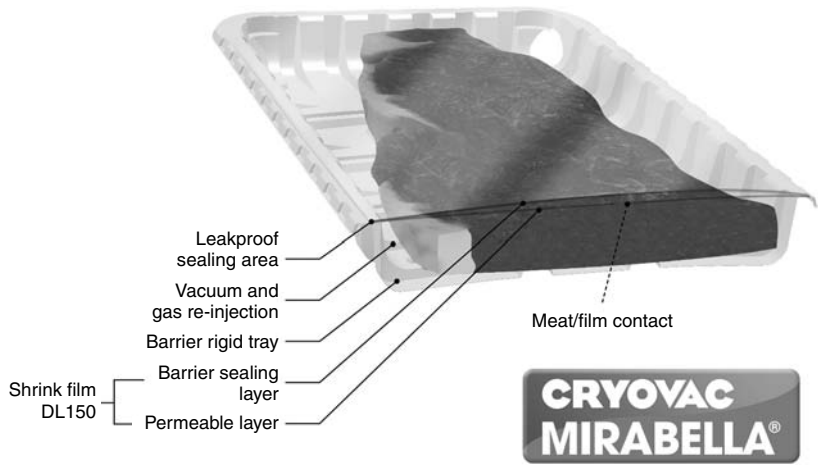

Fig. 6.3 Schematic diagram of the Mirabella MAP system.

to penetrate between the two film layers. This gas space allows the permeable film to touch the meat surface without causing discoloration.

A film that contains sodium nitrite as crystals in the sealant layer to promote bloomed color of fresh meat has been developed by Curwood as FreshCase®. The sodium nitrite (NO_2) level in the film varies with the type of meat to be packaged (3–10 mg/g for beef, 1–3 mg/g for pork and less than 1 mg/g for chicken). It is converted into nitric oxide gas, leaving no residual NO_2 in the meat. The composite film with outer nylon film layer, barrier layer of ethyl vinyl alcohol or polyvinylidene chloride, and sealant layer of ethylene vinyl acetate or polyethylene containing the NO_2 crystals has received approval for use in the United States. Approximately 12 h contact time of the film with the meat is needed for blooming of pork and ground beef and 48 h for whole muscle beef cuts. Currently, use- or

freeze-by code dating of meat with this technology is limited to 30 days for whole muscles and seven days for ground beef and a qualifying statement of 'vacuum packaging with nitrite containing film to protect flavor and color' is required on the product label (Siegel, 2011).

Sealed Air Cryovac has also created a package with separate compartments for meat and for marinade. Marinade on Demand™ is a two-part thermoformed rollstock package that creates an easy, sanitary and controlled marinating process for consumers who only remove the product for cooking after the desired marination time (Fig. 6.4).

Saddle pack-style packaging uses special rollstock material to package individual meat or poultry portions on a rollstock machine. Perforations in the film separate the portions, allowing consumers to easily remove desired quantities (Fig. 6.5). The product format can be flat or folded to create a smaller-sized retail package. The vacuum packaging eliminates a need for repackaging before freezing while preventing leaks and freezer burn, and allows for options of easy-opening sealing and of printing the top web in up to eight colors.

Some companies are developing alternatives to polypropylene, polyethylene or barrier foam trays for use as heatable containers. Crystallized polyethylene terephthalate (CPET) trays from Alcan Packaging Food America have resin 1 recycling code for 100% recyclability and contain an average of 17% post-consumer recycled material. These trays are available in many colors and shapes, have dual ovenability with temperature tolerance of −40°C to 200°C, and provide comparable barrier protection of multilayer polypropylene-ethyl vinyl alcohol trays. Recommendations are to seal the trays with ethyl vinyl acetate-coated lidding films.

Ilapak has developed a flow wrapper that uses in-line chambers for vacuumizing packages that are then discharged into the flow wrapper for introduction of CO_2. This reduces residual O_2 to parts per million and provides for continuous loading and wrapping of meat and poultry in contrast to the racheted cycles

Fig. 6.4 Marinade-on-demand package showing barrier between meat and marinade that allows marinade into the meat cavity when the marinade pouch is squeezed.

Fig. 6.5 Saddle pack packaging for multiple packages of individual meat portions.

while the vacuum chamber completes air removal on conventional form-fill-seal machines (Higgins, 2011a). Other packaging equipment forms film cavities for form-fill-seal applications with air, which allows use of thinner forming films and results in cost savings. These are only a few examples of the proprietary equipment and packaging configurations for different types of products in different companies. Use of company names or brands does not imply endorsement of any product or service, but are provided for credit or identification purposes.

6.4 Effective application of packaging to improve the quality of fresh and processed meat

The following sections discuss the multiple benefits from successful application of various packaging technologies – in terms of quality improvement, increased shelf life and general cost effectiveness.

6.4.1 Quality improvement through packaging

Successful packaging requires the interrelationship of the product and packaging materials with other processing, distribution and display components of the entire fresh meat marketing system (McMillin, 2008). Decisions are required on the desired meat color with high O_2 or low O_2 during transit and display, postmortem age of whole muscle cuts, injected and enhanced products, phosphate types, use of vitamin E, slicing method for bone-in products, bone discoloration, package seal integrity, pre-pricing and dating, freight, cube and tray size issues and productivity measurements (Smith, 2001). Other considerations regarding meat for MAP

include the pigment globin state as raw or cooked, time after harvest, conditions at harvest, temperature of storage, anatomical muscle location, intact or ground, headspace to product volume, exposure to light and heat and anaerobic or aerobic atmosphere (Siegel, 2001). Extension of shelf life with MAP requires matching product and packaging materials through careful selection, proper gas mixes, detection of leaking packages and off-line testing for overall quality control (Stahl, 2007). A major decision in choosing a MAP system is color of meat desired during transit and subsequent display. The packaging systems that provide for retail display of fresh meat with a red color are more highly used because consumers discriminate against beef that is not red during display (Carpenter *et al*., 2001) and will avoid purchasing meat with 20% or more metmyoglobin (MacDougall, 1982). Atmospheres of O_2 above 40% have been used to induce oxymyoglobin color for retail display. Although it has been well established that high O_2 levels cause protein and lipid oxidation before red color diminishes (Lund *et al*., 2007), consumers did not find oxidized flavor to be objectionable even though beef in high-oxygen packaging was tougher and less juicy (Zakrys *et al*., 2009).

Color stability of six beef muscles in a MAP mother pack system with 50% CO_2:50% N_2 and O_2 scavengers had superior redness during 96 h of retail display after six weeks storage in mother packs compared with the muscles under similar conditions without O_2 scavengers (Isdell *et al*., 1999). Sufficient O_2 scavengers are needed to achieve an O_2 half-life of less than 35–40 min in packaging so O_2 remains less than 5000 ppm at any time during storage if scavengers were not used (Tewari *et al*., 2002b). High O_2 systems for beef *Longissimus dorsi* muscles decreased juiciness, tenderness and vitamin E levels and increased objective and subjective evaluations of lipid oxidation compared with anaerobic systems, with decreased tenderness attributed to increased protein oxidation or reduced proteolysis (Clausen *et al*., 2009). Potassium lactate enhancement of beef muscles resulted in less color deterioration than non-enhanced muscles when displayed in high O_2 MAP for five days at 1°C after previous storage of nine days at 2°C (Kim *et al*., 2009). Large beef loin cuts (10 cm long) aged for five or ten days postmortem in high O_2 MAP and vacuum had similar levels of decreased shear force, disappearance of μ-calpain activity, decreased m-calpain activity and increased purge. Aging time in vacuum before packaging in high O_2 MAP did not affect ultimate shear force. Aging for ten days in high O_2 MAP induced higher purge loss and decreased m-calpain activity, but did not influence cooking loss compared with five days of aging (Lindahl *et al*., 2010). Large beef cuts could be aged in high O_2 MAP for five to ten days without negative effect on color stability compared with vacuum aging, but longer aging times decreased color stability (Lindahl, 2011). Beef *M. longissimus dorsi* steaks in vacuum had lower metmyoglobin development, Warner Bratzler shear force and thawing loss after freezing and higher α-tocopherol, color stability, tenderness and juiciness than those in high O_2 MAP after 3, 5 or 15 days of aging (Lagerstedt *et al*., 2011b). Sirloin steaks had longer shelf life in laboratory-packaged MAP than commercially packaged MAP due to high numbers of lactic acid bacteria, while higher levels of O_2 in MAP increased lipid oxidation (Zakrys-Waliwander *et al*., 2011).

It had been previously shown that lactate and phosphate in 10% injection enhancement of *Longissimus*, *Semimembranosus* and *Adductor* muscles improved color stability and star probe objective tenderness while lipid oxidation was decreased, but there were no differences in desmin and troponin-T degradation or myosin oxidative crosslinking between injected and control samples in high O_2 MAP for 16 days (Kim *et al*., 2010b). However, *Longissimus*, *Semimembranosus* and *Adductor* muscles cut at 24 h postmortem had rapid increases in lipid oxidation, decreased color stability and oxidative crosslinking of myosin heavy chain during nine days of display in high O_2 MAP compared with vacuum, while autolysis of μ-calpain activity was not changed (Kim *et al*., 2010a).

Color stability of beef steaks was maintained longer with ultra-low O_2 packaging systems containing CO than for steaks in high O_2 MAP, with no differences in color using 0.4% CO and the remainder gas being argon, nitrogen or CO_2 (Grobbel *et al*., 2008a). Tenderness and juiciness of *Longissimus lumborum*, *Semitendinosus* and *Triceps brachii* was increased with 10% enhancement (solution of beef broth, potassium lactate, sodium phosphate, salt, rosemary). Steaks in high O_2 MAP discolored faster in dark storage at 2°C for 14 days than steaks packaged in VP or ultra-low O_2 with CO MAP (Grobbel *et al*., 2008b). Enhancement of beef *Triceps brachii*, *Biceps femoris* and *Rectus femoris* with ammonium hydroxide, salt and CO resulted in greater color stability and higher total plate counts than non-enhanced steaks. Color stability of the enhanced steaks was improved by packaging in 100% CO_2 compared with packaging in 80% O_2:20% CO_2 (Hamling *et al*., 2008). Beef pretreated with 5% CO:95% N_2 for 24 h before VP was redder than controls without VP. Heat shrinking of the VP resulted in lower drip loss, higher color stability and more intense red color than non-heat-shrunk packaging (Aspé *et al*., 2008). Beef steaks in MAP with 0.2% CO and 30% CO_2 had the highest preservation of color and odor while steaks in 0.4% CO were regarded by panelists to have an 'artificial' color and color stability decreased with storage in 21% O_2 (Venturini *et al*., 2010). Carboxymyoglobin formation in beef steaks packaged in CO, CO_2 and N_2 increased with smaller headspaces and higher levels of CO (Raines and Hunt, 2010). Purge loss was lower in vacuum skin packed retail beef steaks than in VP while vacuum systems produced higher sensory scores and purple color compared with beef in high O_2 MAP (Lagerstedt *et al*., 2011a).

Higher temperatures decreased color, lipid stability and volatile aroma compounds and decreased CO_2 in minced beef through ten days in 30% CO_2:70% N_2 (Limbo *et al*., 2010). Minced beef in 50% O_2:30% CO_2:20% N_2 MAP maintained acceptable color, oxidation stability and microbial loads through 14 days more than other MAP systems (30% O_2:70% N_2, 50% O_2:50% CO_2, 70% O_2:30% CO_2, 30% O_2:30% CO_2:40% N_2) (Esmer *et al*., 2011). Ground beef was more red during 35 days of display after being maintained in packages that were initially MAP with 0.4% CO (balance 30% CO_2:69.6% N_2) than when initial packaging had the CO mix for 48 h at 3°C before the ground beef was repackaged in overwrap polyvinyl chloride film for display (Jeong and Claus, 2011).

Vitamin E in livestock diets has been shown to reduce lipid oxidation of red meats packaged in high O_2 MAP (Álvarez *et al*., 2009). Addition of rosemary extract to minced beef *M. gluteobiceps* controlled lipid oxidation in aerobic storage more than vitamin E added through dietary supplementation to the steers, even though meat redness was not improved. Addition of rosemary extract to beef from the vitamin E-supplemented cattle resulted in the lowest lipid oxidation through a synergistic effect (McBride *et al*., 2007). Ground beef patties with 1% chitosan had lower lipid oxidation than control patties, with lipid oxidation higher in overwrap and high O_2 than in VP or 0.4% CO:19.6% CO_2:80% N_2 MAP (Suman *et al*., 2010). Addition of rosemary extracts to minced pork formed into patties and cooked before storage in anoxic MAP reduced lipid and protein oxidation through six days of simulated lighted retail display at 1°C (Lara *et al*., 2011).

6.4.2 Microbiological improvements through packaging

MAP is widely used to prolong the shelf life of minced meat, with primary dependence on CO_2 for antimicrobial activity. Behavior of CO_2 in MAP packages is influenced by package permeability, convection flux of the gas permeation from the film to the food surface, diffusion into the food, solubility in the food and temperature. Diffusion into a solid matrix like meat is complex and CO_2 has a greater solubility in water than any other gases used in MAP systems (Simpson *et al*., 2009). The CO_2 in MAP is highly soluble in meat due to the high moisture content and is more soluble at colder temperatures. Addition of citric acid or acetic acid decreased counts while cinnamaldehyde had no effect on microbial growth (Schirmer *et al*., 2009). Higher CO_2 inhibited microorganism growth and oxidation, with 70% CO_2:30% N_2 more suitable for frankfurter packaging than vacuum packaging or MAP with 30% CO_2:70% N_2, 100% CO_2 or 80% O_2:20% CO_2 (Gokoglu *et al*., 2010).

Chicken in packaging material with high O_2 transmission rates had the highest bacterial growth and degree of off-odor while chicken in expanded polyethylene terepthalate had similar characteristics to barrier display film even with lower initial CO_2. Storage temperature at 8°C influenced microorganism growth and off-odor more than initial levels of 2 or 4% O_2 in the packages (Pettersen *et al*., 2004b). Pork in MAP with 5–55% O_2, 20% CO_2 and balance N_2 had lower TBARS with 45% O_2 that resulted in minimal differences in color acceptability, overall acceptability and total microbial counts than the other MAP treatments (Zhang and Sundar, 2005).

Breaching of the structural integrity of packaging materials may potentially allow transmission of microorganisms, but there was no evidence of transmission of any pathogenic microorganism across barrier film for VP raw meat, raw meat plastic bags, cooked chicken laminated bags, plastic carrier bags, air-permeable heat shrinkable film or tin foil because the structural integrity was maintained (Moore and Millar, 2006). Films containing antimicrobial agents nisin, food-grade acids and salts, chitosan, plant extracts and lysozyme and lactoperoxidase enzymes reduced microorganisms by 0–9 $\log_{10}$ colony forming units (CFU).

Most films with antimicrobial agents gave 1.5–2 $\log_{10}$ reductions, with combinations of antimicrobials having improved efficacy. Most of the antimicrobial tests limited exposure to less than 12 h and less than seven days of duration (Joerger, 2007). Apple-based edible films with 1.5% or 3% cinnamaldehyde reduced *C. jejuni* inoculated onto chicken breasts by 1.8–6 logs after 72 h at 4°C while 3% carvacrol in the films reduced *C. jejuni* strains by 0.5 logs or more (Mild *et al*., 2011). Oregano and sage essential oils incorporated into cellulose-based filter paper had antimicrobial activity against *Listeria innocua*, *Staphylococcus aureus* and *Salmonella enteritidis*, while only oregano essential oils had antimicrobial activity when incorporated into whey protein films. This was attributed to the higher porosity and diffusivity of the active compounds in the filter paper, showing that interactions of antimicrobial agents and films influenced the antimicrobial properties of the films (Royo *et al*., 2010).

Lactic and acetic acids or their sodium and potassium salts are often used as antilisterial agents in processed ready-to-eat meat and poultry products, which are usually in VP or MAP. Inactivation of *L. monocytogenes* on pork stored aerobically for seven days or in 70% O_2:30% CO_2 for 21 days was greater when nisin, a bacteriosin and lactic acid were used together (López-Mendoza *et al*., 2007). Only one of the two nisin-blend antimicrobial agents incorporated into cellulose coating applied to a barrier film caused *L. monocytogenes* inhibition and its use on beef cubes through 30 days of storage reduced counts by 1 log compared with control film (Matthews *et al*., 2010). Listeria viable counts decreased by 1.5 log units on frankfurters in contact with low-density polyethylene film with a Enterocin 416K1 bacteriocin coating compared with control packaging (Iseppi *et al*., 2008). Inclusion of enterocins to alginate, polyvinyl alcohol and zein films increased presence of voids and pores, but did not change water vapor permeability. Plasticizing of polyvinyl alcohol reduced tensile strength and brittleness and increased strain at break in zein films (Marcos *et al*., 2010). Pullulan, a fungal polysaccharide film containing bacteriocin sakacin A, inhibited *L. monocytogenes* more than 3 $\log_{10}$ cfu/g after three weeks at 4°C (Trinetta *et al*., 2010).

Paper composed of graphene oxide and reduced graphene oxide reduced viability of *E. coli* by 98.5%, but the filtering method of manufacturing the experimental paper is not suitable for mass producing of nanomaterials (Hu *et al*., 2010). The coating of paper by silver nanoparticles, which penetrated the paper surface to a depth greater than 1 μm, caused slightly greater reductions in Gram-negative than Gram-positive bacteria. Initial *E. coli* viability was reduced by 99.97% after 1 h exposure and 100% after 3 h exposure to the coated paper, while initial Gram-positive *S. aureus* viability was reduced by 97.9% after 1 h exposure and 99.91% after 3 h exposure to the coated paper (Gottesman *et al*., 2011).

6.4.3 Processing improvements

Several processing technologies are used to insure precooked or ready-to-eat product safety after processing and packaging, including thermal pasteurization, high-pressure processing and irradiation. All of these require additional packaging

considerations to withstand the process treatment, additional handling and to maintain desired attributes for distribution and display. Packages for post-packaging heating must maintain ink adhesion, gloss and shrink. Irradiation is another method to insure that post-packaged products are free of pathogenic microorganisms. Packaging materials that can be used for irradiated foods in the United States require approval by the Food and Drug Administration. Irradiation is not usually practiced with packaged products because there are only a few generic packaging structures approved for irradiation processing and irradiation may cause undesirable sensory quality by lipid oxidation, off-flavor and pink color of meat in MAP. Production of methylcellulose-based films prepared by casting from 1% aqueous solution of vegetable oil, glycerol and Tween 80 and reinforced with nanocellulose had improved barrier properties after irradiation (Khan *et al*., 2010). Other preservation methods – ultrasonics, pulsed visible light, pulsed electric field, high-voltage ARC discharge, magnetic fields and dense phase carbon dioxide – have not been sufficiently commercialized to evaluate their effectiveness on packaged products or the impacts on packaging materials (Morris *et al*., 2007).

High-pressure processing is a food preservation method that maintains sensory and nutritional attributes while greatly reducing the number of microorganisms and deactivating enzymes by pressurizing the food at 300–800 MPa over several minutes (Caner *et al*., 2004). The intense high pressure (up to 600 MPa) interrupts the cellular activities of microorganisms while the product is minimally affected due to the equal application of the hydrostatic pressure in all directions (Clyma, 2011). Packaging materials must have sufficient flexibility and resilience to compensate for the reduction in product volume during pressurization and then recover to avoid package deformation (Caner *et al*., 2004). High-pressure processing changed the functional properties of polymer packaging films, particularly the heat sealability, except for Surlyn, while layered films were less affected (Dobiáš *et al*., 2004). Laminated package structures that are not vulnerable to high pressures have been developed and coextruded plastics are also being evaluated (Morris *et al*., 2007). VP in polyamide polyethylene blend (1:5 PA:PE) or materials with similar properties of strength and stretchability provide flexibility to withstand high pressure while maintaining a package suitable for sale to consumers. High-pressure processing at pressures of 200, 400 and 600 MPa minimally affected the mechanical strength and water vapor barrier properties of polyamide-polyethylene, polyethylene terephthalate-biaxially oriented polyamide-polyethylene, polyethylene terephthalate-polyvinylidene chloride-polyethylene, polyamide-surlyn, low-density polyethylene and polyethylene-vinyl acetate-polyethylene (Le-Bail *et al*., 2006), so many packaging materials can be used with this food preservation technique. Ultra-high-pressure processing increased solubility of N_2 and O_2 in the polypropylene heat-sealing inner layer and voids and pits form if the pressure is rapidly released (Fairclough and Conti, 2009), which might reduce package seal integrity. Polyester/polyethylene film had minimal changes in properties after high-pressure pasteurization compared with polyethylene/ethylene-vinyl-alcohol/polyethylene, metallized polyester/polyethylene and polypropylene-silicon oxide films (Galotto *et al*., 2010). Packaging factors affecting high-pressure efficiency

and effectiveness are excessive amounts of packaging, the amount of gas inside the package and the sealing ability of the flexible packaging material. MAP with gases are more challenging than VP for high-pressure processing and the amount of gas in a package makes the process more expensive per amount of meat due to the space requirements inside the high-pressure chambers (Clyma, 2011). High-pressure processing differentially affected the different microorganisms on cooked ham, but 400 or 600 MPa for 10 min at room temperature highly inhibited major spoilage bacteria (Han *et al*., 2011).

6.5 Future trends

Economies in terms of lowering costs or increasing value will compel continued meat and poultry packaging changes. Research on shelf life, quality and safety of different packaging system options will be in terms of methodologies to improve product analyses; additional information on pigment chemistry, oxidative mechanisms and microorganism dynamics; understanding of animal genetics and quantitative trait loci; active and intelligent options; and changes in materials with nanotechnology and resin incorporation (McMillin, 2008). Any packaging changes should account for consumer acceptance levels since familiar packaging technologies are preferred over non-familiar technologies (Wezemael *et al*., 2011).

Many specific packaging innovations will be based upon current proprietary technology developments and consumer demographic and cultural indications. Aging populations will require improved easy-to-peel films that provide desired containment and molding of products through processing and handling. Resealable packages that maintain package integrity until opening, that can be opened easily and that can be resealed multiple times will improve convenience for fresh and processed meats. Packaging that is recyclable, of biodegradable materials, creating less waste, produced with less energy and from renewable sources will continue to receive attention (Dressler, 2010b). Transparent recyclable plastic packages for chicken that allow viewing of the product and eliminate foam trays and plastic overwrap will be adapted for some red meat products.

Processors will desire to improve efficiencies and cost effectiveness through puncture-resistant films that do not require thicker film layers or additional protective shielding material to prevent damage from bones. Elimination of trays with packaging that retains rigidity during storage, handling and shelf display without deformation from stacking will improve costs and logistical considerations. Square trays will reduce the amount of scrap material to be recycled after each tray is punched and molded and allow smaller corrugated shipping cases, increasing shipping efficiency by three or more cases per pallet. Improvements in adhesives for laminated films, including less solvent use and more ultraviolet or electron beam binding (Lingle, 2010) and allowing lamination of film to less expensive materials such as paperboard will continue.

Additional developments of CO, including use of marinades containing CO before packaging, films to improve CO transmission to meat in master pack

systems and films that provide preferential CO permeability over other gases will continue to allow display of meat with red color and extended storage. Emphasis on packaging to ensure product safety will include tamper-evident tape as well as packaging that senses meat spoilage, specific quality changes or growth of pathogenic microorganisms by changes in color or printing to warn consumers that the food is unsafe or has less than desired properties. Use of graphics imbedded between film layers or within extrusions to provide print integrity and eliminate ink contamination of products will continue, particularly for lidding materials. Improved technologies will allow imbedding of components into outer, barrier or sealant film layers to provide for maintenance of initial packaging conditions or to cause desired changes during storage of the meat or poultry.

Packaging and tray sealing equipment that can accommodate different tray sizes and depths or different gas compositions simultaneously for different brands, manufacturers or even products (Stones, 2011) are currently available as prototypes or in development. Speed and efficiency of packaging equipment will continue to be concerns of processors.

6.6 Sources of further information and advice

There are many sources of information on packaging for fresh and processed meat. Background information can be obtained through books available from many publishers. Scientific journal sources are less available, but provide generally accepted theories and applications in specialized packaging areas. The rapidly changing technologies generally indicate that the most current developments in equipment, materials, systems and accessories are given in monthly trade magazines and newsletters, either web- or print-based, for the meat and food industries and packaging practitioners.

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7

Advances in vacuum and modified atmosphere packaging of poultry products

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Abstract: Temperature, humidity, light, oxygen and water activity (aw) can affect the behaviour of microorganisms that can result the type and the rate of spoilage in poultry meat. The potential use of traditional preservation techniques such as vacuum packaging (VP) and modified atmosphere packaging (MAP) can be combined with decontaminants, additives (natural or chemical), natural biopreservatives and/or can be integrated to emerging technologies such as active and intelligent packaging, irradiation and high pressure. In addition, the potential use of alternative methods such as metabolomics that correlate microbial growth and chemical changes occurring during meat storage can reveal chemical indicator(s) that may be useful tools for quantifying poultry quality or freshness.

Key words: ecological determinants, spoilage, biopreservation, metabolomics, shelf life, food ecology.

7.1 Introduction

The popularity and consumption of poultry meat has increased globally over the last few years, with worldwide production reaching 91.9 million tonnes in 2009 (FAO, 2010). This increased consumption of fresh and processed poultry products can be attributed to the desirable sensory characteristics and the association that the consumer has made with poultry products being 'healthier' than red meats. As fresh poultry meat is a highly perishable food, due to its biological composition, the high consumption of poultry products leads to concerns pertaining to product safety, shelf life, quality and associated desirable sensory characteristics.

Many interrelated factors can influence the shelf life of poultry meat; such as environmental conditions (temperature, humidity, light, oxygen), water content, indigenous enzymes and, most importantly, microorganisms. Consequently, developing and combining preservation methods to extend shelf life and increase safety of these products is of great importance. Thus, traditional preservation techniques such as vacuum packaging (VP) and modified atmosphere packaging (MAP) can be combined with decontaminants, additives (natural or chemical), natural biopreservatives and/or be integrated to emerging technologies such as active and intelligent packaging.

The development in food processing and preservation technologies makes it evident that the important and urgent task of identifying spoilage indicators is a complicated proposition. Currently, there is no general agreement on what constitutes the primary early quality changes in muscle-based foods, an issue that remains unsolved to this day, despite the abundance of more than 50 chemical, physical and microbiological methods that have been used over decades for the detection and measurement of meat spoilage (Ellis *et al*., 2002; Nychas *et al*., 2008). Traditional methods of quality analysis such as microbial counts, give retrospective information, while sensory analysis, despite the fact that is quick and well accepted, has been traditionally dependent on accessibility to highly trained personnel and consequently, can be very expensive to conduct. Thus, alternative methods that correlate microbial growth and chemical changes occurring during meat storage can reveal chemical indicator(s) that may be useful tools for quantifying poultry quality or freshness (Dainty, 1996; Nychas *et al*., 2008).

7.2 Role of packaging and conventional packaging systems

The main function of poultry meat packaging is to protect the product against bruising, physical and chemical changes, and microbial contamination, which can give rise to deteriorative effects, including discolouration, off-flavour and off-odour development, nutrient loss, textural changes and pathogenicity. Packaging systems are designed to maintain the natural quality of poultry products as they move throughout the food chain in a series of events or stages until final preparation and consumption by consumers. The primary role of packaging for processed poultry products is to prolong shelf life (Totosaus and Kuri, 2007).

Variables that influence shelf-life properties of packaged fresh and processed poultry meat are product type, gas mixture, package and headspace, packaging equipment, storage temperature and additives. Depending on the packaging format and materials used, the product can be stored either ‘aerobically’, or under vacuum or in a modified atmosphere. Factors to take into consideration regarding such packaging materials include the control of oxygen permeability, humidity, hardness and stability, apart from the capacity of impression and sealing properties, together with heat resistance properties, market requirements and costs (Totosaus and Kuri, 2007) (see Chapter 6).

Packaging materials used for meat products are usually plastics, more specifically, laminates, in which polymer layers with good oxygen-barrier properties like polyamide or polyethylene tetrapthalate are adhered to a polymer layer possessing good humidity barrier performance and sealing properties such as polyethylene and polypropylene. Packaging materials with good oxygen-barrier properties can be used for low O_2 VP and MAP applications. An air-permeable packaging is generally characterized as aerobic packaging, though it has been stated that the use of overwrapped packaging materials held within master packs or tray-in-sleeve systems allows for this packaging option to be a component of MAP (McMillin *et al*., 1999). VP involves placing a product in a pack possessing low-oxygen permeability – for example, in a laminated plastic pouch – removing air from the package and applying a hermetic seal. The O_2 level is reduced to less than 1% by drawing a vacuum within the pack prior to sealing.

A modified atmosphere (MA) can be defined as one that is created by altering the natural composition of air (i.e., 78% nitrogen, 21% oxygen, 0.03% carbon dioxide and traces of noble gases) to provide an alternative atmosphere for increasing the storage length and quality of food/produce (Phillips, 1996). This can be achieved by using active or passive MAP. Active modification occurs by the displacement of gases in the package, and their replacement by a desired mixture of gases, while passive modification occurs when the product is packaged using a selected film type, and the desired atmosphere develops naturally as a consequence either of the products' respiration or via the diffusion of gases through the film (Lee *et al*., 1996; Moleyar and Narasimham, 1994; Zagory, 1994). Oxygen (O_2), nitrogen (N_2) and carbon dioxide (CO_2) are mainly used in MAP preservation of meat and poultry. Other gases, with the exception of carbon monoxide, such as, nitrous and nitric oxides, sulphur dioxide, argon and chlorine have been used for experimental purposes only (Phillips, 1996). For safety, regulatory and cost restrictions, the use of these alternative gases have not been applied commercially. In addition to the commonly used gases in MAP, ozone (O_3) is another active gas with established antimicrobial properties against bacteria, yeasts and moulds, and makes a positive contribution to the maintenance of muscle colour (Hotchkiss, 1989; Kim *et al*., 2003). There has been a renewed and increased industrial interest in using this gas to extend the shelf life of MAP meat and poultry products, especially after the recent US Department of Agriculture (USDA) approval for safe use of ozone in food preservation, as well as in sanitation procedures (Kim *et al*., 2003).

The main purposes of meat packaging technology are (Skandamis and Nychas, 2005):

- **Shelf-life extension:** Depending on the type of meat/meat product, the time of display in a supermarket or shop can be doubled using MAP/VP techniques. Thus, waste is minimized and ordering and restocking are more flexible.
- **Enhanced appearance and presentation** (i.e., quality): The use of MAP presents an obvious advantage at both retailer and consumer level, in having meat in a tray.

- **Reducing the need for artificial preservatives:** Fulfilment of consumers' expectations since extra efforts and state-of-the-art technology, represented by the use of MAP/VP without any additional artificial preservatives.
- **Ability to access new markets:** This can be achieved due to the longer shelf life provided using MAP.
- **Minimization of meat waste:** Contribution to the restoration of the international 'image' and competitiveness of the meat sector, by assuring safety, optimizing quality and reducing rejected products.

The quantitative and qualitative differences in microbiota, proportions of unsaturated lipids as well as pigments between poultry and red meats, lead to different requirements in gas mixtures used in MAP (from those used in red meats) to optimize shelf life and visual appearance of poultry and poultry products (Saucier *et al*., 2000). Moreover, from a microbiological perspective, the higher pH of poultry (5.7–6.0 for the breast and 6.4–6.7 for the legs; Marenzi, 1986) should be seriously considered when selecting the gas mixtures required for effective MAP storage.

7.3 Shelf life of fresh and processed poultry products in conventional packaging systems

In the following, we discuss the issues that arise affecting various aspects of the keeping properties of poultry stored using conventional packaging systems, looking at where problems occur and how they can be used to best effect.

7.3.1 Quality aspects of poultry products during storage

Colour, appearance and texture are important factors that consumers will consider before making a decision to buy poultry (see Chapter 4). From a quality standpoint, meat colour is important, and selection of the gaseous atmosphere has a critical bearing on this attribute. The bright red colour associated with freshness can be obtained by packing meat in an MA. To avoid the discolouration of red meat, a high percentage of O_2, or pO_2, is included so that the oxygenated bright red colour of oxymyoglobin is retained, a method called 'high oxygen modified atmosphere' (Gill and Molin, 1991). There is, however, a relationship between O_2 and CO_2 in MAP meat packs with respect to meat quality and shelf life in general. Both gases select for different microbial associations from the initial microbiota (O_2 for aerobic and CO_2 for facultative anaerobic biota) and both influence meat colour in different ways. The function of CO_2 in the gas mixtures is to selectively delay microbial growth (Daniels *et al*., 1985), which, together with oxidation processes, are the primary causes of meat quality deterioration during storage. O_2 favours the development of an increased depth of oxymyoglobin, thereby affecting the red colour of meat (Kropf, 1993). However, the use of high concentrations of O_2 promotes oxidation of lipids, especially in minced meat, which has its cell

structure disrupted, leading to increased exposure of lipids to O_2, enzymes, haeme pigments and metal ions (Jakobsen and Bertelsen, 2000; Jayasingh *et al*., 2002).

Veberg *et al*. (2006) have reported that for minced turkey meat, the *a** values on the surface of high O_2 packaged samples were substantially reduced from day 7 to day 12 of storage, probably due to oxidation and external accumulation of metamyoglobin. Vacuum-packaged samples had intermediate *a** values as a consequence of the presence of purple deoxymyoglobin. The *b** (yellowness-redness) values were higher in high O_2 packs than in vacuum-packaged samples of turkey, while the *L** (lightness) values were only higher in high O_2 samples of turkey at day 7, but not at day 12 of storage. The concentration of myoglobin in the minced turkey thighs was 0.31%. For poultry patties packaged in MAP (Mastromatteo *et al*., 2009), a decreasing trend was observed through storage with regard to *a** values (indicative of red colour) and chroma (colour intensity) together with an increasing trend in *b** and hue values, which were attributed to the gradual oxidation of myoglobin and accumulation of metamyoglobin resulting in meat discolouration. The reduction of colour intensity could be due to the high concentration of carbon dioxide (40%) in MAP. On the other hand, it was inferred that the redness stability for air-packed samples was prolonged due to low concentration of CO_2. Redness (*a**) values related to myoglobin content and chroma for aerobically packaged patties increased at the end of the storage period, with a concurrent decrease in hue values.

Oxidative rancidity is one of the main factors limiting the quality and acceptability of poultry products, affecting the sensory quality relating to flavour and odour. Lipid oxidation is a complex process whereby unsaturated fatty acids react with molecular O_2 via free radicals, and form peroxides or other primary products of oxidation. Secondary oxidation products, such as aldehydes, ketones and esters, are responsible for the increased deterioration and rancid flavour during frozen storage (Pérez-Chabela, 2007). It has been reported that turkey patties stored in high O_2 atmospheres for 7 and 12 days, respectively, showed a high extent of lipid oxidation. Lipid peroxidation, measured in terms of thiobarbituric reactive substances (TBARS) for turkey patties stored for 7 and 12 days in a high O_2 atmosphere, was significantly ($p < 0.05$) higher compared with patties stored in vacuum. The mean rancidity scores for the whole turkey patties stored in high O_2 were also notably higher compared to those packed under vacuum. Moreover, oxidative rancidity was accelerated in the meat of birds fed diets high in polyunsaturated fatty acids, and was more rapid in dark leg meat than in white breast meat from the same carcass (Lin *et al*., 1989). Smiddy *et al*. (2002) have found that cooked chicken patties, prepared from thigh muscle, were more oxidized than the respective breast patties, irrespective of the packaging system (MAP – 70% N_2:30% CO_2 or vacuum packaged). Oxidation levels in MAP thigh patties were probably higher than in breast patties due to the combination of increased susceptibility and higher initial oxygen levels in thigh patty packs. Although slightly higher levels of oxygen were observed in cooked vacuum-packaged breast patties than in thigh patties, lipid oxidation of cooked vacuum-packaged thigh patties was greater than for breast patties. Overall, lipid oxidation appeared to be mainly influenced by the oxygen level in the pack (see Chapter 4).

7.3.2 Precepts of poultry meat ecosystem

From the very early stages of meat science research, spoilage of poultry muscle (thigh and leg) has received special attention (McMeekin, 1975, 1977). Spoilage of poultry muscles encompasses changes of the available low molecular weight compounds (e.g., lactate, glucose, amino acids), during the proliferation of the bacterial population that comprise the microbial association of the stored meat. The dominance of a particular microbial association for poultry muscle depends on factors that persist during processing, transportation and storage in the market. It is well established that in food system(s) five categories of ecological determinants – p that is, intrinsic, extrinsic, implicit, processing and emergent effects (Gould, 1992; Mossel, 1983; Odum, 1993) – influence the establishment of a particular microbial association. Thus, the different hurdles imposed (e.g., packaging, temperature, preservatives), as well as intrinsic factors (e.g., pH, glucose concentration) can influence the succession of the components of the microbial association, particularly the so-called 'ephemeral spoilage microorganisms' (ESO) – that is, those which fill the niche available by adopting ecological strategies (Ercolini *et al*., 2009; Nychas *et al*., 2008). These strategies, developed by ESO, are the consequence of environmental determinants such as the application of stress (abiotic factors, i.e., intrinsic, processing, extrinsic), any destructive or enrichment disturbance of the ecosystem (e.g., sudden event that provides newly available energy resources for exploitation) and the incidence of competitors (for carbon source, O_2 or other substances, e.g., ferric compounds) (Boddy and Wimpenny, 1992). For this reason, scientists and technologists involved in poultry meat production should attempt to control or modify some (e.g., temperature), or all of the parameters noted above, with a view to (a) extending the shelf life of poultry meat and (b) providing poultry products with acceptable shelf life. The use of MA can be regarded as such an approach (Skandamis *et al*., 2005).

Ephemeral spoilage organisms (ESOs)

It is well documented that the physiological status of an animal at slaughter, the spread of contamination during slaughter and processing, the temperature and other conditions of storage and distribution are the most important factors which determine the microbiological quality of poultry meat (Davies and Board, 1998). Indeed, as the inherent antimicrobial defence mechanisms of the live animal are destroyed at slaughter, the resultant meat is liable to rapid microbial decay. In principle, some of these microorganisms will be derived from the animal's intestinal tract and others from the environment with which the animal had contact at some time before, or during slaughter. For example, studies on the origin of the contaminants have shown that the source of *Enterobacteriaceae* on meat is associated with the processing work surfaces and not with direct faecal contamination. Moreover, psychrotrophic bacteria are recovered from hides and work surfaces within an abattoir, as well as from carcasses and butchered meat at all stages of processing (Bolder, 1998).

As mentioned above, a great number of microbiological studies pertaining to meat and poultry have established that spoilage is caused only by an ephemeral

Table 7.1 The genera of bacteria and yeasts most frequently found on poultry

Bacteria	Bacteria	Yeasts
Acinetobacter	*Kluyvera*	*Candida*
Aeromonas	*Kurthia*	*Debaryomyces*
Alcaligenes	*Lactobacillus*	*Trichosporon*
Alteromonas	*Leuconostoc*	
Arthrobacter	*Listeria*	
Bacillus	*Micrococcus*	
Brochothrix	*Moraxella*	
Campylobacter	*Neisseria*	
Carnobacterium	*Pantoea*	
Chromobacterium	*Pediococcus*	
Citrobacter	*Planococcus*	
Clostridium	*Plesiomonas*	
Corynobacterium	*Proteus*	
Enterobacter	*Pseudomonas*	
Enterococcus	*Serratia*	
Escherichia	*Streptococcus*	
Flavobacterium	*Streptomyces*	
Hafnia	*Staphylococcus*	

Source: Stanbridge and Davies (1998).

fraction of the initial microbial association (Nychas *et al*., 1998). The initial microbiota of poultry is similar to that of meat and consists of mesophilic and psychrotrophic microorganisms (Table 7.1; Bolder, 1998; Dainty and McKey, 1992). However, McMeekin (1975, 1977) reported differences in the composition of different parts of chicken, specifically between breast and legs, even with different penetration rates for various bacteria (Thomas *et al*., 1987). Storage conditions affect both the type and rate of growth of dominant microbiota.

A consortium of bacteria, commonly dominated by *Pseudomonas* spp., is in most cases responsible for spoilage of poultry meat which is stored aerobically at different temperatures. It is now well established that under aerobic storage the presence of certain species of *Pseudomonas* (i.e. *Ps. fragi*, *Ps. fluorescens*) is important in terms of microbial spoilage of poultry meat. The population of pseudomonads to an arbitrary level of $10^{7–8}$ cfu/g has been attributed to slime and off-odour formation in muscle foods. However in practice, both these characteristics become evident when the pseudomonads have exhausted the glucose and lactate present in meat and begin to metabolize nitrogenous compounds such as amino acids (Nychas and Tassou, 1997). Cold-tolerant Enterobacteriaceae (e.g. *Hafnia alvei*, *Citrobacter freundii* and *Enterobacter cloacae*) also occur on chilled meat stored aerobically (Zeitoun *et al*., 1994), but in terms of numbers, they do not significantly contribute to the microbial association. Despite the fact that *Enterobacteriaceae* rarely contribute significantly to the spoilage microbiota of meat and meat products, they have been considered as indicators of food safety.

Brochothrix thermosphacta and lactic acid bacteria have been detected in the aerobic spoilage biota of chilled poultry. These organisms have been isolated from

poultry carcasses during boning, dressing and chilling (Bolder, 1998). Both lactic acid bacteria (LAB) and *Br. thermosphacta* are the main, if not the most important, cause of spoilage, which can be recognized as souring rather than putrefaction (Kakouri and Nychas, 1994). This type of spoilage is one of the two distinct situations related to poultry meat which is commonly associated with MAP as the result of competition between facultative anaerobic Gram-positive biota. The second situation is where competition occurs between Gram-negative biota (pseudomonads and *Enterobacteriaceae*). The changes caused by either situation are related to (i) the type, composition and population of the microbial association and (ii) the type and availability of energy substrates in poultry meat. Indeed, the type and extent of spoilage is governed by the availability of low molecular weight compounds (e.g. glucose, lactate) existing in meat (Nychas *et al*., 1998). By the end of this phase changes and subsequent overt spoilage are due to catabolism of nitrogenous compounds and amino acids as well as secondary metabolic reactions. Moreover, induction of microbial competition by deliberate addition of Gram-positive biota (protective cultures of lactic acid bacteria) in order to compete against Gram-negative biota can be considered as a third situation of microbial competition to the benefit of food preservation and termed as 'biopreservation' (Gombas, 1989; Holzapfel *et al*., 1995).

Finally, yeasts and moulds have been reported to be a minor part of the microbial association of poultry products and are occasionally evident in microbial profiles from fresh and processed poultry products approaching the end of their shelf life, especially in the case of aerobically stored products (Balamatsia *et al*., 2007; Ismail *et al*., 2000; Patsias *et al*., 2006a, 2008).

Storage under aerobic conditions

In aerobic conditions, and especially at refrigeration temperatures, the psychrotrophic Gram-negative biota consists primarily of pseudomonads which dominate due to their shorter generation times compared to other members of the microbial association, and in addition to their metabolic pluralism, that allows pseudomonads to catabolize a variety of nutrients, primarily glucose, followed by lactate, gluconate and nitrogenous compounds, such as amino acids, proteins, creatine and creatinine (Skandamis *et al*., 2005).

Balamatsia *et al*. (2006) reported that for fresh chicken breast samples stored aerobically at 4°C, pseudomonads, *Enterobacteriaceae* and LAB were the dominant populations, while the shelf life of the product was 4–5 days. The counts of LAB in chicken samples stored under MAP were comparable to those stored in air throughout storage. In a similar study for precooked chicken stored at 4°C (Patsias *et al*., 2006b), it was shown that pseudomonads counts were generally higher in aerobically stored samples compared with MAP, whereas *Enterobacteriaceae* counts were always below the detection limit. Lactic acid bacteria showed similar counts with pseudomonads until the presence of off-flavours, where the population of lactic acid bacteria started to overcome the counts of pseudomonads. The shelf life of this product was indicated to be between 10 and 12 days. Additionally, *Br. thermosphacta* was detected in chicken fillets in lower populations than those above reported bacteria

(Balamatsia *et al*., 2007), while it has been reported that the numbers of *Br. thermosphacta* along with *Pseudomonas* spp. in fresh poultry fillets increased faster than those of lactic acid bacteria and attained highest numbers in the aerobic atmosphere compared with vacuum and MAP (Nychas and Tassou, 1997). Lastly, yeasts and moulds were found to be less numerous than bacteria in raw chicken fillets, which increased during aerobic storage (Balamatsia *et al*., 2007; Patsias *et al*., 2008), whereas in precooked chicken samples yeasts/moulds populations were detected between days 16 and 20 of refrigerated aerobic storage (Patsias *et al*., 2006a).

Storage under modified atmosphere packaging (MAP) or vacuum

The selection and concentration of gases used in MAP, especially in relation to the use of an increased percentage of CO_2 results in a shift of dominant biota from Gram-negative to Gram-positive, consisting mainly of lactic acid bacteria and *Br. thermosphacta* (Stanbridge and Davies, 1998).

Hotchkiss *et al*. (1985) determined the shelf life of chicken quarters stored at 2°C under increasing concentrations of CO_2 from 0% to 80%. Elevated CO_2 concentrations resulted in lower aerobic counts, and extended organoleptic shelf-life in terms of internal and external colour, odour, flavour, tenderness, juiciness, mouthfeel and appearance. The changes in microbial association during storage between aerobic and MAP stored samples was not only quantitative, but qualitative as well. The initial biota isolated from fresh poultry consisted of *Staphylococcus* sp. (29%), pseudomonads (71%) and *Lactobacillus* sp. (0%), while the contribution of these groups to the biota changed within 35 days of storage in 80% CO_2:20% AIR at 2°C to 0, 1 and 99%, respectively (Hotchkiss *et al*., 1985). The respective contributions in aerobically stored samples were 11%, 89% and 0%, respectively. Similar results were reported for ground chicken stored under MAP (Baker *et al*., 1985). Hotchkiss (1989) also reported that 10–20% CO_2 in a packaging atmosphere is sufficient for short shelf life extension (2–3 days) at refrigeration temperatures, whereas a minimum concentration of 50% CO_2 is essential to achieve longer shelf lives for MAP stored poultry. Furthermore, packaging of chicken breasts or thighs in 100% CO_2 was more effective, than VP, in terms of: (i) delaying microbial growth and (ii) occurrence of physicochemical changes, at 3°C and 10°C, while the least effective gaseous atmospheres were 100% N_2 followed by 20% CO_2:80% O_2 (Kakouri and Nychas, 1994). In this study, lactic acid bacteria were the dominant population in 100% CO_2. Co-dominance of this group and *Br. thermosphacta* was observed in vacuum-packaged breasts and thighs, while pronounced dominance of *Br. thermosphacta* occurred in 100% N_2 and 20% CO_2:80% O_2. Growth of pseudomonads was suppressed under all tested atmospheres. According to this comparative report, there were no significant differences in growth of spoilage association between breasts and legs.

Sawaya *et al*. (1995a) monitored the microbiological, chemical and sensory changes of chicken carcasses stored under MAP (70% CO_2:30% N_2,) at 2°C, 4°C, 7°C and 9°C. They found that the shelf life of carcasses stored in 70% CO_2:30% N_2 was approximately three times longer compared with air-stored samples at all three temperatures. Decreasing the concentration of CO_2 to 30% resulted in

a lower, but still significant shelf-life extension. Overall, MAP storage delayed the growth of all microbial groups associated with chicken. Moreover, MAP suppressed the production of microbial metabolites, especially at lower temperatures. In other studies, Jiménez *et al*. (1997) observed that MAP using 70% CO_2:30% N_2 at 4°C extended the shelf life of chicken breasts up to 21 days compared to 5 days for aerobically stored samples. In contrast, Balamatsia *et al*. (2006) showed that packaging of chicken breast fillets in 30% CO_2:70% N_2 at 4°C, increased the shelf life up to 7–8 days compared with 4–5 days for aerobic storage. Finally, in another MAP with gas mixture of 65% CO_2:30% N_2:5% O_2 (Ntzimani *et al*., 2010), it was found that the shelf life of the fresh chicken meat samples was 9–11 days, as determined by microbiological and sensory evaluation of the product.

Patsias *et al*. (2006a) monitored the microbiological, chemical and sensory attributes of precooked chicken meat stored at 4°C under air and three different MAP conditions; 30% CO_2:70% N_2, 60% CO_2:40% and 90% CO_2:10% N_2. Of all the microbial species enumerated, LAB was dominant and constituted a major part of the microbial population associated with the precooked chicken product, irrespective of the packaging conditions used. The 60% CO_2:40% N_2 and 90% CO_2:10% N_2 packaging treatments suppressed the growth of *Br. thermosphacta* and pseudomonads, while yeasts and moulds were suppressed by MAP for all gas mixtures tested. The use of CO_2-enriched atmospheres, as shown in the present study, resulted in an extension of shelf life of precooked chicken by around 4 days (30% CO_2:70% N_2), and by more than 6 days (60% CO_2:40% and 90% CO_2:10% N_2), respectively, compared to aerobically stored samples (shelf life of 12 days). For a similar product (semi-cooked coated chicken fillets) Ntzimani *et al*. (2010) compared the shelf life of chicken samples stored in air and under vacuum at 4°C. VP extended the shelf life of the product by 5–6 days compared to the control (aerobically stored) samples (which had a shelf life of 10 days).

Pexara *et al*. (2002) monitored the shelf life of cured, cooked, sliced turkey fillets in vacuum packs and in six different MAP (80% CO_2:20% N_2, 60% CO_2:20% O_2:20% N_2, 0.4% CO:80% CO_2:rest N_2, 1% CO:80% CO_2:rest N_2, 0.5% CO:24% O_2:50% CO_2:rest N_2 and 100% N_2). The importance of storage temperature on the growth of bacteria is demonstrated by a higher growth at 10°C than at 4°C. No differences between VP and 80% CO_2:20% N_2 were noted after 25 days at 4°C. In the other five tested MAP stored samples, insignificant differences in growth of LAB were evident, with the exception of the 60% O_2:20% CO_2:20% N_2 mixture which produced the fastest LAB growth rates. The other employed gas mixtures had all the same influence as VP on the growth of the spoilage biota at 4°C. The gaseous mixtures did not affect microbial growth at 10°C in comparison with the growth at 4°C. However, the lowest total counts and LAB counts at 10°C were observed in 100% N_2. In this study, MAP did not markedly reduce the growth rate of LAB compared to VP at both storage temperatures. The five gas mixtures of MA tested seemed to be ineffective in extending the product's shelf life in comparison with VP and 80% CO_2:20% N_2, both employed by the Greek meat industry. The inhibition of the tested modified atmospheres was higher when the gaseous mixture contained 50% CO_2, while higher concentrations of CO_2 had no retarding

effect on microbial growth. The latter is in accordance with the earlier report of Gill and Tan (1979) who shown that little if any increase in effectiveness of modified atmosphere storage is obtained on meat or on complex media by increasing the CO_2 concentration well above 25%. For maximum antimicrobial effect, the storage temperature should be kept as low as possible, because the solubility of CO_2 decreases dramatically by increasing the temperature (Daniels *et al*., 1985). Concluding, the shelf life of vacuum-packaged or MAP cooked cured meats can preferably be defined by unacceptable appearance (slime, drip loss), rather than a certain maximum acceptable bacterial level (Pexara *et al*., 2002).

Growth of pathogens in poultry

Although pathogenic genera do not constitute a part of the spoilage association *per se*, their occurrence is possible due to their presence in the raw meat or transfer during unhygienic processing of a product (see Chapter 1). In other words, they may constitute a numerically minor and 'passive' part of an association.

The principal pathogens of concern are *Aeromonas hydrophila*, *Listeria monocytogenes*, *Yersinia enterocolitica*, *Salmonella* spp., enterohemmorrhagic *Escherichia coli*, *Campylobacter jejuni/coli*, *Staphylococcus aureus*, *Clostridium perfringens* and *Clostridium botulinum* (mainly processed products). *A. hydrophila* was shown to be capable of growing on turkey meat stored at 1°C, or 7°C in air and 100% N_2 (Mano *et al*., 2000). Growth of *A. hydrophila* was also detected at 7°C in 20% CO_2:80% O_2, but not at 1°C, whereas no growth was observed in 40% CO_2:60% O_2 at both temperatures (Mano *et al*., 2000). Likewise, Mano *et al*. (1995) reported growth of *L. monocytogenes* on turkey stored aerobically, or in MA of 100% N_2, or CO_2:O_2 mixtures (20%:80% or 40%:60%) at 7°C, while at 1°C, only aerobic storage and 100% N_2 allowed growth of the pathogen with significantly lower numbers. Wimpfheimer *et al*. (1990) found that *L. monocytogenes* did not grow on raw chicken stored in 75% CO_2:25% N_2 at 4°C, 10°C or 27°C, but grew when held under aerobic conditions and in a 72.5% CO_2:22.5% N_2:5% O_2 atmosphere, at all three temperatures. The tolerance of *Y. enterocolitica* to CO_2 is considered higher compared to other pathogens (Garcia de Fernando *et al*., 1995). For instance, a higher growth potential of *Y. enterocolitica* than *L. monocytogenes* was reported on cooked chicken product stored in 44% CO_2:56% N_2 (Barakat and Harris, 1999). It has been reported that storage of cooked turkey at 4°C in 30% CO_2:70% N_2 delayed toxin production when compared with packaging in 100% N_2, whereas, no marked effect of MAP was evident at higher temperatures, such as 10°C and 15°C (Lawler *et al*., 2000). Nychas (1994) reported that *Salmonella* survived, but failed to grow, on chicken breasts and thighs when stored under vacuum, 100% CO_2, 100% N_2 and 20% CO_2:80% air at 3°C. However, when these poultry products were stored at 10°C, atmospheres, but decreased in a 100% CO_2 atmosphere. Phebus *et al*. (1991) reported that 100% CO_2 allowed the longest survival of *C. jejuni*, followed closely by 100% N_2, while CO_2:N_2 atmospheres (80:20, 60:40, 40:60), O_2:CO_2:N_2 atmospheres (5:10:85), as well as 100% air caused a slightly more rapid inactivation of *C. jejuni*, irrespective of the gas mixture used at two temperatures.

7.3.3 Effective application of packaging systems

For a successful application of any type of packaging, the following issues exhibit a pivotal role:

- The composition and the numbers of the initial microbial association. A large initial population will most likely reduce the shelf life of the product.
- The vital role of strict temperature control and its selective action on the food ecosystem is of great importance. Abuse temperature conditions that may occur through the chill chain may alter the proposed shelf life for each packaging system. Additionally, the time of technological application is important. The earlier the selection of an association by extrinsic factors begins, the better the results that can be anticipated.
- The selection of an appropriate gas mixture suitable for a particular ecosystem is essential. Apart from other ecological (e.g., pH, water activity (aw), nutrients, etc.) factors, the colour of the meat and oxidative rancidity play an important role in the selection of gases for different poultry meat types.
- The permeabilities of the different packaging materials to the gases employed should be selected critically, so that the added gases or those produced *de novo* in a food (meat) ecosystem should be retained.
- Combination processes (e.g., MAP-irradiation) or the packaging technologies alone change the spoilage pattern of the food (meat) ecosystem. Special attention is necessary when different processing technologies are combined with novel packaging systems due to the dominance of different ephemeral spoilage organisms.

7.4 Extension of shelf life and future trends in packaging systems

Extending the shelf life of poultry products is a major concern for the poultry industry. It is well known that the combined use of several preservation methods, termed as the 'hurdle concept' (Leistner and Rodel, 1976) is an efficient means to reduce the tolerance of microorganisms to adverse environmental conditions. Maintaining chilling conditions is the primary key for food preservation; the concept can be applied to control spoilage and safety of fresh meat and poultry, by exploitation of existing and future preservation methods. By adopting the hurdle concept, two or more preservation techniques (e.g., MAP and irradiation) could be combined to extend the magnitude of preservation potential over the use of each method applied singly (see Table 7.2; Skandamis *et al*., 2005).

7.4.1 Irradiation

Irradiation (up to 10 kGy) is a preservation method that has been more extensively investigated for preservation of poultry than red meats, with established regulatory frameworks in place, especially in the United States (Durante, 2002; Farkas, 1998;

Table 7.2 Shelf-life extension combining conventional packaging systems with different preservation treatments

Poultry type	Storage conditions	Treatments	Microbial indices	Treatment effectiveness	Shelf life	Reference
Fresh chicken legs	6°C, MAP[a] (90%CO_2: 10%O_2)	Lactic acid/sodium lactate buffer (2, 5, 7.5, 10%)	TVCs[b], *Pseudomonas* spp., LAB[c], *Enterobacteriaceae*, H_2S-producing bacteria	Log reductions of <1 to >2 depending on the treatment and the microbial group	13 d[d] (control), 14 d (2%), 15 d (5%), 16 d (7.5%), 17 d (10%)	Zeitoun and Debevere, 1992
Fresh minced chicken meat	10°C, Air (0.03%CO_2: 20.9%O_2:79%N_2), MAP1 (20%CO_2: 80%O_2), MAP2 (20%CO_2:10%O_2: 70%N_2)	Irradiation (1.0 or 3.1 kGy)	TVCs, coliforms	Reduction of 0.8 logs (1.0 kGy) and 1.4 logs (3.1 kGy) in MAP2 compared to air, further reductions of 0.3 logs (1.0 kGy) and 0.5 logs (3.1 kGy) in MAP1 compared to MAP2.	2 d (Air), 5 d (MAP1 and MAP2)	Grandison and Jennings, 1993
Fresh chicken breasts	3±1°C, Air	Microwaves (2450 MHz) for 10, 20 or 30s	TVCs, *C. jejuni, E. coli*	No significant effect on the numbers of microorganisms were observed	No significant shelf life increase was observed	Göksoy *et al.*, 2000
Non-conventional poultry patties	0–18°C, Air or MAP (40%CO_2:30%O_2: 30%N_2)	Decontaminants: acetic acid (0.5%), Additives:thymol (0–300 ppm) and carvacrol (0–300 ppm)	TVCs, *Pseudomonas* spp., LAB, *Enterobacteriaceae*	Combination of the antimicrobials and low temperature (0–3) were the most effective (1–1.5 log reductions depending on the treatment and the microbial group)	Depended on the different storage conditions and the combination of the treatments	Mastromatteo *et al.*, 2009

(*Continued*)

Table 7.2 Continued

Poultry type	Storage conditions	Treatments	Microbial indices	Treatment effectiveness	Shelf life	Reference
Fresh chicken chilly	0–3°C	Irradiation (1, 2 or 3 kGy)	TVCs, *Staphylococcus* spp., coliforms, *Clostridium* spp., yeasts and moulds	3 kGy was the most effective on TVCs (4–5 log reductions depending on the storage time), *Staphylococcus* spp., coliforms, *Clostridium* spp. were not detected after 1 kGy treatment, yeasts and moulds were not detected at any of the samples	1 w[e] (control), 2–3 w (1,2 kGy), more than 4 w (3kGy)	Kanatt *et al.*, 2005
Chicken legs	3±1°C	Decontaminants (dipping): trisodium phosphate – TSP (12%), acidified sodium chloride –ASC (1200 ppm), citric acid – CA (2%), peroxyacids – PA (200 ppm)	TVCs, *Pseudomonas* spp., LAB, *Enterobacteriaceae*, coliforms, *Micrococcaceae*, Enterococci, *Br. thermosphacta*, yeasts and moulds	Treatments TSP, ASC and CA were the most effective (1–2 log reductions depending on the treatment and the microbial group)	3 d (control and PA), 5 d (TSP, ASC, CA)	del Rio *et al.*, 2007

Minced chicken breast	4°C, VP[f]	High pressure – HP (400 MPa) for 10 min	TVCs, LAB, GNB[g], coliforms	Reduction of ≈4.5 logs of TVCs, >5 logs of GNB (not detected at HP-treated samples), ≥4 logs of LAB (detected after 4 days at HP-treated samples), ≥2.5 logs of coliforms (not detected at HP-treated samples)	Nd[h]	Rivas-Cañedo *et al.*, 2009
Fresh chicken meat	6°C, MAP (65%CO_2: 5%O_2: 30%N_2)	Additives: Combinations of Nisin (0, 500, 1500 IU/g) and EDTA (0, 10, 50 mM)	TVCs, *Pseudomonas* spp., LAB, *Enterobacteriaceae*	Log reductions <1 to >2 depending on the treatment and the microbial group	Longer preservation with treatments 500 IU/g Nisin-50 mM EDTA; 1500 IU/g Nisin-50 EDTA	Economou *et al.*, 2009
Cooked chicken breast fillets	4°C, 8°C or 12°C, VP	High pressure (400–600 MPa) for 1, 2 or 10 min	TVCs, LAB	600 MPa for 10 min combined with storage at 4°C gave the lowest microbial counts	Nd	Patterson *et al.*, 2010

(*Continued*)

Table 7.2 Continued

Poultry type	Storage conditions	Treatments	Microbial indices	Treatment effectiveness	Shelf life	Reference
Semi-cooked coated chicken fillets	4 ±0.5°C, Air or VP (with or without additives)	Additives: Combinations of EDTA-lysozyme solution -EL(1.50% w/w), rosemary oil –R (0.20% v/w) and oregano oil-O (0.20% v/w)	TVCs, *Pseudomonas* spp., LAB, *Enterobacteriaceae*, *Br. thermosphacta*, yeasts and moulds	Treatments VP+EL+R and VP+EL+O were the most effective against Gram-negative, Gram-positive bacteria, and to a lesser extent on yeasts	Treatments VP+EL+R and VP+EL+O produced a shelf-life extension of 7–8 days, as compared to the control samples	Ntzimani *et al.*, 2010

Notes: [a] MAP: modified atmosphere packagin; [b] TVCs: total viable counts; [c] LAB: lactic acid bacteria; [d] d: days; [e] w: weeks; [f] VP: vacuum packaging; [g] GNB: Gram-negative; [h] Nd: no data available.

Morehouse, 2002). It has been reported that below the limit of 10 kGy doses, chemical and nutritional effects on the amino acids profile were not detectable, while the absence of free radicals in meat at low radiation doses prevented a more significant loss of amino acids (Elias, 1985). The reasons for preferential application of irradiation on poultry are summarized by the following: (1) poultry tissues can be easily irradiated (primarily due to their smaller size) and subsequently studied for efficiency assessment of the treatments, (2) the colour scale of red meats is wider than poultry and, thus, discolouration is less likely to occur, (3) establishment of irradiation as a preservation method for eviscerated chicken would significantly benefit the commercial distribution of poultry (Lee *et al*., 1995).

Irradiation of chicken meat with 2.5–3.0 kGy and subsequent storage under elevated carbon dioxide, or nitrogen atmospheres at refrigeration temperatures has been shown to increase the shelf life of poultry (Grant and Patterson, 1991; Kanatt *et al*., 2005) as well as to sensitize pathogens, such as *L. monocytogenes* (Patterson *et al*., 1993; Thayer and Boyd, 1999), *S. typhi* (Lacroix and Chiasson, 2004), *E. coli* (Lacroix and Chiasson, 2004) and *A. hydrophila* (Stecchini *et al*., 1995). Although irradiation is the best method to ensure the microbiological safety of raw meat, its use has been responsible for causing a number of radiolytic meat quality defects. Irradiation of pork and poultry meat was shown to accelerate lipid oxidation (Katusin-Razem *et al*., 1992). Nam and Ahn (2003a) studied the effects of combining aerobic and anaerobic packaging on colour, lipid oxidation and volatile production to establish a modified packaging method to control quality changes in irradiated raw turkey meat. The same authors (Nam and Ahn, 2003b) also studied the effects of double packaging and antioxidant combinations on colour, lipid oxidation and volatiles of irradiated raw turkey breast during refrigerated storage and after cooking. Lipid oxidation is the major problem with aerobically packaged irradiated turkey breast. They concluded that the combination of double packaging and antioxidants was more effective in reducing sulphur volatiles and lipid oxidation, when compared with aerobic packaging. In another study, Kanatt *et al*. (2005) investigated the effect of irradiation processing on the quality of chilled meat products. They reported that irradiation significantly improved the microbiological quality of the products by reducing total bacterial counts (TBC). Furthermore, the decrease in TBC was dose-dependent in all products. In less than 14 days of storage, non-irradiated chilly chicken had counts greater than 6 log cfu/g, while in irradiated samples (3 kGy) TBC did not reach these numbers even after 28 days of storage. Non-irradiated chilly chicken control samples had initial *Staphyloccocus* spp. counts of 2.32 log cfu/g, which increased to 5.12 log cfu/g by 21 days. In all irradiated chilly chicken samples *Staphyloccocus* spp. was not detected during storage. Faecal coliforms were detected in only one batch of non-irradiated control chicken samples. Lipid peroxidation was also measured in terms of thiobarbituric reactive substances (TBARS). Non-irradiated control samples showed lower TBARS values ($p < 0.05$) than irradiated equivalents. The increase in TBARS values was dose-dependent. However, in the case of chilly chicken the increase in TBARS values of irradiated samples was not significant, probably due to the spices used in its preparation that are known to have antioxidant activity. In

the case of chilly chicken it was found that immediately after irradiation the overall sensory scores of irradiated and non-irradiated samples were not significantly ($p < 0.05$) different. Appearance, flavour and texture of irradiated samples were not different from the non-irradiated control equivalents and all samples were organoleptically acceptable.

With regard to the effect of irradiation on the colour of poultry tissue, it has been shown that in the presence of nitrogen, irradiation resulted in the development of bright colourations in the muscle during storage (Patterson, 1988). Maintenance of ideal meat colour during irradiation can be enhanced by many approaches that can be combined, such as pre-slaughter feeding of antioxidants to livestock, optimizing the condition of the meat prior to irradiation, addition of antioxidants, gas atmosphere, packaging and temperature control (Brewer, 2004). Gomes *et al*. (2003) reported that irradiated, mechanically deboned chicken meat showed higher values for *a** (redness) compared with non-irradiated samples from the fourth day under refrigeration. Considering the sensory analysis, colour, psychrotrophic bacterial counts and TBARS measurements as a whole, irradiated samples using doses of 0.0, 3.0 and 4.0 kGy were acceptable under refrigerated storage for 4, 10 and 6 days, respectively.

Millar *et al*. (2000) studied the effect of ionizing radiation on the colour of leg and breast meat of chicken, turkey and goose. The ionizing radiation was shown to have an effect on poultry meat colour, which was dependent on species, muscle type and the surface measured. The common effect in all species muscles was to make the freshly cut surfaces redder. The most striking species difference was the high *a** values in goose breast and their fairly rapid post-irradiation decline in the non-irradiated samples compared to chicken and turkey breast meat. Comparison of leg muscles is more difficult given the different times of post-slaughter irradiation. The main feature when comparing the leg muscles is not the high *a** values associated with them, but the low *b** values and consequently, the low hue angles in goose leg compared to the other two species. These differences between species are considered to reflect differences in haem pigment content, with chicken breast and goose leg possessing the lowest and highest myoglobin content, respectively. It was postulated that the observed red colour was as a result of the formation of a carboxyhaem pigment, carboxymyoglobin and/or carboxy haemoglobin.

7.4.2 High-pressure processing

High-pressure processing (HPP) is a non-thermal technology, which applies pressures up to 1000 MPa for a variable time. It has been reported as a preservation method as it is able to extend the shelf life of food without modifying its sensory properties or nutrient content (Cheftel and Culioli, 1997; Hendrickx *et al*., 1998). The effectiveness of HPP on suppressing the growth and survivability of spoilage and pathogenic microorganisms, as well as inactivating food enzymes, depends on different factors, such as process parameters utilized, strain and growth stage of microorganisms concerned and food matrix to be processed (Rivas-Cañedo *et al*., 2009).

Rivas-Cañedo *et al*. (2009) demonstrated the effect of high-pressure treatment (400 MPa, 10 min at 12°C) on vacuum-packed minced beef and chicken breast, packaged with or without aluminium foil in a multilayered polymeric bag. They found that Gram-negative bacteria in beef and chicken breast were more affected by HPP than Gram-positive bacteria. Additionally, the effect of HPP on volatile compounds differed. The application of HPP clearly decreased the levels of a number of compounds derived from microbial metabolism, such as ethanol and ethyl esters, or from lipid oxidation, such as aldehydes and 1-alcohols, while favouring the formation of diacetyl and related compounds such as acetoin and 2-butanone. The volatile profiles of minced beef and chicken breast subjected to HPP underwent significant changes, most of which can be associated with the death of microorganisms or the inactivation of enzymes related with the formation of flavour compounds. Finally, these authors noted that the packaging material is an important factor in maintaining the volatile profile of treated meats and the use of aluminium shielding was considered to be appropriate, since lower interactions between package, meat and the environment were observed in both HP-treated and untreated meat samples when wrapped in aluminium foil prior to packaging in a polymeric plastic material.

Additionally, Patterson *et al*. (2010) have investigated the microbiological quality of vacuum-packaged cooked minced chicken meat treated using a range of pressures (400–600 MPa) and holding times (1, 2 and 10 min), followed by storage at 4°C, 8°C or 12°C. They found that as the pressure level and hold time increased, the number of surviving microorganisms decreased significantly. At 400 MPa, the counts for a hold time of 10 min became significantly lower only by day 14 of storage, while at 600 MPa, the counts were significantly lower from day 7 onwards. Overall, a treatment of 600 MPa for 10 min resulted in significantly lower microbial counts throughout storage, compared to other treatments. All counts throughout the 35-day storage period were lower when a processing hold time of 10 min was applied. Overall, a treatment of 600 MPa for 10 min, followed by storage at 4°C provided the lowest microbial counts throughout the 35-day storage period. It needs to be mentioned that in this study, the initial numbers of the cooked chicken, with or without pressure treatment, were at or below the level of detection (2.3 log cfu/g).

7.4.3 Treatments with chemical and natural compounds

Many chemicals, including chlorine (Kraft *et al*., 1982), short-chain organic acids (Zeitoun and Debevere, 1992), trisodium phosphate (Ismail *et al*., 2001), herbs (Ismail *et al*., 2001), electrolysed oxidized water (Fabrizio *et al*., 2002; Park *et al*., 2002) and bacteriocins have been recommended for reduction of microbial load on the surface of poultry carcasses. Although their antimicrobial properties are established in literature, only a few have received considerable attention due to their potential practical application (mainly organic acids), whereas even fewer have been evaluated in combination with other preservation methods, such as MAP. In particular, lactic acid, acetic acid, propionic acid, citric acid and sorbates

have been characterized as 'generally recognized as safe' (GRAS: available at http://www.cfsan.fda.gov/~rdb/opa-gras.html), and hence, gained popularity as decontamination agents for poultry carcasses.

It has been reported that selection of optimal concentrations of CO_2 in combination with sorbates (2.5–5.0%) may reduce the required levels of the latter agent to half, in order to succeed in the total inhibition or inactivation of pathogens, such as *Salmonella enteritidis* and *Staphylococcus aureus* on various food ecosystems, including fresh chicken thighs (Elliot and Gray, 1981; Elliot *et al*., 1982; Gray *et al*., 1984). Later, Elliot *et al*. (1985) found that combining potassium sorbate up to 2.5% with 100% CO_2, even at the abuse temperature of 10°C, resulted in doubling the shelf life of chicken thighs compared to the effect of either agent used alone, as well as in comparison to aerobic storage. Zeitoun and Debevere (1992) demonstrated that decontamination of fresh chicken legs with sodium lactate/lactic acid buffering system (pH 3) at concentrations from 2% to 10%, followed by packaging under 90% CO_2:10% O_2 and storage at 6°C, produced a substantial increase in shelf life by 13 days compared to untreated samples (stored under MAP). Based on this study, the same researchers further investigated the impact of the most effective concentration of lactic acid/sodium lactate buffer – that is, 10% (pH 3.0) – on the growth of each member of the microbial association of chicken legs, whereas they also monitored changes in the composition of *Enterobacteriaceae* during storage (Zeitoun *et al*., 1994). It was concluded that the highest microbial inhibition was effected by the combination of lactic acid buffer with MAP storage, compared to either factor used alone or when compared to aerobic storage. The effect of immersion in 10% lactic acid/sodium lactate buffer on the shelf life of MA-packaged chicken was further established in combination with lower CO_2 concentration – that is, 70% CO_2:5% O_2:25%N_2 and storage at 4°C, or 7°C (Sawaya *et al*., 1995b). The investigators monitored the changes in the spoilage association and with respect to two other spoilage indices, namely, the extract release volume (ERV) (Egan *et al*., 1981) and the concentration of free fatty acids (FFA). The combination of MAP with lactic acid buffer extended the shelf life of chicken by greater than 36 and 35 days at 4°C and 7°C, respectively, compared to only 22 and 13 days shelf-life extension for untreated MAP samples, and 5–7 days for aerobically treated packaged samples. Delay in the reduction of ERV and increase in FFA values correlated well with the shelf life of chicken held under different storage conditions. In another study, the pre-cooking injection of chicken legs with sodium lactate and another commercial antimicrobial, in combination with low storage temperature (3.5°C), significantly delayed the growth of Gram-positive endogenous biota and extended the lag phase of *L. monocytogenes* and *Y. enterocolitica* under a MAP mixture of 44% CO_2:56% N_2 (Barakat and Harris, 1999).

A comparative evaluation of lactic acid (1%), acetic acid (1% and 2%) and potassium sorbate (0–2.5%) as decontamination solutions suggested that acetic acid was the most effective compound in extending the shelf life of chicken carcasses, followed by lactic acid and potassium sorbate (Tessi *et al*., 1993). Based on these findings, Jiménez *et al*. (1999) investigated the combined effect of

immersion in 1% acetic acid, with packaging in 70% CO_2:30% N_2 and storage of chicken breasts at 4°C. Acetic acid treatment of chicken breasts maintained the total viable counts and the populations of pseudomonads, lactic acid bacterial and enterobacteria approximately 2.0–2.5 logs lower than untreated samples. Moreover, sensory evaluation showed that decontaminated samples maintained a pleasant, but slightly acidic smell until the end of storage (21 days) at 4°C, in contrast to non-decontaminated samples that developed strong off-odours early in the same storage period (Jiménez *et al*., 1999).

Natural compounds, such as nisin, lysozyme, herbs, spices and essential oils have been investigated to replace chemical preservatives and, consequently, promote 'green label' products. With regard to bacteriocins, nisin is also an antimicrobial that has been successfully combined with MAP to extend the shelf life of poultry products (Cosby *et al*., 1999). In particular, addition of nisin at levels higher than 50 μg/mL, in combination with 20–50 mM of EDTA, maintained the total viable counts of broiler carcass drummettes packaged in 20% CO_2:80% O_2 approximately 2.0 log cfu/g lower than untreated-MAP, or treated-air packaged samples, after 18 days of storage at 4°C (Cosby *et al*., 1999). Likewise, the combination of sakakin K with MAP exerted strong antilisterial effects on chicken breasts (Hugas *et al*., 1998). Economou *et al*. (2009) have also studied the application effect of nisin and EDTA treatments on the shelf life of fresh chicken meat stored under MAP (65% CO_2:30% N_2 5% O_2) at 4°C. Chicken meat was subjected to the following antimicrobial combinations with Nisin–EDTA treatments (added post-production to chicken samples), including: N1 (no nisin–EDTA added; control sample), N2 (500 IU/g; no EDTA added), N3 (1500 IU/g; no EDTA added), N4 (500 IU/g-10 mM EDTA), N5 (1500 IU/g-10 EDTA), N6 (500 IU/g-50 mM EDTA), N7 (1500 IU/g-50 EDTA), N8 (10 mM EDTA; no nisin added) and N9 (50 mM EDTA; no nisin added). They have shown that the limit for sensory acceptability was reached for (cooked) chicken samples on days 10–11 (N1 samples), day 12 (N2 treated samples), days 13–14 (N3 and N4- treated samples), days 17–18 (N5 treated samples), day 20 (N7 treated samples) and on day 24 (N6 treated samples). The use of MAP, in combination with nisin–EDTA antimicrobial treatments, resulted in a shelf-life extension of fresh chicken by approximately 1–2 days (N2), 3–4 days (N3 and N4), 7–8 days (N5), 9–10 days (N7) and 13–14 days (N6). Overall, acceptability (odour data) of MAP-treated chicken samples under treatments N1 and N2 correlated rather well with microbiological shelf life (TVC data), with the exception of treatments N3–N5, N6 and N7, where a difference in shelf-life of 1–2, 2–3 and 4 days was recorded, respectively. Chicken meat was better preserved under treatments N6 and N7, maintaining acceptable odour attributes, even up to 24 and 20 days of storage, respectively.

Chouliara *et al*. (2007) studied the combined effect of oregano essential oil (0.1% and 1%, w/w) and MAP (30% CO_2:70% N_2 and 70% CO_2:30% N_2) on the shelf-life extension of fresh chicken meat stored at 4°C. Based primarily on sensory data, the shelf life of aerobically packaged fresh chicken meat was around 5 days. Addition of 0.1% oregano essential oil extended the product's shelf life by

around 3–4 days, while MAP extended shelf life by 2–3 days. The combination of both MAP and 0.1% oregano essential oil extended shelf life by around 5–6 days. Microbial populations were reduced by 1–5 log cfu/g for a given sampling day, with the most pronounced effect being achieved by the combination of MAP and oregano essential oil. Colour values were not considerably affected by oregano oil or by MAP, but sensory analysis showed that oregano oil at a concentration of 1% imparted a very strong taste to the product.

Mastromatteo *et al*. (2009) have studied the combined effect of thymol (0–300 ppm), carvacrol (0–300 ppm) and temperature (0–18°C) on the quality of non-conventional poultry patties (a mix of ostrich, chicken and turkey meats) packaged in air and MAP (40% CO_2:30% O_2:30% N_2). In order to reduce initial cell load, before the addition of the above antimicrobial agents, they applied a decontamination treatment by dipping in 0.5% acetic acid solution for 5 min. The obtained results showed that the initial cell load for the total viable counts was 7.04 log cfu/g for chicken meat, 4.97 log cfu/g for turkey meat and 5.12 log cfu/g for ostrich meat. After acid treatment the cell load was reduced by 1.37, 2.25 and 0.41 log cfu/g for chicken, turkey and ostrich meat, respectively. Regarding the active compounds, it was stated that thymol was less effective than carvacrol, while the combination of MAP and thymol had an additive effect on TVC cell load. Increasing amounts of thymol and carvacrol decreased the final cell load of lactic acid bacteria for patties packaged in air. However, for MAP packed samples, the increase in the amount of carvacrol resulted in lower final cell population. The final cell load of *Enterobacteriaceae* decreased with increasing amounts of thymol and carvacrol for both patties packaged in air and MAP, with the effect of active compounds on *Pseudomonas* spp. providing a similar trend. Overall, it was demonstrated that the quality of poultry patties was influenced by storage temperature and the presence of essential oils. For the patties mixed with antimicrobial compounds and stored at low temperatures (0–3°C) a reduction in the cell load of about 1.0– 1.5 log cfu/g was observed. The log reduction was lower at the end of the storage period and decreased with increasing temperature. Most notably, acetic acid treatment did not affect the appearance or odour of the patties, while colour parameters were not influenced by the addition of the active compounds.

In another study, Ntzimani *et al*. (2010) investigated the effect of natural antimicrobial treatments (EDTA, lysozyme, rosemary and oregano oil) and their combinations on the shelf life of semi-cooked coated chicken meat stored in vacuum packages at 4°C. Of the antimicrobial combination treatments examined in this study, the use of treatments EDTA–lysozyme–rosemary oil (VP + EL + R) and EDTA–lysozyme–oregano oil (VP + EL + O) were the most effective against the growth of Gram-negative and Gram-positive bacteria, and to a lesser extent on yeasts. The presence of rosemary oil (0.2% v/w) in cooked VP + EL + R and VP + EL + O samples produced a distinct but acceptable pleasant odour and taste which was well received by the panellists. The application of oregano oil in cooked chicken samples was not as pleasant compared to rosemary oil. Based on both microbiological (TVC data) and sensory analyses, treatments VP + EL + R and VP + EL + O produced a shelf-life extension of 7–8 days compared to control samples.

Several compounds have been found to be capable of retarding or preventing autoxidation processes, thus extending the shelf life of fresh and processed poultry products. However, such compounds cannot reverse the oxidation process or suppress the development of hydrolytic rancidity. Antioxidants have been mainly investigated for effectiveness on turkey meat, probably due to the low content of natural tocopherols and the high oxidative potential of this type of poultry (Boselli *et al*., 2005; Mielnik *et al*., 2003). Mielnik *et al*. (2003) investigated the effect of commercial rosemary antioxidants on oxidative stability of mechanically deboned turkey meat compared with Trolox C (a synthetic water-soluble α-tocopherol analogue) and ascorbic acid (vitamin C). Supplementation of the turkey meat with antioxidants could be an alternative method to prevent oxidative degradation of the meat during frozen storage when vacuum packaging is not feasible. Trolox C possessed the greatest antioxidative activity as reflected by the lowest values of TBARS and volatile compounds. Ascorbic acid was less efficient than Trolox C and Biolox HT-W (rosemary), but was more potent than most rosemary extracts in suppressing lipid oxidation, especially during long-term frozen storage. This investigation showed that oxidative stability of mechanically deboned turkey meat, stored in packages with free-oxygen access, was improved by adding antioxidants.

Batifoulier *et al*. (2002) reported that supplementation of turkeys with α-tocopheryl acetate increased vitamin E content of microsomal membranes and had also a protective effect on lipid oxidation. Supplementation with vitamin E significantly protected free thiols from oxidation but had only a small effect on carbonyl group formation. Beltran *et al*. (2003) reported that salt and mechanical processing had a greater pro-oxidant effect on pressurized samples, while rosemary extract had an antioxidant effect, EDTA strongly inhibited oxidation and hexamethaphosphate also showed antioxidant potential. Oregano supplements to chicken meat protected against stress-induced increases in TBARS in different muscles, with no effect on water-holding capacity (Young *et al*., 2003).

7.4.4 Active and intelligent packaging

Active packaging

Active packaging refers to the incorporation of certain additives into packaging systems (whether loose within the pack, attached to the inner layer of packaging materials or incorporated within the packaging materials themselves) with the aim of maintaining or extending product quality and shelf life (Kerry *et al*., 2006). Active packaging functions and technologies include moisture control, O_2 or CO_2 scavengers or emitters, odour controllers, flavour enhancement, ethylene removal, antimicrobial agents and microwave susceptors, in addition to indicators of specific compounds and temperature control packaging (Kerry *et al*., 2006; Zhou *et al*., 2010) (see Chapter 20).

Briefly, active packaging systems can be divided into the following categories (Coma, 2008; Kerry *et al*., 2006):

- packaging produced by adding a sachet into the package (O_2 scavengers, CO_2 generators, ClO_2 generators)
- packaging with bioactive agents incorporated into the packaging film (O_2 scavenging films, silver ions, triclosan, bacteriocins, spices, essential oils, enzymes and other additives)
- coating of the packaging surface with a matrix that acts as a carrier for the bioactive agent
- utilization of inherently antimicrobial polymers exhibiting film-forming properties, such as cationic amino-polysaccharides or polymers which are chemically modified to produce bioactive properties
- utilization of bioactive edible coatings directly applied onto the food.

Regarding poultry meat products, Sante *et al*. (1993) evaluated a series of packaging atmospheres, including air, vacuum, 100% O_2, 100% N_2, 100% CO_2 plus O_2 scavenger and 25% CO_2:9% N_2:66% O_2 for their potential to increase shelf life and maintain colour stability of chicken breast muscle. Packaging under vacuum or 100% CO_2 with O_2 scavengers significantly improved instrumental colour stability of breast muscle, stored at 3–4°C for 21 days, compared to all other packaging systems assessed. Aerobically stored samples showed the lowest ability to sustain visual appearance. The increase in shelf life due to retardation of microbial growth coincided well with the effectiveness of the above two atmospheres to enhance colour stability. Moreover, Ellis *et al*. (2006) have found that fast- and slow-release ClO_2 sachets reduced total plate counts by 1.0–1.5 log cfu/g in packages of chicken breast meat after 15 days. In this case, no off-odour was detected by sensory panellists, but the colour of chicken adjacent to the ClO_2 was adversely affected. Vermeiren *et al*. (2002) have investigated the feasibility of a low-density polyethylene (LDPE) film containing triclosan to inhibit microbial growth on food surfaces. However, triclosan, incorporated at 500 and 1000 mg per kg of polymer in LDPE films, exhibited antimicrobial activity against pathogenic bacteria in agar diffusion assay, did not effectively reduce microbial growth on chicken breast meat in VP at 7°C.

Intelligent packaging

Intelligent packaging (also more loosely described as smart packaging) is packaging that, in some way, senses some properties of the food it encloses, or the environment in which it is kept, and which is able to inform the manufacturer, retailer and consumer of the state of these properties (Kerry *et al*., 2006). Although distinctly different from the concept of active packaging, features of intelligent packaging can be used to check the effectiveness and integrity of active packaging systems (Hutton, 2003). Smart packaging devices, may include different types of sensors – gas sensors, fluorescence-based oxygen sensors, biosensors – or indicators – integrity indicators, freshness indicators, time–temperature indicators (Kerry *et al*., 2006) (see Chapter 20).

The latest type of indicators, like second-generation time–temperature indicators (TTIs), can be used to help control the realization of an unbroken cold-chain,

since the indicator shelf life is dependent on the time–temperature history of the package throughout the whole distribution chain. TTIs attached to the package surface integrate the cumulative time–temperature history of the product starting from the moment of indicator activation, which is visualized as a colour change or colour movement. Smolander *et al*. (2004) studied the applicability of TTIs for quality control of modified atmosphere packaged (80% CO_2:20% N_2) broiler chicken cuts using various constant and variable temperature conditions. According to obtained results, TTIs appear to be useful tools for the evaluation of the quality of broiler chicken cuts. The rate of colour change of most TTIs generally is correlated well with aerobic plate counts, whereas some TTIs had good correlation with *Enterobacteriaceae* counts and the odour of broiler chicken cuts. Some indicators correlated well with both aerobic mesophilic and psychrotrophic counts as well as with *Enterobacteriaceae* counts. Even if the indicators were selected based on their market availability it was possible to find indicators with endpoints matching the microbiological and sensory shelf life of broiler chicken cuts. During *in situ* implementation of TTIs, the shelf life of the indicator can be tailored to match that of the product's shelf life.

Further studies are needed to investigate the application of TTIs and/or other indicators and sensors in monitoring the quality of fresh and processed poultry.

7.5 Chemical indicators for assessing the quality of fresh and processed poultry

To date the assessment of meat quality and safety is primarily based on sensory and retrospective microbiological analyses (Nychas *et al*., 2008). Sensory analysis is an important and common method used to evaluate the quality of food commodities since the consumer is the ultimate judge of product quality (Lee and O'Mahony, 2004). However, this approach has certain disadvantages as it predominantly relies on highly trained taste panels, a procedural approach which makes it costly and unattractive for daily analysis. On the other hand, microbiological analyses are laborious, time-consuming and costly for retrospective analysis, as well as destructive to products analysed, requiring in most cases a complex process of sample preparation, ultimately failing to deliver the 'immediate answer required' (McMeekin *et al*., 2007).

Apart from the classical microbiological methods, quality and safety of packaged products may also be assessed using specific indicators, which correlate microbial growth to changes in physical and physicochemical properties of packaged foods (Skandamis *et al*., 2005). Monitoring the changes that may occur in the meat substrate during storage can provide useful qualitative and quantitative information on the degree of spoilage and thus indicate the quality and the remaining shelf life of a given poultry product (see Table 7.3). This evaluation could be provided using several analytical tools such as high performance liquid

Table 7.3 Chemical indicators for assessing the quality of fresh and processed poultry

Poultry type	Storage conditions and treatments	Types of analysis	Shelf-life indicators	Concluding remarks	Reference
Chicken breast fillets or thigh meat	3°C or 10°C, VP[a], MAP1[b] (100% CO_2), MAP2 (100% N_2) or MAP3 (20% CO_2:80% O_2)	Microbiological glucose, L-lactate, acetic acid, ammonia	Glucose, L-lactate, acetic acid, ammonia	Glucose and lactate were initially higher in breast than in thigh muscles and both decreased during storage at all conditions. In MAP1 and 3, L-lactate acid was always higher than in MAP2 or VP. In MAP3, L-lactate decreased more rapidly than in MAP1 or MAP2. Acetic acid and ammonia increased during storage at all conditions. The increase of ammonia was always lower in MAP1 or 3.	Kakouri and Nychas, 1994
Fresh poultry fillets	3°C or 10°C, Air, VP or MAP (100% CO_2)	Microbiological enzymatic (glucose, L-lactate) HPLC [c] (water-soluble proteins)	Glucose, L-lactate, water-soluble proteins	Glucose and L-lactate decreased progressively almost in all samples, and this decrease was always delayed in MAP. The profile of water-soluble proteins of samples stored under Air or VP/MAP conditions was significantly different, while the proteolysis was evident regardless of the microbial level.	Nychas and Tassou, 1997

Raw chicken legs	Range of temperatures; 1–7°C, range of MAP (O_2: 1, 2, or 4%, CO_2:20, 50, or 80%, N_2: balancing gas)	Microbiological sensory GC-MS [d]	Butene, ethanol, acetone, pentane, dimethylsulphide, carbon disulphide and dimethyl disulphide	Ethanol and dimethyl sulphide were the compounds detected at the highest levels and were strongly affected by storage time and temperature. Butane, pentane and acetone were detected at the lowest levels, but influenced mostly by CO_2 and O_2 concentration.	Eilamo *et al.*, 1998
Chicken meat breast and thigh muscle, chicken-based meat products	4 ±1°C (Storage of chicken meat breast and thigh muscle)	Moisture content, sulphydric gas, TVB[e] HPLC, LC[f] (bioactive amines)	Quality index based on ratio of spd[g]/spr[h] levels was introduced appropriate for the evaluation of chicken meat quality. Limits suggested: values < 0.50 = fresh product, 0.50 ≤ values ≤ 0.70 = a product for immediate consumption, values ≥ 0.70 = a product in an advanced stage of deterioration	spr and spd were detected in both types of meat. spr was the prevalent amine (70% of total). Low levels of hst[i] were also detected in thighs. During storage there was a decrease in spr, spd levels remained constant, and ptr, cdv[j], hst and trm[k] were formed. The amines were detected at a shorter period of storage in thigh compared to breast. At 15 days, higher levels of amines were found in breast compared to thigh. For most of the chicken-based meat products, there was a prevalence of spd over spr.	Silva and Glória, 2002
Minced chicken breast	Room temperature, Air	Microbiological FT-IR[l]	Monitoring of the metabolic fingerprint/ biochemical changes occurring in the meat substrate	Quantitative interpretation of FT-IR spectra allowed accurate estimates of bacterial loads (total viable counts) to be calculated.	Ellis *et al.*, 2002

(*Continued*)

Table 7.3 Continued

Poultry type	Storage conditions and treatments	Types of analysis	Shelf-life indicators	Concluding remarks	Reference
Broiler chicken cuts	10°C, Air (0.03%CO_2: 20.9%O_2:79%N_2), MAP1 (20%CO_2: 80%O_2), MAP2 (20%CO_2:10%O_2: 70%N_2)	Microbiological sensory GC,GC-MS HPLC	Volatile compounds (hydrogen sulphide, dimethyl sulphide, sulphur dioxide) Biogenic amines (trp[m], srt[n], ptr[o], cdv, hst, trm) TTIs[p]	The microbiological analysis and the TTIs gave the same result and were more critical than either the quality-indicating metabolites or the sensory evaluation.	Vainionpää *et al.*, 2004
Broiler chicken cuts	Different temperature profiles: 3°C to 22°C, MAP (80% CO_2: 20% N_2)	Microbiological HPLC (biogenic amines)	trm, cdv and ptr were shown to be promising indicators	The temperature significantly affected the formation rate of trm, which seemed to be highly consistent with the increase in the aerobic mesophilic viable count. The levels of trm increased during storage and the rate of formation was higher at temperatures above 6.1°C. The levels of ptr and cdv increased during storage, but they were not formed below 6.1°C.	Rokka *et al.*, 2004
Minced turkey thighs	4°C, MAP (30% CO_2: 70% O_2), VP	Sensorycolour and gas, TBARS[q] Fluorescence spectroscopy and imaging GC (fatty acids/fat content)	Fluorescent porphyrins, most likely protoporphyrin and Zn porphyrin	With fluorescence spectroscopy and imaging, it was possible to measure the extent and distribution of lipid oxidation in minced turkey. The spectra showed clear differences between MAP and VP.	Veberg *et al.*, 2005

Broiler chicken cuts	Different temperature regimes (B1: mean + 6.1°C, B2: mean + 2.9°C, B3: mean + 7.4°C), MAP (80% CO_2:20% N_2)	Microbiological Sensory e-nose[r] GC, GC-MS	Dimethyl sulphide, hydrogen sulphide, ethanol and acetone	In B2 the increase of dimethyl sulphide was slower compared to B1 and B3. The amount of hydrogen sulphide in B2 remained at a low level, but increased rapidly in B3. Pentane was also detected in B3 but not in the fresh reference or in B2.E-nose could clearly distinguish broiler chicken packages with deteriorated quality from fresh packages. The counts of *Enterobacteriaceae* and hydrogen sulphide producing bacteria were most consistent with the electronic-nose results.	Rajamäki *et al.*, 2006
Fresh chicken breast	4°C, Air or MAP (30%CO_2:70% N_2)	Microbiological Sensory HPLC (Biogenic amines)	The biogenic amines index (sum of ptr, cdv and trm), may be proposed as a quality index of MAP and aerobically packaged fresh chicken meat. Proposed values: 96–101 mg/kg	Ptr and cdv were found to be the main BAs formed. Ptr and cdv increased, spr and spd decreased in both Air and MAP, with higher levels of the ptr being observed in Air. trm values were low showing an increase, with higher values observed in MAP. hst was only observed after day 11 of storage, with higher values noted in MAP.	Balamatsia *et al.*, 2006

(*Continued*)

Table 7.3 Continued

Poultry type	Storage conditions and treatments	Types of analysis	Shelf-life indicators	Concluding remarks	Reference
Precooked chicken breast	4°C, Air or MAP (30%CO_2:70% N_2)	Microbiological sensory HPLC (biogenic amines)	Values of 14–19 mg/kg for ptr and 1.4 mg/kg for trm may be considered as the limit for spoilage initiation of precooked chicken meat	Spr and spd were found to be the main BAs formed. spr and spd followed a mixed trend and spd values were higher compared to spr. ptr and cdv increased under both Air and MAP, with higher levels of ptr observed under Air and higher levels of cdv observed under MAP. trm levels increased, with higher values being under Air. hst formation was observed under aerobic or MAP and higher hst values were noted under MAP.	Patsias *et al.*, 2006b
Minced chicken breast	4°C, VP, high pressure – HP (400 MPa) for 10 min	Microbiological GC-MS	Hydrocarbons, aldehydes, ketones, alcohols, esters, benzene compounds and others	The abundance of some aldehydes, alcohols, and ethyl esters was significantly lower after pressurization, and that of most ketones and other alcohols significantly higher in HP-treated samples. HP decreased the levels of some compounds coming from microbial metabolism or from lipid oxidation, such as ethanol, ethyl esters, aldehydes and 1-alcohols, while it favoured the formation of diacetyl, acetoin and 2-butanone.	Rivas-Cañedo *et al.*, 2009

Notes: [a] VP: vacuum packaging; [b]MAP: modified atmosphere packaging; [c]HPLC: high performance liquid chromatography; [d]GC-MS: gas chromatography-mass spectroscopy; [e]TVB: total volatile bases; [f]LC: liquid chromatography; [g] spd: spermidine; [h]spr: spermine; [i]hst: histamine; [j]cdv: cadaverine; [k]trm: tyramine; [l] FT-IR: Fourier transform infrared spectroscopy; [m]trp: tryptamine; [n]srt: serotonine; [o]ptr: putrescine; [p]TTIs: time–temperature intigators; [q]TBARS: thiobarbituric reactive substances; [r]e-nose: electronic nose.

chromatography (HPLC), electronic nose and gas chromatography and fluorescence, Raman, FT-IR and NIR spectroscopy. Several studies on analytical techniques and potential shelf-life indicators and their applicability on monitoring the quality of fresh and processed poultry are listed below.

Kakouri and Nychas (1994) monitored the changes in microbial metabolites over time and their possible role as indicators of incipient spoilage in skinless poultry breast fillets or thigh meat stored under MAP with CO_2 (200%), nitrogen (100%), CO_2/O_2 (20%:80%) or vacuum pack at 3°C and 10°C. They reported that when chicken fillets were stored either aerobically or under MAP the concentrations of glucose and L-lactate levels present in the meat were affected. Specifically, the concentrations of glucose and lactate were higher in breast (normal white meat) than in thigh (dark firm dry or red type of meat) on the day the samples were obtained from the processing plant. The changes in the concentration of both substrates was more pronounced at 10°C than at 3°C, while after 13 days of storage under CO_2 the amount of L-lactic acid was always higher compared with samples stored in either nitrogen or under vacuum for both types of poultry muscle stored at 3°C. In samples stored under 20%:80% carbon dioxide:oxygen the L-lactate concentration decreased more rapidly than under carbon dioxide and nitrogen atmospheres or vacuum packing at both temperatures. The decrease of L-lactate in all samples was accompanied by an increase in the concentration of acetic acid and ammonia. The extent of this increase was higher at 10°C than at 3°C. However, it was noted that the increase of ammonia was always lower in samples of breast and thigh muscles stored under carbon dioxide (100%) at 3°C or 10°C.

Nychas and Tassou (1997) also noted that the concentration of glucose decreased progressively for almost in all samples stored under aerobic, vacuum and 100% CO_2 packaging conditions during storage at 3°C and 10°C. This decrease primarily occurred towards the end of storage and was greater at higher storage temperatures. Similar observations were made for L-lactate. Indeed, the final L-lactate content in samples stored under aerobic conditions was significantly lower than those packed under vacuum or 100% CO_2. It needs to be stated that the decrease of these two low molecular weight compounds (glucose and lactate) was always delayed in samples flushed with 100% CO_2. In this study it was also reported that there was a significant difference in the profile of water-soluble proteins of poultry muscles stored under aerobic or VP/MAP conditions, while proteolysis was evident regardless of microbial level.

There are several studies on the possible use of biogenic amines (BA) in meat quality determination. The formation of BA is primarily a consequence of the enzymatic decarboxylation of specific amino acids due to microbial enzyme activity. Numerous bacteria have been reported to possess amino acid-decarboxylase activity (Geornaras *et al*., 1995; Maijala, 1993; Straub *et al*., 1995). The determination of BA is important, not only because of their toxicity, but also because of their potential use as spoilage indicators since they impart putrid odours and off-flavours that can affect food acceptance (Geornaras *et al*., 1995; Halász *et al*., 1994; Ruiz-Capillas and Jiménez-Colmenero, 2004).

Silva and Glória (2002) determined the levels of bioactive amines in chicken meat breast and thigh muscle stored at 4°C for up to 15 days. However, the type of packaging system used in this study was not described. Immediately after slaughter, spermine and spermidine were detected in both types of meat. Spermine was the prevalent amine (70% of total), whereas low levels of histamine were also detected in thighs. During storage there was a decrease in spermine, while spermidine levels remained constant, and putrescine, cadaverine, histamine and tyramine were formed. The amines were detected at a shorter period of storage in thigh compared to breast. At 15 days, higher levels of amines were found in breast compared to thigh. A quality index based on the ratio of the polyamines spermidine/spermine was considered appropriate for the evaluation of chicken meat quality. The limits suggested that values below 0.50 would indicate a fresh product, between 0.50 and 0.70, a product for immediate consumption, and above 0.70, a product in an advanced stage of deterioration. Chicken-based meat products (mortadella, frankfurters, sausage, meatballs, hamburger and nuggets) were also analysed for bioactive amines. There was a prevalence of spermidine over spermine for most of the products, suggesting the incorporation of significant amounts of vegetable protein in the formulations. Nuggets were the only products with amine profiles similar to fresh chicken meat. Sausage contained higher levels of BA than the other products.

The formation of BA (tryptamine, phenylethylamine, putrescine, cadaverine, histamine, serotonine, tyramine, spermidine and spermine) was also studied in broiler chicken cuts stored under MAP (80% CO_2:20% N_2), and several temperature profiles from 3°C to 22°C, representing the extreme temperature abuse encountered during transportation from the retailer to the consumer's refrigerator (Rokka *et al*., 2004). The amines tyramine, cadaverine and putrescine were shown to be promising indicators for both storage time and temperature, as well as for the microbiological quality, of MA-packed broiler chicken cuts. Specifically, the storage temperature significantly affected the formation rate of tyramine which seemed to be highly consistent with the increase in viable aerobic mesophilic counts. The levels of tyramine increased after 5 days of storage if storage temperature was above 6.1°C. Tyramine was also formed during storage at low temperatures, although the rate of formation was lower than at 6.1°C. The concentration of putrescine and cadaverine increased after 7 and 9 days, respectively. Putrescine and cadaverine were not formed below 6.1°C.

Recently, in two similar studies pertaining to fresh chicken breast (Balamatsia *et al*., 2006) and precooked chicken breast (Patsias *et al*., 2006b), six BA, namely, putrescine, cadaverine, spermine, spermidine, tyramine and histamine, have been detected and quantified, whereas agmatine, tryptamine and b-phenylethylamine were not detected in any of the chicken samples stored aerobically or under MAP (30% CO_2:70% N_2) at 4°C. In both studies the levels of putrescine were found to increase for both aerobic and MA-packaged samples, with higher levels of diamine being observed in aerobically stored samples. Cadaverine also showed an increasing trend for both storage conditions, with MA-packaged precooked chicken samples exhibiting higher concentrations of this diamine compared to

aerobically packaged samples. In the case of the fresh chicken samples, values of spermine and spermidine both showed a decreasing trend. Spermine values were similar, irrespective of packaging conditions, and higher than those of spermidine, whereas for spermidine, lower values were obtained for samples stored in air than under MAP. In the case of processed (precooked) chicken samples, both spermine and spermidine production followed a mixed trend (increase followed by a decrease) and spermidine values were found in higher amounts compared to spermine. The diamines, putrescine and cadaverine, were determined to be the main BA formed in fresh chicken samples, whereas in processed samples, concentrations of spermine and spermidine were generally significantly higher as compared to those obtained for putrescine and cadaverine, irrespective of packaging conditions.

Generally, for fresh chicken samples, tyramine values were low, showing an increasing trend throughout storage and presenting higher values in samples packaged under MAP as compared to those held in air. In processed samples, tyramine levels increased, with higher values being observed progressively for samples packaged aerobically as compared to those in MAP. Finally, formation of histamine in fresh chicken meat stored either aerobically or under MAP was only observed after day 11 of refrigerated storage, with higher values noted for samples stored under MAP. In precooked chicken, formation of histamine in aerobic or MAP packaged samples was observed from the beginning of refrigerated storage and higher histamine values were noted for samples packaged under MAP. Based on sensory and microbiological analyses, and also taking into account a BA index (BAI, sum of putrescine, cadaverine and tyramine), BAI values between 96 and 101 mg/kg may be proposed as a quality index for MAP and aerobically packaged fresh chicken meat. Moreover, based on sensory data, after around 8 days of aerobic storage and 12 days under MAP (time to reach initial decomposition stage), the putrescine and tyramine content of chicken samples were around 14–19 and 1.4 mg/kg values that may be considered as the limit for spoilage initiation of precooked chicken meat (respective TVC values for both aerobically and MA-packaged chicken meat were approximately 6.5 log cfu/g).

In another study, Balamatsia *et al*. (2007) evaluated the possible role of volatile amines as indicator(s) of poultry meat spoilage. Fresh chicken meat (breast fillet) was packaged in air, under vacuum and in modified atmospheres of 30% CO_2:65% N_2:5% O_2 and 65% CO_2:30% N_2:5% O_2 and stored for 15 days at 4°C. Based on sensory (taste) analysis and with regard to chicken spoilage and freshness, trimethylamine nitrogen (TMA-N) and total volatile basic nitrogen (TVB-N) values of approximately 10.0 and 40 mg N/100 g, respectively, were proposed by the authors as the upper limit values for spoilage initiation of fresh chicken meat stored aerobically. Final TMA-N and TVB-N values for all chicken samples packaged under MAP were significantly lower ($p < 0.05$) than those packaged in air or under vacuum. Interestingly, the 65% CO_2:30% N_2:5% O_2 gas mixture sample did not reach these values throughout the 15-day storage period. The formation of volatile amines during the chilled storage of chicken meat, under the packaging conditions examined in this study, seemed to be in agreement with the increase in

microbiological counts (TVC) and sensory taste scores, with the exception of that for the 65% CO_2:30% N_2:5% O_2 gas mixture.

Eilamo *et al*. (1998) correlated the increase in volatile compounds with microbiological changes occurring during storage of chicken legs at temperatures ranging from 1°C to 7°C and in range of MAP (O_2:1%, 2% or 4%, CO_2:20%, 50% or 80%, N_2: balancing gas). The volatile compounds were monitored using dynamic headspace gas chromatography-mass spectrometry (HS/GC-MS). The main volatiles identified were: butane, ethanol, acetone, pentane, dimethylsulphide, carbon disulphide and dimethyl disulphide. Ethanol and dimethyl sulphide were the compounds detected at the highest levels and were strongly affected by storage time and temperature. Conversely, butane, pentane and acetone were detected at the lowest levels, but influenced mostly by CO_2 and O_2 concentration, which in turn were highly dependent on film permeability.

Rajamäki *et al*. (2006) investigated the applicability of an electronic nose for the quality control of MA-packaged broiler chicken cuts held at different temperature regimes. The concentration of dimethyl sulphide increased as a function of storage time at all storage temperatures. However, in packages stored in temperature regime B2 (mean 2.9°C), the increase of dimethyl sulphide was slower compared to temperature regimes B1 (mean 6.1°C) and B3 (mean 7.4°C). The amount of hydrogen sulphide in packages stored in the acceptable temperature regime B2 remained at a low level for the entire storage period of 12 days. In contrast, the amount of hydrogen sulphide increased rapidly after 9 days in packages stored in the abusive temperature regime B3. Based on ion chromatograms obtained by HS/GC-MS, dimethyl disulphide (as well as dimethyl sulphide reported above) appeared to be temperature-dependent, as its amount was higher in packages stored in the abusive temperature (B3) than in the acceptable temperature regime (B2). Pentane was also detected in the packages stored in the abusive temperature B3 for 9 days, but not in the fresh reference or in the packages stored in the temperature regime B2 for 9 days. The amounts of ethanol and acetone did not increase notably during the first 9 days of storage. The e-nose could clearly distinguish broiler chicken packages with deteriorated quality from fresh packages, either earlier, or at the same time that sensory quality deteriorated. Concerning the microbiological quality, the presence and numbers of *Enterobacteriaceae* and hydrogen sulphide-producing bacteria were most consistent with electronic nose results, indicating that the e-nose was capable of detecting early signals of spoilage in MA-packed poultry meat.

The effect of HPP on the volatile profile of minced beef and chicken breast was also investigated (Rivas-Cañedo *et al*., 2009). The analysis of the volatile fraction was carried out using HS/GC-MS. HPP had a significant effect on nine volatile compounds in chicken breast. The abundance of some aldehydes (ethanal, octanal, 2-methylpropanal), alcohols (ethanol, 1-propanol, 1-hexanol) and ethyl esters, was significantly lower after pressurization, whereas most ketones (2-propanone, 2-butanone, 2, 3-butanedione) and other alcohols (2-propanol, 2-ethylhexanol and 3-methyl-3-buten-1-ol) were significantly higher in HP-treated samples. Moreover, the effect of HPP on volatile compounds differed. It clearly

decreased the levels of a number of compounds derived from microbial metabolism, such as ethanol and ethyl esters, or from lipid oxidation, such as aldehydes and 1-alcohols, while favouring the formation of diacetyl and related compounds such as acetoin and 2-butanone.

Fourier transform infrared (FT-IR) spectroscopy has been exploited to measure biochemical changes within the meat substrate, enhancing and accelerating the detection of microbial spoilage of minced chicken breast stored at room temperature for 24 h (Ellis *et al*., 2002). Quantitative interpretation of FT-IR spectra was possible using partial least squares regression and allowed accurate estimates of bacterial loads (total viable counts) to be calculated directly from the meat surface in 60 sec.

Veberg *et al*. (2006) investigated the applicability of fluorescence spectroscopy in monitoring the lipid oxidation in minced turkey and pork meat. With fluorescence spectroscopy and imaging it was possible to measure the extent and distribution of lipid oxidation in minced turkey and pork meat. For turkey patties, the spectra showed clear differences between meat exposed to O_2 and meat packed in vacuum, whereas it was feasible to distinguish between turkey patties stored in high O_2 and vacuum already after 7 days. During the storage period, notable lipid oxidation occurred in the turkey patties stored in a high O_2 atmosphere.

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8

Advances in bulk packaging for the transport of fresh fish

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Abstract: This chapter deals with the effective, quality preservative and environmentally sustainable bulk packaging of raw fish products. An introduction to the packaging of highly perishable seafood is presented. The statuses, challenges and advances of bulk packaging are given and discussed as are potential future trends. For example, alternative renewable materials can be used for packaging in addition to the expanded polystyrene (EPS), which currently dominates the market. The use of more efficient methods of packaging, such as only packaging the edible parts of the products – for example, fillets or fillet parts rather than the whole fish – would significantly reduce greenhouse gas (GHG) emissions and energy consumption, as well as reducing distribution costs, without reducing the quality of the fish.

Key words: alternative bulk packaging solutions, modified atmosphere packaging (MAP), sustainable packaging, temperature during distribution, CO_2 emitter.

8.1 Introduction

Fresh or thawed fish is highly perishable and must be correctly handled in order to preserve its quality from the time of processing and packaging until it reaches the consumer. There are several important ante and postmortem factors which affect quality, including freshness and bacterial counts, odour, flavour, juiciness, texture and colour. It is important to ensure environmentally responsible and cost-effective preservation of the fish product. Packaging is, along with external conditions such as temperature and hygiene during processing and storage, the most significant factor in achieving this. Although quality preservation is the chief objective of packaging, many other factors are also essential. All packaging used should be optimalized

with regard to environmental and economic performance throughout the entire value chain. A packaging solution should also have sufficient shock strength and be user-friendly and easy to handle for processors, transporters, handlers, retailers and consumers. Furthermore, the packaging must carry adequate information to ensure proper identification and handling of the product, and this information must be readable throughout the distribution chain. Market acceptance is a significant concern in packaging design since well-designed packaging can increase sales. This is mostly relevant for consumer packaging, however, which is not covered in this chapter. Bulk packaging is, in this context, defined as all packaging elements that fulfil the function of transporting goods in larger quantities within the distribution chain, such as pallets, boxes, ice absorbers, shrink plastic, corner reinforcements, metal bands, etc.

Norway, as a leading exporter of fresh fish, requires a high focus on transport efficiency, quality preservation and environmental distribution. Bulk packaging makes the effective transportation of larger amounts of fish products to distant markets possible. The preferred bulk package unit is currently 10 or 20 kg, but can be bigger, and transport options are via road, rail, sea or air freight. The primary focus of this chapter is on the development of effective, quality preservative and environmentally friendly bulk packaging for the distribution of raw (fresh and thawed) fish products to different markets.

8.2 Status and challenges

A typical bulk packaging solution for fresh fish such as Atlantic salmon consists, at present, of 20 kg units of fish in boxes formed from expanded polystyrene (EPS) containing an additional 3–6 kg of ice. The boxes are placed on Europallets up to nine layers in height and secured by metal bands. The main transport mode is via trucks. A typical truck has a capacity of 33 pallets with 27 boxes on each pallet, which allows a net load of 17.8 metric tons. Atlantic salmon are the main species farmed in Norwegian aquaculture, with a total production of about 800 000 tons (Mt) in 2009 (Statistics Norway, www.ssb.no), which corresponds to the transport of about 40 million EPS boxes. At present, approximately 73% of the salmon are exported as whole, gutted fresh fish, with head on, by trucks to other European countries (Norwegian Seafood Export Council). The heads and bones comprise about 30–40% of the weight of gutted farmed salmon (Rørå *et al.*, 1998), and empirically, 15–20% of the weight in each transport box is ice. Hence, the number of trucks required for fish transportation could be reduced by 30–40% if the salmon were exported as trimmed fillets. Reducing the amount of ice used during transport could further increase transport efficiency.

Different EPS-based container or box format sizings are currently widely used for the transport of fish throughout the world. EPS is water resistant with high thermal-insulating properties making it suitable for the distribution of fresh fish packaged with ice. EPS is manufactured by expanding small polystyrene beads. Each EPS box has a semi-open pore structure consisting of 98% air and therefore, has a low associated weight. This makes EPS cost effective for use with air cargo,

both in pallets and boxes. For this application, versions without drainage holes are used, which is a prerequisite for aeroplane transport.

The normal transport time from the slaughterhouse to the market, when using trucks, is 2–5 days. Today, all transport of fresh fish or thawed raw fish in bulk packaging is made with ice added in the box or container, normally placed on top of the fish. As previously stated, approximately 3–6 kg is the usual quantity of ice used per box of fillets (10–20 kg units). During storage, low temperatures are essential and are commonly provided by refrigeration systems. In the event of the refrigeration system working poorly, it is important that the ice is there to maintain the low temperature. Another common, but probably unintentional, function of the ice is to cool down fish which has not been properly cooled during processing or prior to packaging. When the core temperature of the packaged fish is above zero, some of the ice will melt and mix with residues from the fish. Some of this contaminated melt water spills out of the boxes into the interior of the trucks and later into terminals, boats, railway carriages, etc. The consequences are a corrosive attack on the metal surfaces and the need for frequent cleaning.

During distribution the goods are often transloaded once or sometimes several times between various transportations (trucks, boats, planes and in distribution hubs). This represents a challenge for keeping a stable low temperature. The insulation properties of EPS can counteract the external fluctuating temperatures, as long as the chilling load inside the box is sufficient.

A stable and low temperature cold chain is necessary to ensure adequate preservation of fish quality. To achieve this, both the core temperature of the fish at the time of processing and packaging and the temperature during transport and transloading have to be low and stable. Reducing the core temperature of the fish from 5°C to 0°C for a typical EPS/ice packaging solution takes at least 12 h. Figure 8.1 shows how the EPS, both with and without drainage (one pallet each, consisting of nine layers or 27 boxes) successfully maintained the core temperature of gutted

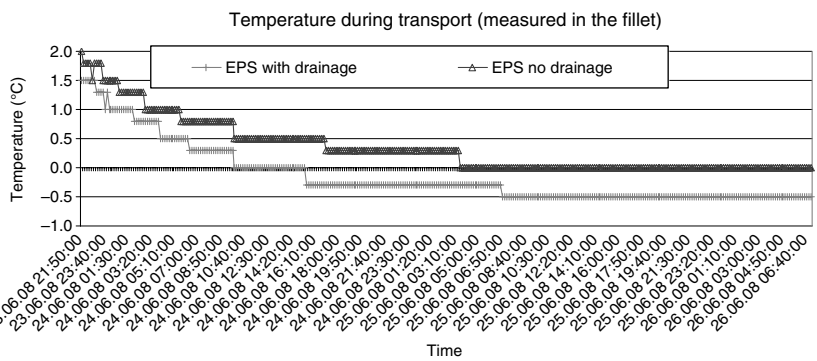

Fig. 8.1 Temperature in the fillet of whole, gutted salmon during transport, packaged in EPS with/without drainage. Six gutted fish (20 kg and 7 ± 1.4 kg of ice) in each box, and both types of boxes were placed on the same pallet. The air temperature inside the truck ranged from −2°C to 2°C.

salmon during transport (from the top layer of the pallet). The air temperature inside the truck ranged from −2°C to 2°C during the two and a half-day transport journey. EPS packaging without drainage required about three times longer to reduce the initial temperature to 0°C than EPS containers with drainage. Whether this affected the salmon products during further repackaging was not studied in this case.

Raw fish products are highly perishable because of their high a_w, neutral pH and the presence of autolytic enzymes, all of which provide very good growth conditions for micro-organisms. A low storage temperature is therefore crucial. Studies show that fresh fish stored at 0°C has double the shelf life of that stored at 4°C (Ratkowsky *et al*., 1983; Storey, 1985). Consequently, an initial temperature of 4°C (which is the typical temperature quoted for refrigerated or chilled foods) is too high for fish. This is further compounded by the issue of mounting melt water levels in packs from ice, which will arise during transport, that reduces the amount of ice inside the package.

The long-distance transport of raw fish in open distribution systems is almost exclusively done using packaging made of disposable materials, like EPS. In more closed distribution systems (one to one distribution) and over shorter distances, reusable boxes are often used as an alternative. Reusable boxes made of polypropylene (PP) or high-density polyethylene (HDPE) have lower insulation properties, but during transport without transloading and with a properly controlled and stable chilling chain, such packaging materials are valuable for fresh fish distribution. Reusable boxes typically have a conical shape and therefore occupy little space when they are transported and stored empty.

HDPE trays, used both for MAP, as well as in a traditional format with ice, have been tested both during transport and for further refrigerated storage. The package unit in the presented test was 5 L for MAP and the oxygen transmission rate (OTR) was measured to be 0.7 cm^3/package.day at 0°C and 100% humidity (method described in Larsen *et al*., 2000), which is a sufficient OTR level for the refrigerated storage of raw fish during the transport period. The trays, both for the traditional packaging using ice and for the MAP packages, contained —three to four layers of Atlantic salmon fillet pieces (fillet halves). After 10 h transport and a further 7 days of storage at 0°C, the MAP fillets had significantly lower bacterial counts compared to the air- and ice-stored fillets (Hansen *et al*., 2009a). The MAP consisted of a gas mixture of 60% CO_2 and 40% N_2 with a gas volume to product volume ratio (g/p ratio) of ½, including a CO_2 emitter that ensured there was a sufficient amount of the bacteria-inhibiting CO_2 gas inside the package despite this low g/p ratio. This study also showed that the use of a packaging material with a lower degree of insulation compared to EPS preserved fresh fish quality equally well, even after prolonged storage, but at a stable storage temperature (0°C).

The environmental impact and resource use for the bulk transport of fish has been calculated for a number of cases using either complete or simplified life cycle assessments (LCA). In simplified LCAs only the energy consumption and greenhouse gas (GHG) emissions have been assessed. These assessments show that bulk transport significantly contributes to the total environmental impact of

seafood products when considering the whole life cycle – for example, up to 10% of the total GHG emissions. The relative importance of the packaging on the total environmental impact of the product is especially high in cases where the product transported has a relatively low environmental impact from fishing and processing, or if the amounts of product waste from distribution are relatively low.

In a study examining the effects of distributing fresh fish from Norway to the European market either as gutted whole fish, whole fillets or as fish loins, and where the loins were either packed in bulk packaging with 5 kg boxes or directly in smaller MAP consumer packaging (HDPE trays, with sufficient barriers for the refrigerated storage of raw fish), it was shown that if the filleting and packing of the fish occurred in Norway, it was more economical to pack loins in 5 kg boxes and repack the fillets in France before distributing to Paris. However, it was also shown that if the distance between the packing plant and the final destination was less than about 1500 km, it then became more economical to pack the fillets directly into MAP trays in Norway, even for product destinations in southern Europe. The main reasons for this are that only one packing process is involved, that only the high-quality part of the fish is distributed over long distances, and that the bulk packaging step is excluded. This might also have had an impact on fish quality, as the risk for exposure to bacteria was reduced when the amount of contact with process equipment and humans was minimized.

In a study of GHG emissions (Fig. 8.2) from the packaging and distribution of fresh fillets compared to the distribution of whole gutted fish, it was shown that GHG emissions were reduced by more than 40%. This is due both to the fact that

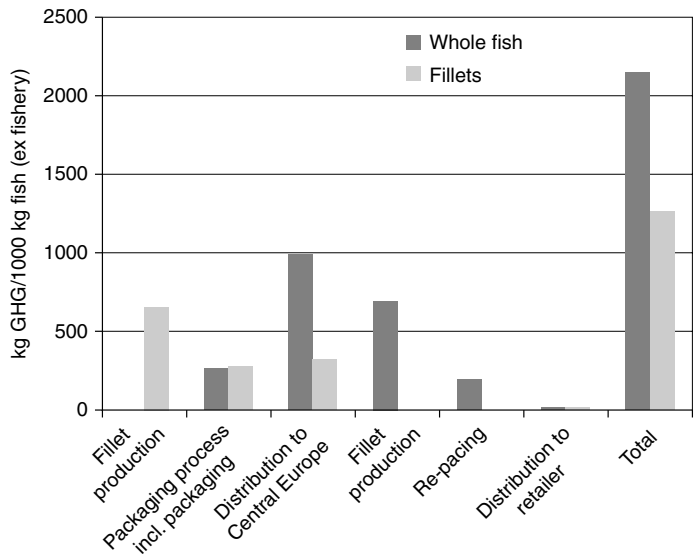

Fig. 8.2 Greenhouse gas emissions from distribution of whole gutted fresh fish and fresh fish fillets from Norway to Central Europe. (From Vold and Hanssen, 2010.)

skins, bones and heads were removed before distribution, and because the fish fillets did not have to be repacked at the destination (Vold and Hanssen, 2010).

The environmental impact from fish transport can therefore be reduced by transporting only the parts of the fish, which are fit for human consumption. The remainder of the fish is normally utilized as animal feed. These by-products are transported very efficiently in compact blocks and are also normally transported over short distances.

Several studies have investigated the environmental impacts of packaging systems for the bulk transportation of fish. These studies have employed whole life cycle evaluations and have considered the entire distribution chain from a bulk packaging perspective. Packaging may also have an indirect environmental impact by reducing or increasing product wastage. This effect was also taken into consideration where possible for the evaluations.

8.3 Advances in bulk packaging for the transportation of processed fish

Alternative packaging solutions for the bulk packaging of fresh or raw fish have been examined over the last few years, to meet new demands in the market for packaging solutions that are based in renewable materials and, which are easy to recycle. The main focus has been on the development of boxes manufactured from fibre materials made of both corrugated and solid paperboard. Quality preservation during transport was evaluated on the basis of temperature control during transport/storage and product quality at the time of arrival at market.

Fibre materials made of corrugated boxboard for raw fish packaging have been shown to maintain low fish temperature during refrigerated distribution. The fibre boxes and EPS boxes containing wild captured Atlantic cod loins packaged with ice maintained a similar storage temperature during a period of 4 days of transport from the processing plant until they reached market. However, the high insulating property of EPS makes such boxes more resistant to fluctuating temperatures surrounding the boxes (air inside trucks, etc.).

In one case study, the impact of cod loin distribution from western Norway to a customer in the EU was calculated. When testing with an alternative chilling method (after thawing), it was found that the fish fillets were much colder during processing and packaging than in the regular thawing/chilling process (0°C vs. 4°C). With good temperature control within the distribution chain, no ice was needed in the boxes for those with 0°C core temperature at the time of packaging. Studies of the fish quality could not find any differences in quality between the fillets distributed in boxes with ice and those without ice. Replacing the ice with cod loin would result in a substantial reduction in the environmental impact of the distribution of fish fillets. In fact, GHG emissions would be lowered by 18%.

HDPE is a low-cost polymer with gas barrier properties that are relatively poor, but are still high enough for the transport and storage of raw fish products. HDPE has been widely used in portion packaging for perishable foods stored in

MAP for less than 21 days, which is much longer than is needed for most types of bulk distribution.

HDPE trays also exist in larger units, with capacities of up to 18 kg (www.promens.com) for bulk MA packages. These packages have ten times less pre-packaging storage volume than similar EPS boxes, but have no insulation properties. One of the benefits of HDPE packages is that they can be used for modified atmosphere packaging as well as for traditional packaging with ice and air (Hansen *et al*., 2009a). Gel ice can be added to ensure a low temperature inside the package, also when using MAP. Modified atmosphere has, during several trials, shown an inhibitory effect on the growth of micro-organisms in a chilled environment (Hansen *et al*., 2007, 2009a, 2009b). These studies show that spoilage bacteria were inhibited by initially adding CO_2 gas, and then adding a CO_2 emitter pad inside the package that developed CO_2 during storage.

One case study used bulk HDPE trays for a shipment of pre-rigor filleted salmon fillets. Fillets weighing 15 kg were packaged in 24 L trays (gas to product ratio of 0.6) and filled with 60% CO_2 and 40% N_2 before sealing the top web. Forty trays were put on a pallet and transported on a chilled truck from Norway to a plant in central Europe for repackaging and further transport out to the retail stores. To counteract the poor insulating properties, ice gel was added to the packages. The transport took 3 days. By using a 100 × 120 cm pallet two and three trays can be used per layer, which can be alternated in orientation, thus interlocking the layers. Gas composition in the head space was measured upon arrival, and the CO_2 level had decreased to 26.8%, which is caused by the diffusion of CO_2 into the fillets. The diffusion of CO_2 is a lengthy process, and it is likely that there is a gradient of CO_2 concentration from the fillets on top to the fillets at the bottom of the tray although this has not been quantified. Dissolving CO_2 into the fillets during shipment would, however, have a positive impact on the amount of CO_2 in the portions when packaged in MA. This increased CO_2 in the portions packages would increase the shelf life of the fillets. The cold chain was never broken in this trial, and the gel ice was still frozen when the packages were opened. The average temperature in the fillets was 0.5°C upon arrival. Transport in MA packages had no effect on the drip loss during shipment. Concluding the trial: HDPE trays are a good alternative to traditional EPS as long as the cold chain is well maintained.

The EPS boxes used for the fish industry have been further developed in recent years resulting in increased strength and easier handling (Bewi Produkter AS and Vartdal Plastindustri AS, Norway). The EPS top lid and the use of straps can be replaced by a sealed plastic top film that reduces the material costs of the lid. The use of a film lid increases the number of boxes by one layer per pallet, which allows more efficient transport. There is also potential for packaging in modified atmosphere with a barrier film inside the box. Studies of MAP with a CO_2 emitter pad, of farmed Atlantic cod loins in such packages showed an optimal preservation of quality. Tests have also been performed for salmon fillet, which show improved inhibition of bacterial growth and lower intensity of negative associated sensory attributes when adding CO_2 emitter to MAP (Hansen *et al*., 2009a, 2009b).

8.4 Effective application of bulk packaging for transportation of raw fish products

There is increasing interest in the pre-rigor filleting of farmed salmon because such early processing makes it possible to supply the market with superior-quality fresh products, improves the logistics at the processing plant and eliminates costs associated with the transportation of unwanted product components such as heads and bones (Mørkøre *et al*., 2008). Pre-rigor salmon fillets are also thicker, firmer and often the colouration is more intense compared with their post-rigor counterparts (Skjervold, 2002). It is well documented that a low storage temperature and MAP inhibits bacterial growth and biochemical degradation, thus facilitating a prolonged product shelf life (Fletcher *et al*., 2002; Hansen *et al*., 2004, 2007; Randell *et al*., 1999; Sivertsvik *et al*., 2002). Partly freezing the outer fillet layer from −0.5°C to −2.0°C (superchilling), has proven to be more efficient in inhibiting biochemical changes and bacterial spoilage compared to traditional chilling (Chang, 1998; Duun and Rustad, 2007, 2008; Rosnes, 2000; Sivertsvik *et al*., 2003). By superchilling the fillets prior to packaging and chilled distribution, the refrigerating capacity is stored into the product sufficiently to avoid the requirement for ice during a certain period at refrigerating temperature. Superchilling technology has been used for decades in the seafood industry (Huidobro *et al*., 2002; Merritt, 1965; Nowlan *et al*., 1974; Partmann, 1965), but so far no study has explored the possibility of combining pre-rigor filleting, superchilling, MAP using CO_2 emitters and the subsequent cold storage of fish fillets packaged in transport boxes containing several layers of fillets. A CO_2 emitter maintains the partial pressure of CO_2 inside a package during storage by generating CO_2 gas and thereby replacing the gas absorbed into the product. Consequently, a CO_2 emitter enables a lower gas volume to product volume ratio, as mentioned earlier. This is demonstrated in the MAP of farmed pre-rigor filleted cod and pre-rigor filleted Atlantic salmon (Hansen *et al*., 2007, 2009b). Because MAP packages have a slow chilling rate, it is more efficient to superchill the fillet products before packaging (Torstveit *et al*., 2001). MAP using a CO_2 emitter can increase the transport efficiency compared to ordinary MAP, because a significantly lower headspace volume is required (Hansen *et al*., 2007). The combination of superchilling prior to MAP of pre-rigor fillets could thus contribute to improved sustainability throughout the whole production of salmon fillets, provided that the quality of the salmon meets the expectations of the industry and consumers alike. An investigation of the quality evolution in farmed Atlantic salmon shows that combining short-term superchilling and MAP with a CO_2 emitter prolonged the shelf life during 0°C storage of pre-rigor salmon fillets compared to traditional chilling or packaging in air/with ice, which can improve the sustainability throughout the value chain (Hansen *et al*., 2009a).

Studies have also been made of the GHG emissions of the distribution of fresh fillets from Norway to central Europe using conventional packing with ice, compared to superchilled fresh fillets. The results show that although the pre-treatment process use a significant amount of energy, which causes GHG emissions, the total emissions throughout the distribution chain is reduced by approximately

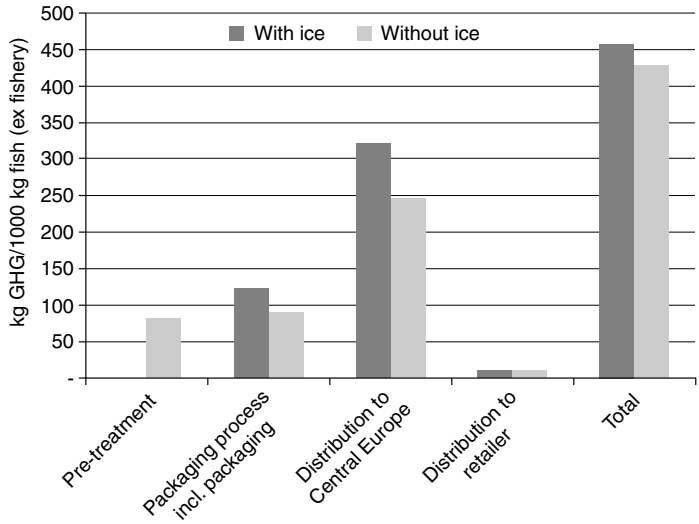

Fig. 8.3 Greenhouse gas emissions from distribution of fresh fish fillets from Norway to Central Europe by conventional packing with ice and with pre-treatment with super-chilling without ice. (From Vold and Hanssen, 2010.)

6% due to lower emissions in the packing and distribution processes (Fig. 8.3). The reason is that the ice was substituted with the same weight of fish, making the distribution more efficient (Vold and Hanssen, 2010).

8.5 Future trends in seafood packaging and distribution

To discuss some possible future trends in seafood packaging and distribution, it is first of all interesting to identify some key driving forces that could influence the choice of future packaging and distribution solutions. We will regard the following factors to be among those that will have the most influence in seafood packaging and distribution during the next ten years:

- The need for more sustainable solutions, that reduce consumption of fossil resources both for energy purposes (e.g., transport) and for material production, reduce GHG emissions and reduce waste of products and of packaging materials.
- The need for healthy and fresh food, which also is sustainable with respect to resource management (e.g., fish stocks, food waste reduction) and environmental burdens.

Based on these two driving forces, we assume that packaging and distribution of seafood in the next ten years will be changed in the following ways:

- There will be a significantly increased focus on methods of minimizing food waste from seafood throughout the whole value chain, from fishing or farming,

towards the final consumer. A great deal of food is currently lost over the whole value chain, and the most important driving force towards packaging and distribution is to protect fish quality over the whole distribution chain. It is also important to secure a high level of quality, that lasts long enough for the retailer to sell the fish and for the consumer to use the fish before the quality is lost. Packaging that can protect the quality of the fish during distribution is likely therefore to be even more important in the future, especially in areas with long distances between fishing areas and consumers, and where high temperatures are a challenge. This might lead to an increased use of superchilling as a preparation before transport, and to a high focus on temperature control systems in distribution. It will also favour packaging materials which preserve the quality of the fish. Eventually, more seafood will be distributed as frozen fillets, because this makes it easier to preserve quality from fillet production to the consumer.

- An increased focus on sustainable solutions should be a driving force towards optimal packaging and distribution – that is, packaging materials and solutions that minimize environmental burdens over the distribution chain. This means the use of renewable materials (e.g., fibre materials), materials from certified forestry, packaging that is produced with low emissions to air and water, and with a low consumption of energy (especially fossil energy) and which is easy to recycle and use in new products. Minimizing material use in relation to the function of the packaging is also an important element in this strategy.
- Minimizing transport work is also a trend that will be more important in the future, due to increased transport costs, increased time loss in high traffic density and the need to reduce fossil energy consumption. The present method of seafood transport is quite ineffective, with lots of low-value by-product and ice being transported over long distances together with the fish. It has been estimated that the transport capacity per 1000 kg fillet delivered to customers in Europe could be increased by 50% if fillets were distributed instead of whole fish, and if superchilled fillets were used instead of boxes filled with ice. The distribution of fillets with minimal ice in efficient boxes will thus probably be a trend for the future, to minimize transport work.
- Packaging solutions for the more effective distribution of raw fish using CO_2 gas, either by soluble gas stabilization (SGS) treatment or by adding CO_2 emitter into package.

SGS has been shown to be an interesting pre-treatment for products undergoing packaging in a modified atmosphere (SGS is discussed in Chapter 11). Dissolving CO_2 into the product prior to packaging in modified atmosphere affects the gas volume reduction, rendering no changes in gas volume feasible. This means that the packaging size can be reduced and transport efficiency increased significantly. SGS has shown promising results for fresh Atlantic salmon (*Salmo salar*) fillets (Sivertsvik 2000, 2003), cooked peeled shrimp (Sivertsvik and Birkeland, 2006), chicken breast fillets (Rotabakk *et al*., 2006), Atlantic halibut (*Hippoglossus hippoglossus*) (Rotabakk *et al*., 2008), farmed gilthead sea bream (*Sparus aurata*)

and European sea bass (*Dicentrarchus labrax*) (Mendes and Goncalves, 2008). Transportation of seafood in bulk packages can also be used as a SGS step, where the transport in MAP is used to dissolve CO_2 into the product prior to portioning and retail packaging. The distribution of food inside the package must then be optimized, to secure a uniform distribution of dissolved CO_2 in the product. A CO_2 emitter, as previously mentioned, can be used to improve transport efficiency without compromising quality by the development of CO_2 gas inside the package after it has been sealed. It can be used both in transport packages with layers of fillets and in small consumer packages and it acts as a liquid absorbing pad. Improved quality preservation, increased shelf life and improved transport efficiency were shown by use of this packaging method for fillet parts of both farmed Atlantic cod and Atlantic salmon (Hansen, 2007, 2009a, 2009b), thereby proving it a promising future as a packaging method.

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9

Advances in vacuum and modified atmosphere packaging of fish and crustaceans

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Abstract: Advances published between 2000 and 2010 on vacuum and modified atmosphere packaging (VP and MAP) of fish and crustaceans are reviewed, building on an earlier review of literature up to 1999 by Sivertsvik *et al*. (2002). Although over 180 studies were reviewed, most apply current technology to new species and products. Key advances include the use of soluble gas stabilisation and CO_2 emitters and the development of antimicrobial films. Little research has been commercialised, showing that more targeted research is needed. The major useful advances have been in better understanding and modelling safety and quality of MAP.

Key words: modified atmosphere packaging (MAP), vacuum packaging, lactic acid bacteria, crustacea.

9.1 Introduction

Recently, while holidaying in rural Fiji, we purchased freshly caught fish sold by a fisherman at the side of the road. His display consisted of having his gutted fish hanging on a pole from a flax string through the gill cavity and we had to hunt around to find a plastic bag to put it in so that it did not mess up the rental car too much. This, and the fresh fish market, reflects the way many of world's people still purchase their fish. For thousands of years fish has been sold without any packaging and, in many parts of the world, it still is. Even in technologically advanced countries, in my father's day, fish packaging usually meant that it was wrapped in paper by the fishmonger at the point of purchase. These days, for most

of us, traditional retail packaging invariably means some form of plastic bag and it is almost inconceivable to think of bringing fish home in anything but some form of plastic packaging. Crustacea, whether sold live, chilled or frozen, now also typically come home to the consumer in simple plastic bags with or without packaged outer layers of labelled cardboard. Changes similar to those in retail packaging have occurred with bulk transport of fish, where wooden crates have been replaced, so that traditional packaging now means plastic totes and polystyrene bins, but advances in this area are discussed further in Chapter 8.

Vacuum packaging (VP) has been commonly applied to some processed fish products, where product is placed in a package, air is evacuated and the package is sealed. Apart from separating the product from the external environment, VP has the benefits of limiting the package volume and preventing oxidative spoilage, particularly when the films used have high O_2 barriers. Modified atmosphere packaging (MAP) typically starts by using a vacuum to remove air but then adds gas of a chosen composition (different from air), which is then sealed in the pack. For chilled seafood, the gas used typically contains elevated levels of CO_2 which takes advantage of the antimicrobial effect of this gas and which has been applied to seafood since the 1930s (Coyne, 1932, 1933; Killeffer, 1930; Stansby, 1935).

In this chapter, I will review advances in VP and MAP published in the scientific literature between 2000 and 2010. During that time one detailed review focusing on microbiology of seafood under MAP (Sivertsvik *et al*., 2002) and another general review of seafood MAP (Hansen and Eie, 2005) have been published. The first thoroughly reviewed literature up to 1999 and identified the range of factors contributing to the success of MAP. It concluded that while MAP increases shelf life compared with air storage it confers little increase compared with vacuum packaging. Research in the last 10 years does not support this view. The second review outlined the basic principles of MAP, highlighting temperature control, gas to product ratio (*g/p*) and CO_2 concentration as key parameters to success, and pack collapse and liquid loss as important considerations.

9.2 Innovations in packaging technology

The basic technologies of VP and MAP have largely remained unchanged since they were first introduced in the early part of last century: product is placed in a flexible bag, air is removed by vacuum pump and either the bag is immediately sealed and the external vacuum released (VP) or the gas of the chosen composition is released into the bag, which is then sealed and any remaining vacuum released (MAP). Usually MAP is carried out at the level of retails packs but occasionally MAP is applied to whole or gutted fish before processing. MAP of bulk red fish was shown to have negative effects on sensory characteristics compared with air (Lauzon *et al*., 2002), while bulk MAP had little effect on salmon quality (Randell *et al*., 1999). In contrast, controlled atmosphere storage of hake before retail MAP or air packaging did extend product shelf life (Ruiz-Capillas *et al*., 2001). Recent innovations and modifications to basic MAP and VP technology have included the following.

9.2.1 Reduced gas: product ratios

Here small amounts of 100% CO_2 are used in MAP to produce an end package more similar to VP. One of the disadvantages of MAP is that the packages are bulky, typically containing twice the amount of gas as product and, because of the insulating properties of the gas, difficult to chill. Applying only a small amount of 100% CO_2 means that this will all dissolve into the product, leaving it without any surrounding gas as in VP but still benefiting from the antimicrobial benefits of the CO_2. Schirmer *et al*. (2009) showed that this extended the shelf life of salmon without the bulk of normal MAP. Work in our laboratory had also explored this concept. Initially when packages were under vacuum, we added CO_2 up to a pressure that was still less than atmospheric pressure so that when the vacuum was released from around the pack there was no visible gas in the pack; this was shown to extend shelf life (Scott *et al*., 1984, 1986). Subsequently, like Schirmer *et al*., we added CO_2 at levels such that there was a visible amount of gas around the product immediately after packing but all of this subsequently absorbed into the product, giving a VP appearance (Fletcher *et al*., 2004). We informally termed this process MAPVac, signifying that it was VP achieved through MAP. Although advantageous from the volume and chilling point of view, one of the disadvantages of this approach is that, like VP, it tends to cause higher drip loss from raw fish than MAP (Fletcher *et al*., 2004). Storage of bulk fish in MAP before filleting is also likely to give similar benefits, although this was not noted in a study on redfish (Lauzon *et al*., 2002).

9.2.2 Soluble gas stabilisation (SGS)

This term was coined by Sivertsvik (2000) for the process of dissolving CO_2 into seafood usually under low pressure (ca. 2 bar) before packaging either in MAP, VP or, perhaps, air. Because CO_2 concentrations near the targeted equilibrium are reached before packaging, this has advantages over MAP in preventing pack collapse caused by CO_2 dissolving into the product after packaging. It also allows the desired equilibrium CO_2 levels to be reached with lower *g/p* with the benefits of less bulky, easier to chill packages. Although SGS successfully extended shelf life when followed by VP (Mendes and Gonçalves, 2008a), it was not beneficial when followed by packaging in air (Mendes and Gonçalves, 2008b). This is most likely due to the effect of O_2 but also possibly indicates that the CO_2 might have moved out of solution under air storage conditions. The technology has been successfully applied to shrimp (Sivertsvik and Birkeland, 2006), sardines (Mendes *et al*., 2008), sea bream and sea bass (Mendes and Gonçalves, 2008a) and halibut (Rotabakk *et al*., 2008a).

9.2.3 Moisture and oxygen absorbers and carbon dioxide emitters

VP and MAP with high levels of CO_2 can result in liquid loss from raw seafood, with this being more pronounced when pre-rigour fish are packaged (Hansen and Eie, 2005). As this is unsightly, packs often include a moisture absorber

(Hansen and Eie, 2005). Although MAP is usually achieved by adding a gas of the desired concentration to the pack, modified atmospheres can also be generated within the packs with absorbers and emitters. Oxygen absorbers can prevent oxidation and limit growth of aerobic spoilage microflora (Abe and Kondoh, 1989). Although some early studies showed that oxygen absorbers can have beneficial effects without traditional application of MAP (Suzuki *et al*., 1985; Takiguchi and Aminaka, 1990) and are widely applied in other products (Charles *et al*., 2006) this field has been little studied with regard to seafood. As all films have some oxygen permeability, we used oxygen absorbers designed to operate at low temperature and high moisture to ensure that MAP experiments were carried out under strictly anaerobic conditions (Fletcher *et al*., 2004).

Hansen *et al*. (2009a, 2009b, 2009c) and Hansen and Eie (2005) have investigated using a CO_2 emitter (sodium bicarbonate and citric acid) to maintain the level of CO_2 in the headspace of MAP packages of salmon. The reported benefits of this are the same as SGS: reduced package volume and preventing pack collapse. These researchers determined optimal CO_2 emitter capacity and demonstrated that packs with reduced *g/p* could perform as well as ordinary packs.

9.2.4 Packaging films

Most MAP and VP is carried out in packs made of plastics with high barriers to the gases of interest, O_2 and CO_2. Recently, many innovations in films have been investigated. In an attempt to prevent growth of *C. botulinum*, films with high permeability to oxygen have been developed and applied (Arritt *et al*., 2007). However, such films have a negative effect on shelf life (e.g. Anelich *et al*., 2001) and do not always reduce the time to toxigenesis (Dufresne *et al*., 2000), probably because *C. botulinum* is capable of growing in the presence of oxygen when the redox potential (E_h) is low. Because oxidation of lipids in packaged fish is mediated by light (Choubert *et al*., 2005), light-impermeable packaging materials such as metallised films (Fletcher *et al*., 2002) improve the storage quality of fatty fish. However, one of the benefits of MAP and VP is that customers can see the product and this is lost when light-impermeable materials are used.

A lot of effort has recently gone into the development and testing of antimicrobial films. These can potentially control microbial growth in VP products without the need for additives or CO_2 and without having to declare that antimicrobial additives have been added to the product. Antimicrobial films generally only affect the surfaces of the seafood but for most products the dominant spoilage organisms are found on the surface. To function well the films need to be in direct contact with product so antimicrobial films are usually best used in VP rather than MAP. Most antimicrobial films are being developed for their ability to control pathogenic bacteria rather than spoilage organisms, although many will be effective against these as well. Films have been developed containing the following antimicrobial agents: nisin (Cha *et al*., 2002; Grower *et al*., 2004; Neetoo *et al*., 2008b) and other bacteriocins derived from bacteria (Ercolini *et al*., 2006), chitosan (Begin and Van Calsteren, 1999; Coma *et al*., 2002; Möller *et al*., 2004),

cinnamaldehyde (Ouattara *et al*., 2000) and other essential oils (Oussalaili *et al*., 2007), lysozymes (Appendini and Hotchkiss, 1997; Cha *et al*., 2002; Mecitoglu *et al*., 2006), lactoperoxidase and lactoferin (Chantarachoti *et al*., 2009; Min *et al*., 2005a, 2005b), organic acids (Ouattara *et al*., 2000), grape seed extract and EDTA (Cha *et al*., 2002). Rather than using oil-based polymers, antimicrobial films are often developed in polymers perceived to be more environmentally friendly, such as alginates (Cha *et al*., 2002; Oussalaili *et al*., 2007), carrageenan (Cha *et al*., 2002), whey protein (Min *et al*., 2005b), corn zein (Mecitoglu *et al*., 2006) and chitosan, which is itself antimicrobial (Begin and Van Calsteren, 1999; Coma *et al*., 2002; Möller *et al*., 2004; Ouattara *et al*., 2000). Some of the films are even edible (Coma *et al*., 2002) but only a few antimicrobial films have been applied to seafood (Chantarachoti *et al*., 2009; Neetoo *et al*., 2007).

As well as the potential antimicrobial activity of films, an antioxidant film containing polyphenols has been shown to be useful in preventing lipid oxidation in coho salmon (Rodriguez *et al*., 2009a) and Hoshino *et al*. have patented odour-absorbing film technology claimed to be useful in extending the storage life of fish (Hoshino and Osanai, 1986; Hoshino *et al*., 1990).

9.3 Advances in understanding spoilage processes in packaged fish

The following sections study in detail the different types of spoilage that can take place during the period of time during which seafood travels between point of catch and the consumer.

9.3.1 Microbial spoilage

Major advances have been made in understanding the spoilage process occurring in packaged fish. Bacteria are commonly recognised as the main spoilage agent of chilled seafood and whereas bacteria have traditionally been identified by phenotypic characteristics, they are now largely identified by direct reference to their genotype such as 16S RNA sequencing (Rudi *et al*., 2007). Such techniques have the potential to identify many bacterial species that were previously non-culturable. In practice, because fish is a nutrient-rich medium, easy to mimic with microbiological media, the main spoilage microflora of fish are readily culturable bacteria. What genotypic identification schemes do is to provide much greater consistency and accuracy in identifying the spoilage microflora. The specific spoilage organism (SSO) concept, that there is a single main spoilage organism for particular products under particular storage conditions, has been widely applied to packaged fish. Different SSO groups have been reported for different fish species: *Photobacterium* in coalfish (Rudi *et al*., 2004); *Photobacterium* spp., *Shewanella* spp. and *Pseudomonas* spp. in cod (Hovda *et al*., 2007a, 2007b); *Photobacterium phosphoreum*, *Pseudomonas* spp. and *Brochothrix thermosphacta* in farmed halibut and *Streptococcus* species in sea bass (Poli *et al*., 2006) and

lactic acid bacteria in eels (Arkoudelos *et al*., 2007). Product form also affects the spoilage microflora so in cold-smoked vacuum-packed salmon (CSS): Dondero *et al*. (2004) identified *Lactobacillus* as major spoilage organisms, Olofsson *et al*. (2007) found both *Lactobacillus* and *Photobacterium* dominating, while in MAP salmon it was *Brochothrix* and *Carnobacterium* species but not *Photobacterium* (Rudi *et al*., 2004). Such studies have generally not been repeated by different laboratories so it is not known to what extent microflora differences are dependent on particular catch histories or laboratory methodologies. Although the original intention was that once SSOs were known, targeted controls could be devised for them, research has seldom achieved this, with the exception of controls for *Photobacteria* by freezing (Bøknæs *et al*., 2000, 2002). Knowledge of SSOs has led to the use of more relevant media for monitoring microbial spoilage but these have not always been successful. Tryfinopoulou *et al*. (2001) found that the commonly used *Pseudomonas* CFC-selective medium was ineffective for enumerating *Pseudomonas* spp. in MAP. Biogenic amine production, the basis of some bacterial spoilage indices, was highest in sardine and herring stored in air followed by VP and MAP (Özogul and Özogul, 2006; Özogul *et al*., 2002a, 2002b). Vacuum packaging was also shown to inhibit the formation of biogenic amines in carp, although these were poorly correlated to spoilage in this freshwater species (Krizek *et al*., 2004). Total volatile base nitrogen (TVBN), another spoilage indicator based on microbial metabolites, was shown not to correlate with spoilage in trout, another freshwater species (Azizishirazi and Shahram Shekarforoush, 2010).

9.3.2 Non-microbial spoilage

As well as better understanding microbial spoilage, knowledge of non-microbial spoilage factors has also increased for packaged fish. MAP and VP were shown to inhibit protein (Payap *et al*., 2004; Wan-Chul *et al*., 2003) and collagen (Payap *et al*., 2005a) degradation, thereby protecting product firmness. However, drip loss was shown to be higher in MAP than air (Rosnes *et al*., 2006). Because the gas present in MAP increases thermal resistance, it results in lower chilling rates of fish than vacuum-packed fish. This can increase spoilage unless thorough chilling is applied during the MAP process (Torstveit *et al*., 2001). MAP has also been shown to inhibit the breakdown of ATP derivatives, which has led to the suggestion that breakdown products, particularly hypoxanthine, might be suitable as spoilage indices in packaged fish (Özogul *et al*., 2007). However, the ATP derivative-based K value was not suitable in MAP seer fish (Yesudhason *et al*., 2009).

9.3.3 Modelling spoilage

Predictive modelling of seafood has made great progress in the last years and, through implementation of popular software products (e.g., Dalgaard, 2009), is now a well-utilised tool in managing seafood safety, quality and shelf life (Fletcher, 2010). A number of models have been designed to predict spoilage of packaged fish under various conditions. Models based on the growth of *P. phosphoreum* in

cod fillets, plaice fillets and salmon steaks have been incorporated into the freely available Seafood Safety and Spoilage Predictor (SSSP) software (Dalgaard, 2009), but these models do not include the effect of using O_2 in the gas mixes, which is recommended for some of these species (Sivertsvik, 2007). Corbo *et al.* (2005) have developed a kinetic model based on total bacterial count and total coliforms to evaluate the shelf life of cod under different MAP treatments (high and low oxygen) and temperatures. Koutsoumanis *et al*. (2000) used red mullet (*Mullus barbatus*) as a case study, to develop models combining the effect of temperature with the level of CO_2 in a modified packaging environment (Koutsoumanis *et al*., 2000). The growth of a range of spoilage flora (*Pseudomonas* spp., *Shewanella putrefaciens*, *B. thermosphacta* and lactic acid bacteria) was monitored and combined models developed. The models were judged satisfactory when assessed on three different fish species. Simpson *et al*. (2003) developed a model to predict the consequences of temperature abuse on shelf life of Pacific hake in MAP. This model predicts the effects of temperature, gas concentration, and relative humidity on shelf life. Recently Dai and Weng (2010) used the kinetics of *P. phosphoreum* to predict shelf life of large yellow croaker under MAP at 4°C or −1°C. Tsironi and Taoukis (2010) developed a model on the effect of temperature on gilthead sea bream (*Sparus aurata*) under MAP with osmotic pre-treatment and nisin. The combined treatment extended shelf life from ten to 48 days. No doubt other species-specific spoilage models that incorporate packaging considerations will continue to be developed.

9.4 Advances in understanding food safety implications of packaging

As well as advances in understanding spoilage, a lot of work has gone into understanding the microbial safety risks of packaged fish. Since early outbreaks of botulism from vacuum-packed smoked fish in 1960 (Thatcher *et al*., 1962), the anaerobe *Clostridium botulinum* (particularly the marine psychrotrophic Type E strain) has been recognised as a potential hazard of packaged seafood. This organism will grow and produce toxin in oxygen-reduced packaging but the key question is under what conditions this will happen before the product is rejected as a result of spoilage. Dufresne *et al*. (2000) recently found that MAP trout stored at 12°C spoiled before toxin production. Reddy *et al*. (1999) found the same for MAP packed marine-caught cod and stored at 4°C, 8°C and 16°C, as did Lyon and Reddmann (2000) for crawfish tails stored at 4°C and 10°C. However, aquacultured tilapia, catfish and salmon stored at 8°C and 16°C sometimes developed toxin at the same time or before spoilage (Reddy *et al*., 1999). Reddy *et al*. (1999) suggested that this might be related to the higher fat content of the aquaculturally produced fish.

Understanding has increased for the problem of bacterial-induced histamine poisoning from fish such as tuna under refrigerated packaging. Decreasing levels of histamine are generally formed in air, VP and MAP, respectively (Özogul *et al*.,

2004) and different bacterial species produce different levels of histamine under the different packaging types (Özogul and Özogul, 2005). However, Cai *et al*. (2010) found minimal differences in histamine levels in sliced tilapia treated with liquid smoke when stored under VP or air, although the levels of putrescine, another biogenic amine, were lower under VP. MAP (especially combined with 5% NaCl) inhibits the growth of the mesophilic histamine producer *Morganella morganii* (Aytac *et al*., 2000). Although usually caused by mesophilic bacteria, it has long been recognised that some psychrotrophic bacteria can also produce histamine (Okuzumi *et al*., 1981). *P. phosphoreum* and particularly *Morganella psychrotolerans* have recently been identified as contributing to histamine levels in packaged refrigerated fish (Dalgaard *et al*., 2006; Emborg *et al*., 2002, 2005, 2006). Growth and histamine production by *M. psychrotolerans* under MAP with different levels of CO_2 has been included in the SSSP software (Dalgaard, 2009), thereby allowing users to predict the times that susceptible products can be safely stored under MAP at different temperatures.

Much recent food safety work has related to *Listeria monocytogenes*, another psychrotolerant pathogen capable of growing in reduced oxygen conditions. MAP inhibits, but does not prevent the growth of *Listeria* in trout, but it also extends shelf life (Yilmaz *et al*., 2009). Fresh trout was shown to spoil before high *Listeria* counts developed in vacuum or MAP (without CO_2) but smoked trout developed high counts before spoilage (Joong-Han *et al*., 2008). A number of researchers have shown the effect of MAP in combination with other treatments on *Listeria* (Duffes *et al*., 2000; Payap *et al*., 2006; Tassou *et al*., 2004; Vogel *et al*., 2006; Zuckerman and Ben Avraham, 2002). The process of cold smoking was shown to reduce the growth rate of *Listeria* in CSS (Ribeiro Neunlist *et al*., 2005). However, one of the biggest advances in understanding has come with the development of mathematical models predicting the growth/no-growth boundaries for *L. monocytogenes* in lightly preserved seafood (Mejlholm and Dalgaard, 2007), which is readily available in the SSSP (Dalgaard, 2009). This allows users to predict combinations of MAP atmosphere, smoke concentration, salt, pH, nitrites, organic acids (acetate/diacetate, benzoic, citric, lactic and sorbic) and temperature that will not allow the organism to grow. In combinations where growth occurs, the SSSP also models growth of *L. monocytogenes* under combinations of factors, including the amount of lactic acid bacteria present. These recently released models have proven to be of great benefit to seafood producers wanting to assure safety in their packaged products. However, it is important to recognise the limitations of the models in that, although validated in a number of food products, they have largely been developed in model broth systems and producers need to validate them in their own product before totally relying on them for food safety.

Some studies have looked at other pathogens as well as psychrotolerant bacteria. Payap *et al*. (2006) showed that pyrophosphate had a synergistic action with MAP against *Escherichia coli* O157 and Tassou *et al*. (2004) showed that pretreatment by immersion in a sorbate solution and/or heating (60°C for 1 min) enhanced control of *Salmonella* Enteritidis by MAP.

9.5 Applying and modelling different gas configurations for different fish

One of the first questions users ask when wanting to apply MAP is 'What gas mix should we use?' Although there have been recommendations available for many years (e.g., Cann, 1984), often the basis for those recommendations or literature to evaluate their validity was not available. A lot of work has recently been published investigating the effect of different gas mixtures on fish of different species (Table 9.1). Different mixes give different shelf lives for different products but because of the range of methods of defining shelf life this data can usually not be compared across studies so is not included in Table 9.1. Most studies show MAP to perform better than VP, which in turn is better than air. The exceptions to this order of packaging performance were highlighted in several studies. Muratore and Licciardello (2005) found that VP smoked swordfish slices stored better than MAP (5% O_2, 45% CO_2, 50% N_2) equivalents and Corbo *et al*. (2005) showed that VP performed better than a high O_2 (80%) MAP at controlling bacteria in cod. These exceptions where VP performs better than MAP probably relate to oxidative spoilage caused by the O_2 in the MAP. Recommended gas mixes for MAP usually contain CO_2 for its bacteriostatic effect. Some contain O_2 to inhibit biochemical changes although, to restrict oxidation, O_2 is not usually included with fish containing high levels of lipid. The remainder of the gas mix usually consists of the relatively inert N_2, although some have used argon (Ar) with variable results. Giménez *et al*. (2002a) found no difference between using 20–30% Ar or N_2 while Choubert *et al*. (2008) found 40% Ar to perform better than 40% N_2.

Many studies identify some MAP combinations that perform better than others when used under similar conditions, but few optimise the effect of different gas configurations on shelf life and quality. As this effect has not been modelled, results can be applied outside of the experimental conditions. Key to modelling the effect of MAP on shelf life is to understand the diffusion of gas (particularly the highly lipid and water soluble CO_2) into fish flesh. Devlieghere *et al*. (1998) identified that, as well as the gas mix, the gas to fish ratio (*g/p*) has a major bearing on the equilibrium concentration of CO_2 in the product and this in turn is an important parameter for microbial growth (Devlieghere *et al*., 1998). Any particular gas mixture will give different equilibrium CO_2 concentrations for each *g/p*. Devlieghere *et al*. (1998) developed an equation to calculate CO_2 equilibrium, which requires the calculation of Henry's constant and the solubility of the gas in the product at a given temperature. Simpson *et al*. (2001) determined Henry's constant for a lean fish (Pacific hake) as 12.76 atm kg/mol (3.45 g CO_2/kg fish) at 0°C. They developed a mathematical model for gas transfer in MAP based on Fick's second law, but noted that postmortem variations of pH could affect CO_2 solubility. Finally, Rotabakk *et al*. (2008b) developed a model for solubility of three gases (CO_2, N_2 and O_2) in MAP or SGS that included the effect of temperature, but ignored film permeability. This was validated using their own data, but when applied to data found in the literature, the model gave good correlations only

Table 9.1 Recent (2000–10) publications applying different gas mixes to different fish species

Fish	Species	Reference	Criteria	Product form	g/p[a]	Temp. (°C)	Gas mix (%)				Best mixes[b]
							CO_2	O_2	N_2/Ar[c]	Air	
Bass	*Dicentrarchus labrax*	Torrieri *et al.*, 2006	Sensory, microbiology	Gutted	2	3	70	0	30	0	
					2	3	70	20	10	0	
					2	3	60	30	10	0	
					2	3	60	40	0	0	
					2	3	50	30	20	0	
					2	3	0	21	79	0	1
		Reale *et al.*, 2008		Gutted	?	2	0	0	0	100	3
					?	2	40	20	40	0	2
					?	2	60	5	35	0	1
		Provincial *et al.*, 2010	Sensory, microbiology, chemical, physical	Fillets	3	4	0	0	0	100[f]	1
					3	4	60	40	0	0	4
					3	4	50	50	0	0	3
					3	4	40	60	0	0	2
Bonito	*Sarda sarda*	Alak *et al.*, 2011	Biogenic amines	Fillets	—	4	0	0	0	100[f]	4
					—	4	0	0	0	100[g]	1
					0	4	0	0	0	0	3
					2	4	100	0	0	0	2
Carp	*Cyprinus carpio*	Babic *et al.*, 2009	Sensory, colour	Fillets		3	40	60	0	0	2
						3	100	0	0	0	1
		Hudecova *et al.*, 2010	Microbiology	Portions	—	4	0	0	0	100	3
					?	4	30	0	70	0	2
					?	4	20	80	0	0	1
		Jezek and Buchtova, 2010	Sensory, chemical	Fillets	—		0	0	0	100	2
					?		25	5	69[h]	0	1
Cod	*Gadus morhua*	Corbo *et al.*, 2005	Microbiology	Fillets	?	4	0	0	0	100	7

					0	4	0	0	0	0	1
					?	4	65	5	30	0	2
					?	4	20	80	0	0	8
					?	8	0	0	0	100	10
					0	8	0	0	0	0	3
					?	8	65	5	30	0	4
					?	8	20	80	0	0	5
					?	12	0	0	0	100	11
					0	12	0	0	0	0	9
					?	12	65	5	30	0	6
					?	12	20	80	0	0	12
		Hansen *et al*., 2007	Sensory, microbiology	Fillets	0[a]	0	0	0	0	0	2
					3.9	0	60	40	0	0	1
					1.3[e]	0	60	40	0	0	1
		Sivertsvik, 2007	Sensory, microbiology, TMA	Loin portions	2	0	100	0	0	0	
					2	0	0	100	0	0	
					2	0	0	0	100	0	
					2	0	50	50	0	0	
					2	0	50	0	50	0	
					2	0	0	50	50	0	
					2	0	67	17	17	0	
					2	0	17	67	17	0	
					2	0	17	17	67	0	
					2	0	33	33	33	0	
					2	0	0	63	37	0	1[f]
Eel	*Anguilla anguilla*	Arkoudelos *et al*., 2007		Whole	?	0	0	0	0	0	2
					?	0	0	0	0	100	3
					?	0	40	30	30	0	1
Hake	*Merluccius merluccius*	Ordonez *et al*., 2000	Sensory, microbiology, biochemical	Steaks	3	2	0	0	0	100	
					3	2	20	0	0	80	
					3	2	40	0	0	60	1

(*Continued*)

Table 9.1 Continued

Fish	Species	Reference	Criteria	Product form	g/p[a]	Temp. (°C)	Gas mix (%) CO_2	O_2	N_2/Ar[c]	Air	Best mixes[b]
		Ruiz-Capillas *et al.*, 2003	Sensory, Enterobacteriaceae, biochemical	Slices	—	2?	0	0	0	100	1
					?	2?	60	15	25	0	1
					?	2?	40	40	20	0	1
					?	2?	60	40	0	0	1
Halibut	*Hippoglossus hippoglossus*	Ruff *et al.*, 2003	Oxidation, drip, microbiology, colour	Fillets	0	−20	0	0	0	0	1
					0	2	45	25	30	0	1
Herring	*Clupea harengus*	Özogul *et al.*, 2000a, 2000b	Sensory, microbiology, biochemical	Whole	0	2	0	0	0	0	2
					2	2	0	0	0	100	3
					2	2	60	0	40	0	1
		Özogul *et al.*, 2002a, 2002b	Biogenic amines								
Mackerel	*Scomber japonicus*	Erkan *et al.*, 2007	Sensory, microbiology, TMA	Fillets	0		0	0	0	0	2
					—		0	0	0	100	2
					?	4	70	5	25	0	1
		Goulas and Kontominas, 2007a	Sensory, biochemical	Fillets	0	2	0	0	0	0	2
					2	2	70	0	30	0	1
					2	2	50	20	30	0	2
	Scomber colias japonicus	Stamatis and Arkoudelos, 2007	Microbiology, fatty acids, NH_3,	Fillets	0	3	0	0	0	0	2
					0	6	0	0	0	0	3
					—	3	0	0	0	100	3
					—	6	0	0	0	100	4
					?	3	50	0	50	0	1
					?	6	50	0	50	0	2

Mullet	*Mullus surmuletus*	Pournis *et al.*, 2005	Sensory, microbiology, biochemical	Whole	—	4	0	0	0	100	3
					?	4	20	10	70	0	2
					?	4	40	10	50	0	1
					?	4	60	10	30	0	2
Pearl spot	*Etroplus suratensis*	Lalitha *et al.*, 2005	Sensory, microbiology, pathogens	Gilled and gutted	—	0–2	0	0	0	100	3
		Ravi Sankar *et al.*, 2008			?	0–2	40	60	0	0	2
					?	0–2	50	50	0	0	2
					?	0–2	60	40	0	0	1
					?	0–2	70	30	0	0	2
					?	0–2	40	30	30	0	3
Salmon	*Salmo salar*	de la Hoz *et al.*, 2000	Sensory, microbiology, biochemical	Steaks	3	0	0	0	0	100	3
					3	2	20	0	0	80	2
		Fernández *et al.*, 2009	Sensory, microbiology, biochemical	Fillets	—	2	0	0	0	100	7
					1.2	2	0	0	0	100	7
					2.2	2	75	0	25	0	5
					1.2	2	25	0	75	0	6
					1.2	2	40	0	60	0	6
					1.2	2	75	0	25	0	4
					2.5	2	60	0	40	0	3
					2.5	2	75	0	25	0	2
					2.5	2	90	0	10	0	1
		Fernández *et al.*, 2010	Sensory, microbiology, biochemical	Fillets	—	2	0	0	0	100	6
					1.2	2	0	0	0	100	6
					1.2	2	25	0	75	0	5
					1.2	2	40	0	60	0	4
					1.2	2	75	0	25	0	3
					2.5	2	60	0	40	0	3
					2.5	2	75	0	25	0	2
					2.5	2	90	0	10	0	1
		Hansen *et al.*, 2009a	Microbiology, drip loss	Fillet	1[e]	2	60	0.06	40	0	1
					1[e]	2	60	0.06	40	0	1

(*Continued*)

Table 9.1 Continued

Fish	Species	Reference	Criteria	Product form	g/p[a]	Temp. (°C)	Gas mix (%) CO_2	O_2	N_2/Ar[c]	Air	Best mixes[b]
		Hansen *et al.*, 2009b	Microbiology	Fillets	0.5	−1	0	0	0	100	
					0.5	0	0	0	0	100	
					0.5[e]	0	60	40	0	0	
					0.5[e]	−1	60	40	0	0	1
		Hansen *et al.*, 2009c	Sensory, microbiology, texture	Fillets	1[e]	1.2	0	0	0	0	3
					1	1.2	60	40	0	0	1
					3	1.2	60	40	0	0	2
		Schirmer *et al.*, 2009	Sensory, microbiology	Pieces	0	4	0	0	0	0	2
					0.2	4	100	0	0	0	1
	Oncorhynchus tshawytscha	Fletcher *et al.*, 2002	Sensory, microbiology, biochemical	Fillets	4.2	0	0	0	0	100	3
					4.2	9	0	0	0	100	4
					4.2	0	40	0	60	0	1
					4.2	0	0	0	100	0	2
		Fletcher *et al.*, 2004	Sensory, microbiology	Portions	0	0	0	0	0	0	
					0.36	0	100	0	0	0	
					0.73	0	100	0	0	0	
					1.09	0	100	0	0	0	
					1.46	0	100	0	0	0	
					2.18	0	100	0	0	0	
					2.91	0	100	0	0	0	
					3.64	0	100	0	0	0	1[d]
					3.64	0	40	0	60	0	2
Sardine	*Sardina pilchardus*	Özogul *et al.*, 2004	Sensory, microbiology, biochemical	Whole	0		0	0	0	0	2

		Özogul and Özogul, 2006	Biogenic amines		—		0	0	0	100	3
		Özogul *et al.*, 2007			2	4	60	0	40	0	1
		Erkan *et al.*, 2006	Sensory, microbiology, biochemical	Headed and gutted	—	C	0	0	0	100	2
					?	A	35	5	60	0	2
					?	B	70	5	25	0	1
	Sardinella brasiliensis	Teodoro *et al.*, 2007	Microbiology, biochemical	Whole	0	2	0	0	0	0	
					15	2	0	0	0	100	
					15	2	100	0	0	0	
					15	2	50	50	0	0	1
Seabream	*Sparus aurata*	Giménez *et al.*, 2002b		Fillets	0	1	0	0	0	0	3
					—	1	0	0	0	100[f]	5
					?	1	50	0	50	0	1
					?	1	50	10	40	0	2
					?	1	50	20	30	0	4
					?	1	50	30	20	0	4
Silver carp	*Hypophthalmichthys molitrix s*	Jezek *et al.*, 2010	Colour, A_W, pH	Whole	0	2	0	0	0	0	1
					—	0	0	0	0	100[f]	2
Swordfish	*Xiphias gladius*	Pantazi *et al.*, 2008	Sensory, microbiology, biochemical	Slices	0		0	0	0	0	2
					—	4	0	0	0	100	3
					?	4	40	30	30	0	1
Tilapia	*Oreochromis niloticus*	Heidmann Soccol *et al.*, 2005	Safety and spoilage microbiology	Fillets	0	1	0	0	0	0	
					On Order	1	60	40	0	0	
		Peng *et al.*, 2009	Sensory, drip, colour, microbiology, TVBN	Fillets	3	−0.7	100	0	0	0	1
					3	−0.7	70	0	30	0	1
					3	−0.7	50	0	50	0	2
					3	−0.7	30	0	70	0	3

(*Continued*)

Table 9.1 Continued

Fish	Species	Reference	Criteria	Product form	*g/p*[a]	Temp. (°C)	Gas mix (%) CO_2	O_2	N_2/Ar[c]	Air	Best mixes[b]
Trout	*Onchoryncus mykiss*	Gobantes *et al.*, 2002	Colour	Fillet	0	0–2	0	0	0	0	2
					2	0–2	0	0	0	100	3
					2	0–2	80	0	20	0	1
		Aras Hisar *et al.*, 2005	Microbiology, colour	Fillet	0	4	0	0	0	0	3
					—	4	0	0	0	100	4
					?	4	40	30	30	0	2
					?	4	90	2.5	7.5	0	3
					?	4	100	0	0	0	1
					0	10	0	0	0	0	3
					—	10	0	0	0	100	4
					?	10	40	30	30	0	2
					?	10	90	2.5	7.5	0	3
					?	10	100	0	0	0	1
		Arashisar *et al.*, 2004	Microbiology, biochemical	Fillets	0	4	0	0	0	0	3
					—	4	0	0	0	100	3
					2	4	100	0	0	0	1
					2	4	90	2.5	7.5	0	2
					2	4	40	30	30	0	4
		Giménez *et al.*, 2002a	Sensory, microbiology, biochemical	Fillets	0	1	0	0	0	0	3
					—	1	0	0	0	100[f]	4
					?	1	50	10	40	0	1
					?	1	50	10	0	0	1
					?	1	50	20	30	0	2
					?	1	50	20	30 Ar	0	2
					?	1	50	30	20	0	2
					?	1	50	30	20 Ar	0	2

		Azizishirazi and Shahram Shekarforoush, 2010	Sensory, microbiology, TVBN	Gutted	0	3	0	0	0	0	2
					2	3	0	0	0	100	2
					2	3	60	0	40	0	3
					2	3	40	10	50	0	3
					2	3	60	10	30	0	1
		Choubert *et al.*, 2008	Microbiology, biochemical, colour	Fillet	—	2	0	0	0	100	3
					1.5	2	60	0	40	0	2
					1.5	2	60	0	40 Ar	0	1
Tuna	*Thunnus albacares*	Emborg *et al.*, 2005	Histamine, histamine-producing bacteria		0	1–3	0	0	0	0	3
					3		60	0	40	0	2
Wolf-fish	*Anarhichas minor*	Rosnes *et al.*, 2006	Sensory, microbiology	Portion	—	−1	0	0	0	100	3
					1	−1	60	0	40	0	1
					—	4	0	0	0	100	4
					1	4	60	0	40	0	2

Notes: Literature up to 1999 was reviewed by Sivertsvik *et al.* (2002).

[a] gas: product ratio of 0 = vacuum pack; ? means this parameter is not published;— refers to product held in open air with a theoretical infinite gas:product ratio; [b] 1 = best, 2 = second best etc.; [c] inert gas is always N_2 unless otherwise indicated; [d] gas mix supported by CO_2 emitter; [e] calculated rather than measured best gas mix; [f] air through an O_2 permeable film overwrap; [g] chitosan film; [h] 1% gas was CO.

with 12 of 19 tested datasets. They suggested that the differences might relate to fish pH, NaCl or lipid levels, which affect solubility but which were not included in their model.

With the expansion of aquaculture allowing shorter time between harvesting and packaging, a lot of packaging work has been carried out on pre-rigour fish (Adland Hansen *et al*., 2009; Bøknæs *et al*., 2002; Hansen *et al*., 2007, 2009a, 2009b, 2009c; Rosnes *et al*., 2003; Sivertsvik, 2007). Sivertsvik *et al*. (2004a, 2004b) determined Henry's constant at 0°C for cod, anglerfish, wolffish, tuna and salmon. Henry's constant was determined as averaging 42, 45, 49, 46 Pa ppm^{-1} (mg CO_2 per kg fish) (2.41, 2.25, 2.07 and 2.20 g CO_2/kg fish) mg CO_2/kg fish for the white fish with three different fat levels, giving Henry's constants of 49, 44 and 44 Pa ppm^{-1} (2.20, 2.07 and 2.07 g CO_2/kg fish) for the salmon, respectively, containing 9.9, 15.6 and 21.1% fat. The constant increased with increasing temperature (tested at −2°C, 0°C, 2°C and 4°C for cod and low lipid salmon). The researchers concluded that Henry's constant could be predicted by the solubility of CO_2 in water corrected for the total water and fat content of the fish. Sivertsvik *et al*. (2007) also modelled and optimised MAP for the extension of shelf life of pre-rigour farmed cod fillets. Like many earlier workers (e.g. Cann, 1984), they found that it was beneficial to include O_2 in the gas mix for such white fish species. This is because O_2 levels above 10% inhibit the reduction of trimethylamine oxide (TMAO) to trimethylamine (TMA), the main spoilage compound in cod and other gadoid species. They concluded that at 0°C with *g/p* of 2, the optimum gas mix was 37:63 CO_2:O_2, which they predicted would give lowest drip loss, TMA and TVBN while inhibiting microbiological growth and maintaining high odour scores and TMAO content in packaged farmed cod fillets. However, this combination would only be optimal for low pH (ca. 6.0) pre-rigour cod. Thus, in a different species (seabream), Giménez *et al*. (2002b) found that gas mixes containing 20% or more O_2 performed worse than MAP containing lower O_2 levels or VP, due to yellowing of the fillets in the gas mixes containing high levels of oxygen.

Ross and Dalgaard (2004) published a simplified equation for determining the equilibrium of CO_2 that takes account of the effect of temperature on Henry's constant. This does not account for the effect of pH or fish composition on the solubility of CO_2 in the fish. Although both of these can have a significant effect on CO_2 solubility (Gill, 1988), Dalgaard claims that this equation provides realistic predictions for concentrations of dissolved CO_2 (Dalgaard, 2009). An ideal model would include the effect of temperature, fish composition and pH on the equilibrium of gas composition. Recently, Fernández *et al*. (2010) developed equations for solubility of CO_2 in salmon held under different starting concentrations and *g/p*.

The solubility of CO_2 corresponded well with sensory and microbiological results, and was proposed as a scale-up factor for comparing experimental results with commercial packaging configurations. In our laboratory, we determined Henry's constant experimentally in high-fat king salmon at our experimental temperature (0°C) as 3.44 g/L atm (3.11 g CO_2/kg fish) and applied Devlieghere *et al*.'s

equations (1998) using the gas laws to make adjustments for the gas temperature at the time of packaging. We found that although increasing CO_2 decreased microbial growth, too much dissolved CO_2 gave raw and cooked salmon a carbonated mouthfeel (Fletcher *et al*., 2004). We modelled this effect and found the optimum level of dissolved CO_2 to be 0.5–1.0 (mL/g). Corbo *et al*. (2005) modelled the effect of storage temperature on bacterial counts under VP and two MAP conditions (Table 9.1), but they did not record *g/p*, thereby making it difficult for others to apply the results without using the same equipment and packaging materials. The SSSP also contains models for predicting shelf life of cod, plaice and salmon under different MAP regimes (0–100% equilibrium headspace CO_2 at 0–15°C) based on earlier publications (Dalgaard, 1995; Dalgaard *et al*., 1997). The models are based on the growth of *P. phosphoreum* in a laboratory medium under different CO_2:N_2 mixtures at different temperatures. The SSSP also includes models on the growth/no growth boundary for *L. monocytogenes* under MAP based on the work of (Mejlholm and Dalgaard, 2007). The software provides the ability to calculate headspace CO_2 based on equations published by (Ross and Dalgaard, 2004) but SSSP models do not yet include the effect of O_2 on shelf life.

9.6 Applying packaging technologies to products other than fresh fillets

Another area of change is the range of seafood products that are being subjected to MAP and VP. As well as fresh fillets, VP and MAP have now been successfully applied to a wide range of other fish products. MAP of molluscan shellfish will be reported in Chapter 10. Although little recent work has specifically addressed the packaging of crustaceans, crayfish have been stored under MAP (Gong *et al*., 2008), crab and crawfish under VP (Kannapha *et al*., 2008; Lyon and Reddmann, 2000) and SGS shrimp under both MAP and VP (Sivertsvik and Birkeland, 2006). Fresh (Hyung-Taek *et al*., 2002) and smoked roe (Jong Hyuk *et al*., 2006; Seung Hwa *et al*., 2009) has been packed under MAP and VP. Predictably, the storage of frozen fish (Dragoev and Balev, 2006; Malota and Halamcikova, 2007; Rodriguez *et al*., 2009a), frozen fish mince (Leelapongwattana *et al*., 2005; Sanchez-Alonso *et al*., 2007, 2008) and frozen mince balls (Ersoy and Yilmaz, 2005) have been improved by VP as this reduces oxidation of lipids and limits other oxygen-mediated biochemical deterioration. The shelf life of thawed fish can be improved by MAP (Bøknæs *et al*., 2001, 2002; Dalgaard *et al*., 2006; Fagan *et al*., 2004). Salted and desalted fish have improved storage lives when subjected to MAP (Erkan *et al*., 2002; Fernandez-Segovia *et al*., 2006; Magnusson *et al*., 2006) and VP (Erkan *et al*., 2009; Escriche *et al*., 2003; Fernandez-Segovia *et al*., 2003; Rodriguez *et al*., 2009b). Although not vacuum packaged in the USA owing to concerns over botulism, smoked fish products are commonly vacuum packed in many countries, with beneficial effects (Dondero *et al*., 2004; Leblanc *et al*., 2000; Ribeiro Neunlist *et al*., 2005). Smoked fish have also been successfully stored under MAP (Bugueno *et al*., 2003; Cakli *et al*., 2006; Muratore and Licciardello,

2005; Quinones *et al*., 2003). Processed products subjected to MAP include: semi-dried flounder (Seung-Taek and Hyun-Sook, 1999), fish cakes and burgers (Corbo *et al*., 2009; Del Nobile *et al*., 2009; Jeya Shakila *et al*., 2009; Metin, 2001, 2002) and other cooked products (Dragoev, 2008a, 2008b; Turkkan *et al*., 2010). Ready-to-cook products subjected to MAP include stuffed fish (Metin, 2003), while ready-to-eat products include fish salad (Metin *et al*., 2002a), gravid trout (Michalczyk *et al*., 2008) and maatjes herring (Lyhs *et al*., 2007). Ready-to-eat 'carpaccio' can also be vacuum packed (Paleari *et al*., 2009). Clearly MAP and, in many circumstances, VP will extend the shelf life of most seafood products. At this stage, the size of the extension and the best packaging system needs to be determined for each configuration. The only attempt at producing a generic model for products other than fresh fish has been that of Mejlholm and Dalgaard (2007) (see Section 10.5) for lightly preserved fish, but this study addressed only the food safety issue of *L. monocytogenes* and not shelf life. To make such a generic model for spoilage of fish and shellfish would not be possible at the moment because of the wide variety of SSOs that would need to be modelled for the different products. Additionally, the spoilage flora of many of these products have not yet been identified.

9.7 Combining packaging technologies with other treatments

One of the big pushes in MAP research over the last ten years has been to combine MAP (or sometimes VP) with other treatments, particularly other antimicrobial treatments. Most of these attempts have resulted in synergistic effects on microflora, with the combination resulting in longer storage life than either of the treatments on their own.

9.7.1 Heat

Combining vacuum packing with heat in the sous-vide process is a well-established technology, although the ability of the process to protect from botulism has been challenged (Hyytiä-Trees *et al*., 2000). The technology has recently been successfully applied to fish cakes (Jeya Shakila *et al*., 2009) and a range of other seafood products (Fagan and Gormley, 2005). Although heat processing usually results in a cooked product, the combined instantaneous heat treatment of fillet surfaces with vacuum packaging has been investigated with a view to extending the shelf life of a raw product (Perez-Alonso *et al*., 2004). This increased the shelf life of Atlantic pomfret from seven to 12 days when stored at 4°C.

9.7.2 Superchilling

As the freezing point of seafood is about −1.5°C, the temperature of product can be taken below 0°C without freezing it and a number of studies have combined this with MAP with commensurate increases in shelf life (Fernández *et al*., 2009, 2010;

Hansen *et al*., 2009b; Lauzon *et al*., 2009; Reynisson *et al*., 2009; Rosnes *et al*., 2006; Shu-Lai *et al*., 2010; Sivertsvik *et al*., 2003; Wang *et al*., 2008).

9.7.3 Irradiation

Although successful at inactivating micro-organisms without cooking in research trials, this technology has never achieved widespread commercial implementation, probably due to the cost of the equipment and resistance to the technology. However, as a post-packaging pasteurisation process, it is often applied in combination with VP and research continues to be published on the benefits of this approach (Chouliara *et al*., 2005; Mbarki *et al*., 2009; Moini *et al*., 2009; Panchavarnam *et al*., 2003). MAP has not been used in combination with irradiated fish, although a study comparing the two treatments showed MAP to have an advantage over irradiation that was due primarily to changes in colour, odour and texture of irradiated sea bass (Reale *et al*., 2008).

9.7.4 High-pressure processing (HPP)

This emerging post-packaging technology for inactivating micro-organisms in raw products is often applied in combination with vacuum packaging – for example, cold-smoked salmon (Lakshmanan *et al*., 2005) and fresh crab meat (Kannapha *et al*., 2008). However, the effect of the packaging is seldom investigated separately from the HPP, and only one study combining MAP with HPP on fish has been published (Amanatidou *et al*., 2000). Although HPP and MAP applied alone increased the shelf life of fresh salmon by two and four days, respectively, compared with VP, when HPP was applied to MAP, microbiological shelf life was increased by 12 days. Cruz-Romero *et al*. (2008) carried out a study on Pacific oysters (*Crassostrea gigas*) that had been treated with HPP and then stored under air, MAP or VP. Oysters stored under MAP or VP had reduced bacterial counts and thiobarbituric acid-reactive substance (TBARS) values were lower than those stored in aerobic packaging. Both of these studies show a synergistic effect on bacteria that is likely to apply to other finfish and crustaceans. However, neither study reported on the sensory properties of HPP products stored under MAP.

9.7.5 Carbon monoxide

Treatment of some fish with CO, particularly fish featuring red colours, enhances their appearance by eliminating brown colour development in products (Chi-Ching *et al*., 2001; Kristinsson *et al*., 2007). Minimal research has been carried out on the effect of combining CO treatment with packaging technologies in seafood. Jezek and Buchtova (2010) included 1% CO in MAP of carp and found better quality than air-stored controls, although no comparison was made with MAP without CO. Research including CO as part of the gas mix used in MAP of beef found that while it improved colour and slowed microbial growth, CO did not mask microbial spoilage in MAP (Hunt *et al*., 2004).

9.7.6 Active packaging

Apart from moisture and O_2 absorbers noted in Section 7.2.3, a TMA scavenger has been used in combination with MAP (Franzetti *et al*., 2001). The absorber was embedded in an expanded polystyrene tray and increased shelf life of products by delaying growth of Gram-negative and sulphide-producing bacteria, scavenging most of the TMA from the headspace and favouring growth of microbial strains that were not involved in the generation of off-flavours.

9.7.7 Biopreservatives and their bacteriocins

Biopreservatives are benign micro-organisms that prevent unwanted bacteria from growing, while bacteriocins are short poly-peptides produced by biopreservative organisms as antagonists to competing microflora. Biopreservative *Bifidobacteria* (Altieri *et al*., 2005) acted synergistically with MAP on plaice and *Carnobacteria* to improve storage life of VP cold-smoked salmon (Duffes *et al*., 2000). The approved bacteriocin nisin enhanced the storage life of fish in combination with MAP (Cabo *et al*., 2005; Tsironi and Taoukis, 2010) or VP (Neetoo *et al*., 2008b; Zuckerman and Ben Avraham, 2002). Microgard® is another bacteriocin successfully applied with VP (Zuckerman and Ben Avraham, 2002) and others have been considered for sous-vide products (Rodgers, 2004).

9.7.8 Additives

Many different additives have been added to seafood to improve its storage quality in combination with VP or MAP. Most of these were incorporated for their antimicrobial properties and include traditional additives either alone or in combination: NaCl, organic acids (acetic and citric acid), salts of organic acids (citrates, acetates, diacetates, lactates, sorbates, phosphates, pyrophosphates), sodium benzoate and EDTA (Aytac *et al*., 2000; Boskou and Debevere, 2000; Cabo *et al*., 2005; Fernandez-Segovia *et al*., 2003; Frangos *et al*., 2010; Goulas and Kontominas, 2007a; Heidmann Soccol *et al*., 2005; Lauzon *et al*., 2009; Magnusson *et al*., 2006; Manju *et al*., 2007a, 2007b, 2008; Metin *et al*., 2002b; Neetoo *et al*., 2008a; Park et al., 2009; Payap *et al*., 2005a, 2005b; Poulose *et al*., 2010; Sallam, 2008; Schirmer *et al*., 2009; Shalini *et al*., 2000, 2001; Tassou *et al*., 2004; Vogel *et al*., 2006), respectively. As well as synthetically produced chemicals, a range of natural preservatives have also been applied in combination with MAP and VP, with varying degrees of success. These include dispersion coatings of the fish itself (Panchavarnam *et al*., 2003) and herbs and natural oils with oregano (Frangos *et al*., 2010; Giatrakou *et al*., 2008; Goulas and Kontominas, 2007a; Kostaki *et al*., 2009), rosemary (Dragoev, 2008a, 2008b) and thymol (Altieri *et al*., 2005; Corbo *et al*., 2009; Kykkidou *et al*., 2009) successfully improving storage life while others had minimal effect (Fernández *et al*., 2009). Rather than antimicrobials, other additives were investigated in MAP and VP as antioxidants to control oxidation (Dragoev, 2008a, 2008b; Ruff *et al*., 2003; Sajid and Soottawat, 2010; Sanchez-Alonso *et al*., 2008; Seung-Taek and Sang-Woo, 1999; Yi-Chen *et al*., 2008) and with maltodextrin as an osmoregulator (Tsironi and Taoukis, 2010).

9.8 Conclusions

Although VP and MAP have been used to improve the storage life of chilled seafood since the 1930s, they remain the subject of much research effort as indicated by the 180 publications that have emerged from the area over the last ten years and which have been cited in this review. Many of these studies are simply the result of applying the established technology to new fish species or to new product forms and quite a number are the result of seeking synergisms between VP and MAP and other technologies to improve storage life. Despite appearing in the scientific literature, few of these combinations of MAP or VP with other technologies and few of the new fish species and product forms are actually being sold commercially. There is a relatively large gap between a successful scientific study and a successful product. The reasons for this gap need to be clearly identified. Given the effort required for, and the cost associated with the carrying out of such studies, scientific studies should be carried out only on products that are likely to succeed and more effort should be focused on establishing the business case for the product before doing the science unless truly novel technologies are envisioned. In the last decade, there have been a few notable technological advances in MAP such as the use of SGS and CO_2 emitters. However, again these advances have yet to be commercialised, indicating that there are barriers other than science that are holding them back. These barriers may include:

- difficulties in having new technologies accepted by regulatory bodies
- concerns from producers about consumer reaction to chilled products with very long shelf lives
- the cost of implementing new technologies outweighing the benefits.

Similarly, there have been many advances in the development of antimicrobial films, but very little if any commercialisation of these, probably relating to the cost escalations and technical challenges of scaling up from a successful laboratory implementation to a commercial system for producing films or in some cases to regulatory restrictions. For seafood products that are already being successfully marketed, advances in the ability to model changes in gas composition during storage and to model the response of micro-organisms to different packaging conditions are making it much easier to select optimal packaging conditions to assure quality and safety.

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Advances in vacuum and modified atmosphere packaging of shellfish

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Abstract: This chapter reviews the state-of-the-art in the application of modified atmosphere packaging (MAP) and vacuum packaging (VP) of bivalves, cephalopods and other shellfish. Studies that have advanced our understanding of the efficacy of combining these technologies with other conservation techniques are documented and detailed. Combining technologies stabilizes fresh and cooked shellfish and can lead to a longer shelf life and improved quality of packaged products.

Key words: bivalves, cephalopods, shellfish, EAM, VP, alive, raw, cooked.

10.1 Introduction

Demand is increasing for foodstuffs which have a long shelf life yet are minimally processed, free from additives and safe for consumption, as consumers consider them to be more healthy or natural. Some consider shellfish (molluscs, crustaceans and cephalopods) to be products of this type. The increased demand has led to changes in methods of production and processing of foodstuffs to safeguard public safety, since these foods may also pose a greater risk to consumers.

Crustaceans differ in composition and therefore have different spoilage patterns that make them highly susceptible to decomposition caused by postmortem microbial growth and biochemical reactions which occur under refrigerated storage. Therefore, the food industry needs to seek alternative methods to maintain product freshness.

Crustaceans, like shrimps and prawns, are rich in proteins, minerals and vitamins and have a high content of free amino acids, some of which contribute to

their characteristic flavor (Finne, 1992). Because of this unique composition, their quality and flavor deteriorates rapidly, even under cold storage, due to the presence of spoilage bacteria and enzymes, but also due to melanosis (Martínez-Álvarez *et al.*, 2005). Nonprotein nitrogen compounds contribute to the specific aroma and flavor, and the increase or decrease of these compounds could be related to their spoilage and overall quality. The same can be said for bivalve molluscs like blue mussels, oysters and scallops, which possess a similar composition to crustaceans but can contain high glycogen levels which can assist in extending product shelf life as glycogen slowly breaks down to form lactic acid (Martino and da Cruz, 2004). Other molluscs, such as cephalopods, constitute an important marine resource and are increasingly appreciated by consumers. Furthermore, they are currently recognized as the most promising resource due to their abundance and rapid population growth (Atrea *et al.*, 2009). However, cephalopods contain large quantities of endogenous enzymes that promote rapid protein and nitrogen-containing non-protein (such as ammonium, urea) degradation when fresh (Seibel *et al*., 2004). Their high proteolytic activity also favors the proliferation of altering microorganisms which leads to a rapid loss of quality (Hurtado *et al.*, 1999). Consequently, the majority of cephalopods are stored and marketed in frozen form.

Microbial activity plays a major role in the deterioration in quality of refrigerated shellfish. The presence of microorganisms in molluscs is strongly related to the environmental conditions at the harvesting site, as well as those encountered throughout the cold-chain distribution system. Factors such as the microbiological quality of the water, its temperature and salinity, the presence of natural bacterial communities in the mollusc, the food it has ingested, the capture methods and refrigeration conditions are particularly important (Sivertsvik *et al*., 2002). The microbiota can vary, and may include *Serratia* spp., *Proteus* spp., *Clostridium* spp. and *Bacillus* spp. among others. The bacteria *Vibrio parahaemolyticus* and *Vibrio vulnificus* are noteworthy pathogens that can be transferred from the environment to the public through the consumption of raw shellfish, especially bivalve molluscs (Gram and Huss, 2000; Mahmoud, 2009). Viruses are probably more important than bacteria in terms of transmitted illnesses. Bivalve molluscs are especially important in this regard since they are mainly filter feeders and selectively accumulate bacteria and viruses from the surrounding water (Richards, 1988). A greater incidence of infections and outbreaks attributed to food-transmitted viruses has been observed world-wide during the last decade (Baert *et al*., 2009).

Conventional purification and gentle heat treatment, before or after packaging, can eliminate the majority of bacteria in shellfish. However, the inactivation of viruses ought to be pursued using more rigorous methods. The European Union recommends effective heat treatment in addition to improved control of purification systems and waste water discharge in the vicinity of the cultivation areas. Heat treatment at 90°C for 1.5 min appears to be effective to inactivate Norwalk-like viruses (EC, 2002). In addition, HP processes can control the presence of *Vibrio* spp. (Calik *et al.*, 2002).

The efficacy of food conservation involving active gases or the absence of air is well established. For decades, food technologists have reported how foodstuffs packaged under vacuum or modified atmospheres can reduce respiratory processes and inhibit the growth of microorganisms. Modified atmosphere packaging (MAP) and vacuum packaging (VP) are useful techniques for prolonging the shelf life of perishable products. Despite their advantages, caution is nonetheless advised since inadequate handling and/or prolonged storage may favor the proliferation of hazardous microorganisms and pose a risk to public health. MAP combined with refrigeration is widely employed to delay the deterioration of refrigerated fresh food. The shelf life of shellfish products can be increased by storage in MAP, but this is dependent on the quality of the raw material, the temperature control employed, the mixture of gases used and packaging materials selected. For example, an extension in the shelf life of cooked shellfish by 100–200% can be obtained when optimal storage conditions are achieved (Sivertsvik *et al.*, 2002). The effectiveness of MAP in increasing the shelf life of shellfish has also been enhanced in the last decade with the development of modern packaging materials and systems (e.g., films with improved permeability, etc.).

The principal aim of MAP is to conserve shellfish in good condition during distribution and sale. Coupled with a renewed consumer interest in fresh shellfish, MAP is a promising technology from a commercial and economic perspective. The format of the MA retail package is also advantageous since it is easy to manipulate, attractive and resistant, protects the product from external contamination and safeguards against the release of exudations or smells. Major advances in the application of MAP and VP in the conservation of fresh and cooked shellfish have occurred when they are combined with other conservation treatments or technologies (traditional or emergent). Leistner and Gould (2002) argued that safety and quality is achievable by combining technologies. The following section discusses this area. Section 10.3 then discusses the effective application of traditional, vacuum and MAP packaging to improve shellfish quality and Section 10.4 outlines likely future trends.

10.2 Combination of modified atmosphere packaging (MAP) and vacuum packaging (VP) with other treatments

Efforts in combining MAP and VP technology with other treatments prior to or after packing have increased during the last decade with encouraging results, either to improve the shelf life of raw products, or as an attempt to produce a unique range of ready-to-eat products. Yet, caution is still advised and more research is needed before these methods can be definitively applied to shellfish in general and to bivalves and cephalopods in particular. Documentation in this area is scarce and relates mainly to bivalves packaged under vacuum and presented as final processed and prepared products. The vacuum packaging technique may, in particular, be used together with pasteurization (gentle heating) followed by product storage at refrigeration temperature. A longer marketable period for

fresh shellfish is obtained when both are combined as conservation techniques. Vacuum packaging prevents the development of disagreeable smells and flavors by delaying the onset of oxidative processes and the appearance of altering volatiles. However, due to the physicochemical properties associated with shellfish, shelf life extension is more limited than for other foodstuffs, even when correct handling measures are adopted up to the point of sale.

10.2.1 Bivalves

The conservation of raw molluscs under MAP or VP can be prolonged when these packaging methods are combined with prior treatments. Accordingly, washing of shelled mussels with ozonated water before packaging under vacuum and storage at 5°C led to a shelf life of 12 days compared to only 9 days for a control without the pre-treatment (Manousaridis *et al.*, 2005). Similarly, the packaging of shucked scallops (*Pecten alba*) under vacuum with the addition of 0.1% potassium sorbate provides an acceptable product during 28 days storage at 4°C (Bremner and Statham, 1983). Aside from the microbiological viability, sensorial studies suggest that a product with a higher-quality texture is obtained when bivalves are packaged under vacuum using a flexible material which is impermeable to oxygen and combined with some other form of preservation. For example, before subjecting the VP to heat treatment, mussel meat (*Perna perna*) was pre-treated with different combinations of sodium chloride and lactic acid. After 90 days, the durability and consistency of the mussels were inversely proportional to the quantity of lactic acid employed (Souza *et al.*, 2008).

Proposals for the commercialization of mussels using VP and thermal treatment are occasionally presented though patents. For example, mussels packaged under vacuum using flexible and thermo-retractable material can be cooked within the package. This ensures that the meat remains well-adapted inside the shell and reduces the loss of intervalval fluid and contamination during handing (Boylan, 1987). In other cases, partial cooking inside the packages is employed (Mulloy, 2008). Packages formed by a tray and film and sealed in the upper part that are subjected to thermal treatment have also been proposed for the commercialization of mussels (Keohane and Murnane, 2008).

Studies in the scientific literature employed different conditions of heat transmission for the pasteurization of molluscs under vacuum. For example, mussels were refrigerated at 5.5°C for 21 days following pre-treatment at 100°C for 17–35 min (Skipnes *et al*., 2002). The process of preparing ready-to-eat fried mussel meat also allowed the product to be maintained in a good condition even at ambient temperature (Bindu *et al.*, 2004), although in this case the temperature applied was 121°C. This same period was achieved with clams using thermal treatment under vacuum (Bindu *et al.*, 2007). The combination of preservation methods leads to ready-to-eat products. For example, Haamer (2000) described the microwave cooking of mussels packaged under vacuum using hermetic flexible plastic material. This study reports how vapor produced during steaming can be released through an orifice in the upper part of the package, which closes when heating is

over. The vacuum increases inside the container as the steam condenses. Kyriazi-Papadopoulou *et al.* (2003) obtained a shelf life of 70 days at 2–3°C for mussel meat (*Mytilus galloprovincialis*) packaged under vacuum by combining different technologies. After steaming the mussels at 80°C, the meat was submerged in brine (4%), dried (60–65°C) and smoked at 80°C prior to packaging, which influenced the characteristics of the final product.

Recent studies with oysters (*Crassotrea gigas*) have demonstrated the effectiveness of MAP or VP technology combined with high pressure (HP). Cruz-Romero *et al.* (2008) suggested that the treatment can inhibit or reduce changes in refrigerated oysters. The authors state that HP treatment in combination with MAP/VP and an adequate refrigeration storage temperature can extend the shelf life and improve the safety of oysters, leading to an acceptable product for up to 21 days at 0°C.

10.2.2 Cephalopods

The shelf life of refrigerated raw cephalopods is relatively short. Therefore, an increase is desired through the combined effect of packaging and additives or through the specific characteristics of the packaging materials. The combination of MAP with trays containing absorbers of volatile amines and liquids liberated from the food has been studied in cuttlefish (*Sepia fillouxi*). The results showed that the trays sequestered a large fraction of the trimethylamine (TMA) that accumulated in the headspace and reduced the level of disagreeable odors by delaying microbial development, especially that of spoilage bacteria (Franzetti *et al.*, 2001). Furthermore, the use of a new moisture-adsorbent material for the conservation of squid in MAP led to lower values of chemical indices analyzed after 11 days of storage (Albanese *et al.,* 2005). Other authors have studied the combination of VP with oregano essential oil (EO) on the stability of octopus (*Octopus vulgaris*) refrigerated at 4°C. The sensorial results with regard to odor demonstrated a marketable period of 3 days for the VP product, 11 days for VP with EO (0.2%) and 13 days for VP with EO (0.4%). In agreement with other sensorial characteristics, a product with a marketable period of 6 days was obtained for octopus packaged in air, 9 days under vacuum and 17 and 23 days under vacuum and EO (0.2% or 0.4%), respectively (Atrea *et al*., 2009).

10.3 Effective application of traditional, VP and MAP to improve shellfish quality

The application of packaging technologies such as VP and MAP to shellfish has proved to be very effective. Studies have shown that shellfish in VP and MAP are better quality than control products conserved by refrigeration. The areas explored by current studies of MAP and VP for the preservation of the quality of raw and/or cooked shellfish are similar to those explored by the first experiments in this area: the selection and optimization of the gas or gas mixture and the

refrigeration temperature. Recent studies have shown that the packaging material characteristics, such as the permeability of the packaging film to different gases (Pastoriza and Bernárdez, 2010), influence the product quality. It remains the case that more studies focus on the applications of MAP and VP for fish preservation than shellfish preservation. In general, advances in understanding of the application of MAP and/or VP technologies for shelf-life extension have come about more slowly for shellfish than for finfish.

10.3.1 Live bivalves

Mussels

The mussel sector has shown little innovation in procedures or techniques for commercialization of the live product. It presently continues to employ very similar techniques to those used by its predecessors, which only entail the extraction of the mussel from the area of cultivation, separation, purification, occasional removal of the byssus, superficial cleaning of the valves and packing. The major differences lie with the substitution of manual labor with mechanization in some stages of the process – for example, in brushing or byssus removal. In general, the technology is fairly artisanal.

Live mussels are usually packaged for commercialization in plastic or jute mesh bags and wooden or plastic boxes of various sizes. These packaging methods are impractical and need to be improved. The major disadvantages of mesh bags are their inconvenience and lack of hygiene due to continual drainage of the intervalval fluid from the molluscs. Wooden boxes are heavy and cumbersome which prevents easy handling during commercialization. They are also not hermetic and present similar problems to the mesh bags, which are tackled by placing them in cardboard boxes for distribution. Although plastic boxes are completely closed and free from inconvenient dripping, the mussels are in contact with air which is also not optimal for quality maintenance once the live bivalves have been removed from their natural habitat.

Live molluscs are increasingly commonly presented to the marketplace in hermetic packages. Interest is focused on achieving stability and increasing their life span, thus maintaining their capacity to respond to stimuli and the sensorial qualities that the consumer demands. The results of the majority of studies on the conservation of live bivalves (mainly mussels) in hermetic packages are patent-protected. In general, they describe procedures that take place before packaging such as shell brushing, washing and byssus removal (Guillen, 1999; Pastoriza *et al*., 2006). Each of these pre-treatments affects the stability of mussels, implying that posterior conditioning of the mussels is required prior to packaging to allow them to recover from stress incurred during harvest. Conditioning entails placing the mussels for a time in salt water at refrigeration temperatures followed by closed and refrigerated conservation (Pastoriza *et al*., 2006; Vette *et al*., 1994).

MAP for live mussels requires an environment different from air so that the product maintains a natural and fresh appearance and its quality is guaranteed

during a longer period than is possible with standard packaging. Pastoriza *et al.* (2004) documented greater survival in mussels (*Mytilus galloprovincialis*) during commercialization by using gas mixtures that favored respiration and which were less harmful toward bivalves than other mixtures which contain CO_2. Despite a large bacteriostatic potential, the majority of studies demonstrate that CO_2 in the package can be prejudicial for bivalve conservation, since it can penetrate even closed mussel valves. The high solubility of CO_2 in water can produce changes in the pressure inside the package and lead to acidification. Therefore, in the majority of cases this gas is maintained below the toxicity threshold (of 1%) or eliminated completely (Joseph and Blaizat, 1990; Keizer, 2000; Pastoriza *et al*., 2006; Vos and Nijhof, 2001). Pastoriza *et al.* (2004) observed that the mortality rate of molluscs increased with higher concentrations of CO_2in the initial gas composition. Furthermore, CO_2 generated by respiration of the molluscs in a closed and humid environment may accelerate the build-up of CO_2 to toxic levels.

In many cases, the bivalves are forced to keep their valves closed, yet this compels them to adopt a near anaerobic metabolism while avoiding the leakage of the intervalval liquor (Keizer, 2000; Pastoriza *et al*., 2006; Vos and Nijhof, 2001). It is also beneficial to create an atmosphere in the interior of the package that decreases the metabolism of the bivalve and the ejection of waste products that can stimulate bacterial growth and shorten the conservation period (Vos and Nijhof, 2001). The metabolic rate decreases as oxygen concentrations increase above the background air content of 20.8%. The oxygen content must not exceed 80% to avoid possible explosion hazards at the packaging station or plant (Vos and Nijhof, 2001). For prolonged conservation of live mussels, high concentrations of oxygen in the interior of the package are recommended (Kosaka, 2006; Pastoriza *et al*., 2006).

The shelf life of live mussels varies between 5 and 15 days, depending on the treatment prior to packaging, the gas mixture and the refrigeration temperature during the commercialization. A low temperature has been recommended in order to maintain the bivalves in a lethargic state (Joseph and Blaizat, 1990). Other factors, such as the period of collection or capture, the species or the environmental conditions, can cause variations in the shelf life of molluscs packaged in modified atmospheres. Harding *et al.* (2004) found that the average shelf life of mussels harvested in Canada was influenced by the season and the type of storage, with late autumn to spring being the period where the mussels were in the best physiological condition for posterior processing. The viability of MAP mussels captured in the northwest of Spain also varied as a function of the season and commercial size. Accordingly, results obtained in the authors' laboratory (IIM-AECSIC, Vigo, Spain) within the framework of the AquaGair Project (Fig. 10.1) indicate that small mussels harvested in March had a significantly greater percentage mortality after 7 days compared to medium-sized mussels. Moreover, the medium-sized mussels showed a higher mortality in spring.

Clams

The effect of an oxygen-enriched storage atmosphere was studied in live clams (*Ruditapes decussatus*). Comparison of a batch with MAP and a control stored in

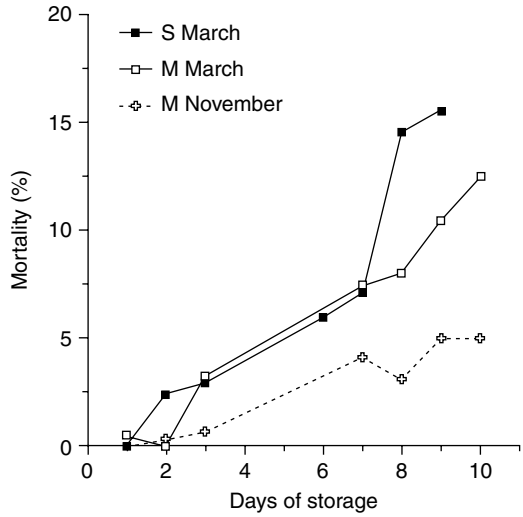

Fig. 10.1 Percentage mortality in small (S) and medium (M) Mediterranean mussels in different seasons packaged with high oxygen concentrations (80%).

air, both at 6.0 ± 0.7°C, revealed that storage under high O_2 did not promote the growth of generic microorganisms or pathogenic bacteria and maintained the characteristic sweetness of taste for a longer period. This led to an acceptable sensory quality in live clams at the end of a 6-day storage period (Gonçalves *et al.*, 2009).

10.3.2 Raw and cooked bivalves

The stability and quality of shelled raw mussels (*Mytilus galloprovincialis*) packaged in modified atmospheres increases with higher concentrations of carbon dioxide in the gas mixture (Caglak *et al.*, 2008; Goulas, 2008; Goulas *et al.*, 2005). These authors compared the conservation of mussel meat stored under refrigeration (2–4°C) in air, VP and MAP. Based on an evaluation of odor and flavor, Goulas *et al.* (2005) reported a marketable period of 14–15 days with 80% CO_2, 11–12 days with 40–50% CO_2, 10–11 days for VP and 8–9 days for the control in air, concluding that MAP increased the shelf life of the product by 5 days compared to air-packaged samples. In addition, Caglak *et al.* (2008) and Goulas (2008) documented a longer shelf life with MAP than VP for fresh mussel muscle.

Results for packaged and refrigerated raw scallop differ according to experimental conditions. Fewer bacterial numbers were again reported by Kimura *et al*. (2000) in scallop muscle packed under modified atmospheres with higher concentrations of CO_2 in the mixture. However, this study recommended the use of 100% O_2 in order to delay the development of *rigor* and the accumulation of octopine, as well as smaller changes in ATP and pH. These effects, in addition to the reported microbial count, prolonged shelf life by nearly 2 days for scallop adductor muscle

at 5°C (Kimura *et al*., 2000). In fresh scallop (*Argopecten purpuratus*), using different temperatures and initial gas mixtures, a predictive and statistically validated mathematical model was used to evaluate alternative package materials and formats (size, material and thickness) of MAP products. The optimum shelf life conditions for scallops were 21 days at 0°C with a gas mixture consisting of 60% CO_2:10% O_2:30% N_2 and 5–7 days for a refrigerated control at the same temperature (Simpson *et al.*, 2007). On the other hand, defrosted scallop meat (*Pecten alba*) packaged with 100% CO_2 was maintained at acceptable conditions for 22 days of storage at 4°C (Bremner and Statham, 1987). Sang-Moo (1996) found that the use of either vacuum packaging in combination with steam cooking for 10 min or packaging with nitrogen only, provided a longer shelf life (36 days) for vieira (*Patinopecten yessoensis*) at 5°C when compared to controls (30 days). However, this author concluded that no significant differences were observed in the bacteriostatic effect between the two packaging formats employed.

10.3.3 Raw cephalopods

Information concerning the packaging of cephalopods with MAP and VP is scarce and, furthermore, the behavior of different species during storage can be very different. Accordingly, bulk storage of gutted lesser flying squid (*Todaropsis eblanae*) and gutted octopus (*Eledone cirrhosa*) in controlled atmospheres showed that, during spoilage, the content of TMA-N and TVB-N at the end of storage were notably higher in the squid than octopus. For both species, the gas mixture with the higher content of CO_2 was more effective in reducing spoilage (Ruiz-Capillas *et al*., 2002).

Packages containing a low content of CO_2 (20%) were efficient in prolonging the shelf life of fresh squid (*Sepia officinalis*) at 3°C. The microbiological results were always lower for the packaged sample and the total viable count of psychotropic microorganisms on day 9 was at least ten times lower than the control (Albanese *et al*., 2005). For the same species, Speranza *et al.* (2009) observed that high concentrations of CO_2 (95%) influenced shelf life negatively, giving rise to dramatic changes in cephalopod color. They recommended using no greater than 40% of this gas. CO_2 concentrations of 45% were used by the authors of the IIM-AECSIC laboratory (Vigo, Spain) for the packaging of gutted and defrosted Patagonian squid (*Loligo gahi*). From a sensorial standpoint, MAP samples stored at 4°C increased product shelf life by 4 days when compared to the equivalent sample held in air. As can be observed in Fig. 10.2, the total viable counts of mesophilic microorganisms were always higher in the control sample. On day 5, the aerobic mesophiles in the control sample increased by more than 1.5 log units compared to the sample held under modified atmosphere (unpublished results).

10.3.4 Other shellfish

One can find other shellfish, such as gastropods, in local markets which often have been vacuum packed following thermal pre-treatment and presented naturally in

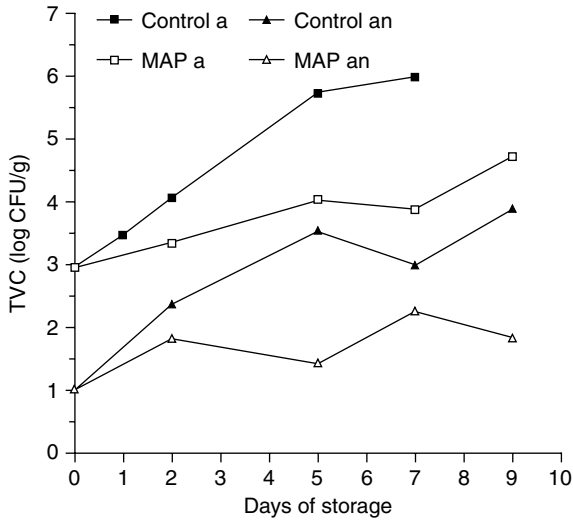

Fig. 10.2 Total viable count (TVC) of aerobic (a) and anaerobic (an) mesosphilic microorganisms in Patagonian squid stored at 4°C in air and MAP.

a sauce. However, scientific information on combined pre-treatment and VP or MAP packaging is scarce with regards to, for example, periwinkles, conches and whelks. A comparative study with abalone (*Haliotis asinina* Linnaeus) during refrigerated storage at 2 ± 1°C for different packaging demonstrated the effectiveness of conservation under modified atmospheres. After 5 days in air and vacuum, the total counts reached 6 log CFU/g sample, whereas this limit was registered after 15 days in MAP. The sensorial results were well correlated with the microbiological results, indicating a shelf life of 3 days for the control and vacuum packed abalone meat and up to 15 days for the batch packaged with 40% CO_2:30% O_2:30% N_2 (Sanguandeekul *et al.*, 2008). Abalone is a very appreciated and sought-after product due to the unique flavor and texture associated with its meat. Abalone cultivation and consumption is increasing and, thus, an opportunity exists for its sale as a fresh product under modified atmospheres. Studies on the quality of packaged abalone meat have led to proposals for an alternative method to quantify the freshness index based on the results of biochemical and instrumental analyses (Siripatrawan *et al.*, 2009).

10.4 Future trends

The application of combined preservation techniques is considered as a future alternative in the stability of foods in general and shellfish in particular. The study and application of combined techniques has already shown promising results in shellfish (Goulas, 2008; Rong *et al.*, 2010; Shengmin, 2009), which is reflected by the tremendous interest being expressed in this area. The application of pre- and

post-treatment on packaged shellfish may be an interesting solution in the attainment of higher-quality products through their direct effects on bacteria, fungi and viruses.

Alternative decontamination/disinfection treatments have demonstrated a great potential to decrease pathogenic bacteria, yet their effect against viruses is questionable (Baert *et al.*, 2009; Lees, 2000; Murchie *et al*., 2005). Recent studies have indicated the global preoccupation of increasing infections and outbreaks attributed to viruses transmitted by food. This reinforces the need to acquire knowledge of the effect of conservation methods toward different viruses (Baert *et al*., 2009). Of particular interest for molluscs is the understanding of the effectiveness of disinfection methods for fresh products and the purification procedures of live bivalves to reduce the viral load. The studies indicate that viruses persist in refrigerated, acidified and frozen food and in packages with modified atmospheres or dry conditions (Baert *et al.*, 2009; Bidawid *et al.*, 2001). Alternative purification systems or decontamination technologies are, therefore, considered necessary to decrease the viral load in bivalve molluscs since current depuration is insufficient to avoid outbreaks of illness (Omura, 2007; Tian *et al.*, 2007). Preservation methods which lead to microbial inactivation, such as thermal treatment, processing at high hydrostatic pressure and irradiation are intervention strategies for both bacteria (Arvanitoyannis *et al.*, 2009; Büyükcan *et al.*, 2009; Kanatt *et al.*, 2006; Mahmoud, 2009) and viruses (Kingsley *et al.*, 2007).

Table 10.1 lists easy-application technologies that could be used individually or combined, and before, during or after packaging, to provide products with a higher guarantee of quality, stability and security.

Once the effectiveness of each of the procedures has been determined, the adequate choice of treatment, or combination thereof, will depend on the characteristics of the raw materials and/or products to package and on the need to secure

Table 10.1 Technologies that could be applied either individually or combined for the conservation of shellfish packaged as MAP and/or VP

	Effective procedures for increasing shelf life
Before packaging	Superficial decontamination of raw materials or prepared products, for example, pretreatment with treated water or germicide (chlorinated, electrolyzed, ozonated) or pulsed light
During packaging	Additives incorporated in packaging material or food, for example, natural compounds with antimicrobial or antioxidant activity
	Aseptic control of the process and packaging material
	Packages with materials that protect from aromas and absorb undesirable compounds and odors
After packaging	Refrigeration during transport and storage and their control to the point of consumption
	Application of high pressure
	Application of irradiation

quality and safety. The synergistic combination of different disciplines to achieve an optimum result in delivering food quality and safety marks a new era in the evolution of food science and technology as it specifically pertains to shellfish products.

10.5 Sources of further information and advice

Legislation

Garrett *et al*. (1996) recommend consulting the criteria established by the National Advisory Committee on Microbiological Criteria for Foods (NACMCF) from the assessment of safety issues related to packaging under vacuum and/or MAP of refrigerated raw seafood products. They address the conditions which should be considered before packaging molluscs and to the control of time/temperature and other monitoring criteria prior to commercialization. They also contribute supporting documentation related to increased safety of seafood products.

The FDA in 2001 licensed ozone as antimicrobial agent for food processing and preservation.

Directive 1991/CEE and FDA in 2003 concerns the microbiological quality of live bivalve molluscs and will be evaluated on the basis of the level of *E. coli* and fecal coliforms.

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Council Directive 91/492/CEE of July 15, 1991 lays down health conditions for the production and placing in the market of live bivalve molluscs destined for direct human consumption or transformation prior to consumption. With the exception of the direction relating to depuration, the present Directive shall apply to echinoderms, tunicates and to marine gastropods. Available at: http://www.euseafood.com/legislation/Directive%20_492EN.pdf. Accessed December 28, 2009.

Council Directive 91/493/CEE of July 22, 1991 lays down health conditions for the production and placing in the market of fishery products destined for human consumption.

Royal Decree 1111/1991 of July 12 (BOE July 17, 1991) modifies the Technical Health Regulations of food additives approved by the Royal Decree 3177/1983 of November 16 and modified by the Royal Decree 1339/1988 of October 28. Concerns whether packaging gases should be considered as food additives.

Royal Decree 1334/1999 of July 31 (BOE August 24, 1999) approves the general rules for labeling, presentation and advertising of foodstuffs. Concerns the obligation of highlighting on the package 'packaged in a protecting atmosphere'.

Royal Decree 121/2004 of January 23 (BOE 5 February 2004) concerns the identification of the fishery, aquacultural and live, fresh, refrigerated or cooked shellfish products.

Regulation (EC) No. 852/2004 of the European Parliament and of the Council of April 29, 2004 concerns the hygiene of foodstuffs and lays down the general rules for the operators of food businesses in the hygiene of foodstuffs.

Regulation (EC) No. 853/2004, article 3, of the European Parliament and of the Council of April 29, 2004 lays down specific rules of hygiene of foodstuffs of animal origin. Section VII addresses bivalve molluscs.

Regulation (EC) No. 854/2004 of the European Parliament and of the Council of April 29, 2004 lays down specific rules for the organization of official controls of products of animal origin destined for human consumption. Annex II, pages 96–104, addresses bivalve molluscs.

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11

Solubility of carbon dioxide in muscle foods and its use to extend the shelf life of packaged products

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Abstract: The effect of CO_2 on spoilage bacteria is usually directly related to the partial pressure of CO_2 and the amount of dissolved CO_2 in the product containing the bacteria. The successful application of modified atmosphere (MA) packaging of muscle food products therefore relies on knowledge of the solubility of CO_2 in the product. Amounts of dissolved CO_2 in MA packaged foods are dependent on several factors, including temperature, pressure, packaging material and ratio between gas and product. In addition, CO_2 can be added either prior to packaging or produced in the package, which affects the amount of dissolved CO_2.

Key words: carbon dioxide, shelf life, solubility, models.

11.1 Introduction

The first recorded experiment on using CO_2 to extend shelf life of foods was performed in 1848, when Bethell patented a method for preserving milk using CO_2 (Valley, 1928). However, no results are available concerning the effectiveness of this method. During the last decades of the nineteenth century, various experiments showed that CO_2 has inhibitory effects on some bacteria, whereas others were unaffected (Valley, 1928). The first scientific paper to discuss the effect of CO_2 on the storage quality of food was 'Antiseptische Eigenschaften der Kohlensäure' (Kolbe, 1882), which recorded an extended storage life for beef placed inside a steel cylinder filled with a CO_2 atmosphere. During the 1920s and 1930s, several publications confirmed the effect of atmospheres with elevated carbon dioxide levels on shelf life. However, it was not until the development of cheap barrier

plastic materials and chilled distribution that the technique was used commercially. Marks & Spencer was a notable early user, launching MA-packaged products around 1980. Since then, CO_2 and MA-packaged products have been used worldwide for a large range of foods, becoming the leading mild preservative method.

The effect of CO_2 on spoilage bacteria is usually directly related to the partial pressure of CO_2 and the amount of dissolved CO_2 in the product containing the bacteria. Hence, the understanding of how CO_2 affects microorganisms and how this relates to the partial pressure and amount of dissolved CO_2 is of vital importance in optimizing modified atmosphere (MA) packages. This chapter will summarize current knowledge about how CO_2 dissolves and acts in muscle foods, and look briefly into alternative and novel methods for generating and using CO_2 as a preservative agent.

11.2 The principle of modified atmosphere packaging (MAP)

The principle of MA packaging is the replacement of air inside a hermetically sealed package with a different gas mix, normally using increased levels of CO_2 (25–100%), balanced with nitrogen (N_2) and/or oxygen (O_2). After its introduction into the pack, the gas mix is not controlled or manipulated further. However, the concentrations of the different gases will inevitably change as they respond to the product; CO_2 dissolves into the water and fat phase of the product, while the partial concentration of the less soluble gases (N_2, O_2) increases. When CO_2 dissolves into the food, a volume contraction termed 'snug down' occurs in flexible and semi-rigid packages, while a slight vacuum builds up in a rigid package. During storage, O_2 is consumed by bacterial respiration and oxidative processes. CO_2 is the most important gas used in MA packaging due to its bacteriostatic and fungistatic properties (Sivertsvik *et al*., 2002). In addition, removal of O_2 also contributes to extending shelf life, particularly for oxygen-sensitive products. The effectiveness of MA packaging in extending shelf-life relies on several factors, including initial raw material quality, handling and hygiene, degree of filling (DF), gas mix, storage temperature and gas permeability of the packaging material. Raw material quality is of great importance, as is hygienic handling. MA packaging inhibits bacterial growth, but high initial bacterial loads drastically shorten shelf life. However, the limiting factor for shelf life is widely reported to be growth of specific spoilage bacteria, upon which CO_2 has limited or no inhibitory effect (Dalgaard *et al*., 1993; Devlieghere and Debevere, 2000; Gill and Tan, 1980; Sivertsvik *et al*., 2002).

MA packaging can increase shelf life by 50–100%. Shelf-life extension is the most apparent advantage of this technique, but it has several other positive effects. Costs are reduced because longer distribution distances are achievable and fewer deliveries are required, thus allowing centralized packaging and portion control. MA packaging also eases separation of sliced products, and can improve presentation by providing a clear view of the product and all-around visibility. It reduces or removes the need for chemical preservatives, too, and the sealed package acts as a barrier against recontamination, prevents drip loss from package and renders

the product odourless and convenient. However, this packaging method also has a number of disadvantages, including the need for temperature control, different gas formulations for different product types and special equipment and training of staff. MA packaging represents an additional cost, and the increased package volume raises transport costs and the retail display space required. In addition, one loses the benefits once the package is opened or a leakage occurs, and dissolved CO_2 into the food can cause packages to collapse.

MA packaging is used to increase the shelf life of a wide range of foods. The use of MA packaging to preserve meat and fish products has been extensively studied, and has been shown to provide considerable shelf-life extensions in comparison to chilled storage in air at similar temperatures (Hotchkiss and Langston, 1995; Lambert *et al*., 1991; Rao and Sachindra, 2002; Sivertsvik *et al*., 2002). Several review articles have been published on the effect of non-respiring food over the last three decades (Church and Parsons, 1995; Daniels *et al*., 1985; Davies, 1995; Hotchkiss *et al*., 2006; Jakobsen and Bertelsen, 2002; Jones, 1986; Parkin and Brown, 1982; Rao and Sachindra, 2002; Reddy *et al*., 1992; Sivertsvik *et al*., 2002; Soccol and Oetterer, 2003; Stammen *et al*., 1990; Tewari *et al*., 1999). These reviews cite numerous studies wherein dramatic shelf-life extension is reported, though in some cases little or no extension of shelf life was observed.

11.3 Effect of CO_2 on microorganisms

MA packaging is conditioned by low storage temperatures, high-quality raw materials, and availability of carbon dioxide (CO_2) as expressed by the partial pressure of CO_2 and the DF. DF is often not mentioned in published papers on MA packaging; usually only the initial gas composition is stated (Sivertsvik *et al*., 2002), and observed effects of the package atmosphere are attributed to the initial gas composition rather than the amount of dissolved CO_2.

The effect of CO_2 on bacterial growth is complex. Four activity mechanisms of CO_2 on microorganisms have been identified (Daniels *et al*., 1985; Dixon and Kell, 1989b; Farber, 1991; Parkin and Brown, 1982), including alteration of cell membrane function effecting nutrient uptake and absorption; direct inhibition of enzymes or decrease in the rate of enzyme reactions; penetration of bacterial membranes leading to intracellular pH changes; and direct changes in the physicochemical properties of proteins. It is likely that a combination of all these activities accounts for any bacteriostatic effect observed.

Strict aerobic Gram-negative organisms cause spoilage of muscle food under aerobic conditions. CO_2 inhibits the growth of normal spoilage flora (e.g., *Pseudomonas* spp., *Shewanella putrefaciens*). During storage, CO_2-tolerant microorganisms (mainly Gram-positive), such as *Lactobacillus* spp., *Photobacterium phosphoreum* and *Brochothrix thermosphacta*, among others, will dominate the spoilage microflora (Dalgaard, 2000). Many common food pathogens are inhibited by CO_2 atmospheres, though *Listeria monocytogenes* and *Clostridium botulinum* are examples of pathogens that are less affected (Table 11.1).

Table 11.1 Effect of CO_2 atmospheres on growth of common food pathogens, including growth limiting factors and type of growth

Microorganism	Effect on growth in CO_2 atm.[2]	Type of growth	Minimum growth limits[1]		
			Temperature (°C)	pH	a_W (or max. % NaCl)
Aeromonas spp.	Inhibited (weakly)	Facultative	0–4	4.0	>4-5% NaCl
Bacillus cereus	Inhibited	Facultative	4	4.3	0.95
Campylobacter jejuni	Inhibited, survival[3]	Microaerophile	32	4.9	0.99
Clostridium botulinum proteolytic (A,B,F)	Unaffected[4]	Anaerobic	10	4.6	0.93
*C. botulinum*non-proteoloytic (B,E,F)	Unaffected[4]	Anaerobic	3	5.0	0.97 (or ≥ 5.5% NaCl)
C. perfringens	Inhibited	Anaerobic	12	5.0	0.95
Escherichia coli	Inhibited (weakly)	Facultative	7	4.4	0.95
E.coli O157:H7	Inhibited (weakly)	Facultative	6.5	4.5	0.95
Listeria monocytogenes	Unaffected/inhibited[5]	Facultative	0	4.3	0.92
Plesiomonas spp.	Inhibited[6]	Facultative	8	4.0	>4–5% NaCl
Salmonella	Inhibited[4]	Facultative	7	4.0	0.94
Staphylococcus aureus	Inhibited (weakly)	Facultative	6 (10 for toxin)	4.0 (4.5 for toxin)	0.83 (0.9 for toxin)
Vibrio cholerae	Inhibited	Facultative	10	5.0	0.97
V. parahaemolyticus	Inhibited	Facultative	5	4.8	0.94 (Halophile)
Yersinia enetrocoloitica	Inhibited	Facultative	−1	4.2	0.96

Source: Edited and adapted from Sivertsvik *et al*. (2002).

[1] From European Chilled Food Federation cited from Martens (1997) and Huss *et al.* (1997).

[2] From Farber (1991) if not otherwise stated. Growth and/or survival of pathogen as relative to growth and/or survival in air.

[3] The bacteria survive better in CO_2 as compared to air, but growth is (weakly) inhibited.

[4] One report of growth stimulation under CO_2, however, several reports on delayed neurotoxin production under atmosphere exceeding 45% CO_2 (Daifas *et al*., 1991; Doyle, 1983; Lambert *et al*., 1991a, 1991b).

[5] Unaffected of CO_2 atmosphere if at least 5% O_2 present, inhibited under 100% CO_2.

[6] From Kirov (1997).

Strains of nonproteolytic *C. botulinum* types B and E, despite having increased lag time and reduced growth rate (Fernandez *et al*., 2001; Gibson *et al*., 2000), display an increase in both neurotoxin gene expression and neurotoxin formation at higher CO_2 concentrations (Artin *et al*., 2008; Lövenklev *et al*., 2004). No such effect was observed for proteolytic *C. botulinum* (Artin *et al*., 2010). Understanding of how CO_2 affects the individual bacterium's gene expressions is incomplete.

A single microbial species is usually responsible for the primary sensory spoilage, and such organisms are described as specific spoilage organisms (SSO). The numbers of SSO and the concentration of their metabolites can be used as objective indices of spoilage in shelf-life determinations. Where the microorganisms responsible for spoilage are known, a close relationship between log numbers of SSO and remaining shelf life can be identified (Dalgaard, 2000). At high CO_2 concentrations *P. phosphoreum* has been found to be the SSO in both MA-packaged chilled cod (Dalgaard, 1995; Dalgaard *et al*., 1993) and salmon (Emborg *et al*., 2002), while *Brochothrix thermosphacta* is the major contributor to spoilage of meat with pH > 5.8 (Gill, 1995). Application of knowledge about SSOs for the determination, prediction and extension of shelf life is of vital importance in order to extend product shelf life and to obtain a high raw material quality over a longer period.

The inhibition is proportional to the concentration of dissolved CO_2 in the product (Devlieghere *et al*., 1998a, 1998b) and, therefore, is also proportional to the partial pressure of CO_2 above the product, in accordance with Henry's law (see Section 11.3.1). The specific spoilage organism for MA-packaged fish from temperate waters is *P. phosphoreum,* which is more CO_2 tolerant than the SSO observed for iced fish, *S. putrefaciens*. However, *P. phosphoreum* also shows an increased growth inhibition with increasing CO_2 levels in the atmosphere (Dalgaard *et al*., 1997) or increasing levels of dissolved CO_2 (Devlieghere and Debevere, 2000). It is therefore critical to know the solubility of CO_2 in the product if MA packaging is to be successfully used for muscle food products.

11.3.1 Solubility of CO_2 and other packaging gases in muscle foods

CO_2 is many times more soluble in water than any other atmospheric constituents: under a 100% CO_2 atmosphere (101.3 KPa) at 0°C, solubility is 3.3 g of CO_2 per kg of water (3300 ppm). CO_2 is 25–35 times as soluble as oxygen and 50–60 times as soluble as nitrogen in water (Mitz, 1979). Because of its natural abundance and biological importance, the solubility of CO_2 in water at low temperatures has been the subject of considerable research, beginning with the early experiments of Bunsen in 1855 (Crovetto, 1991), and this system has been reviewed thoroughly (Carroll *et al*., 1991; Crovetto, 1991; Wilhelm *et al*., 1977). The relationship between the level of CO_2 surrounding a product and the amount of dissolved CO_2 is described by Henry's law (Schumpe *et al*., 1982), which states that the solubility of a gas in a product is proportional to the partial pressure of that gas above the product:

$$P_{CO_2}^{t=\infty} = H_{CO_2,\text{prod}} \times C_{CO_2}^{t=\infty} \qquad [11.1]$$

where $P_{CO_2}^{t=\infty}$ is the equilibrium partial pressure of CO_2 (Pa), $H_{CO_2,prod}$ is Henry's constant (Pa ppm^{-1}), and $C_{CO_2}^{t=\infty}$ is the equilibrium concentration (ppm) of CO_2 in the product.

Henry's constant is given for a specific gas in a specific solvent or product – for example, Henry's constant for various gases in water (Table 11.2) and CO_2 in various food stuff (Table 11.3). The temperature dependency of Henry's constant for CO_2 in water has been provided by Carroll *et al*. (1991), and converted to Pa ppm^{-1} from mol-fraction basis (Sivertsvik *et al*., 2004a):

$$\ln H_{CO_2,H_2O} = -7.7278 + \frac{12.817\times10^3}{T} - \frac{3.7668\times10^6}{T^2} + \frac{0.2997\times10^9}{T^3} \qquad [11.2]$$

where T is the temperature (K).

In terms of traditional MA packaging, CO_2 dissolves into the aqueous part of the product, resulting in volume contraction of flexible packages. For a semi-rigid tray, the major part of this contraction will take place in the most flexible part – that is, the top web. Packaging collapse is usually reduced by lowering the CO_2 partial pressure through the introduction of gases that possess significantly less solubility in product components – that is, N_2 or O_2. Once dissolved, the CO_2 reacts with water and dissociates, forming bicarbonate. The reaction may be written as Knoche (1980):

$$CO_2(aq) + H_2O \leftrightarrow H_2CO_3 \leftrightarrow HCO_3^- + H^+ \leftrightarrow CO_3^{2-} + 2H^+ \qquad [11.3]$$

Table 11.2 Henry's constant (Pa ppm^{-1}) for CO_2, N_2 and O_2 in water

	Temperature (°C)				
	0	5	10	15	Reference
CO_2	30.41	36.28	42.82	50.03	Carroll *et al*. (1991)
N_2	3179	3819	4422	4971	Prini and Crovetto (1989)
O_2	1466	1652	1845	2043	Prini and Crovetto (1989)

Table 11.3 Henry's constant (Pa ppm^{-1}) for various products

Product	Temperature (°C)	Henry's constant	Reference
Chicken breast fillet	2	42.8 ± 3.7	Rotabakk *et al*. (2010)
Farmed cod	3	43.3	Rotabakk *et al*. (2007)
Cod	2	46.1 ± 9.6	Sivertsvik *et al*. (2004b)
Farmed salmon	2	47.8 ± 2.3	Sivertsvik *et al*. (2004b)
Cooked ham	4	64.9 ± 6.5	Sivertsvik and Jensen (2005)
Pacific hake	0	29.0	Simpson *et al*. (2001b)
Lamb meat	2	44.5	Gill (1988)
Water	0	30.41	Carroll *et al*. (1991)

At pH values below 8, the concentration of carbonate ions may be ignored (Dixon and Kell, 1989a), giving the following:

$$CO_2(aq) + H_2O \leftrightarrow H_2CO_3 \leftrightarrow HCO_3^- + H^+ \quad [11.4]$$

However, more than 99% of dissolved CO_2 in pure water, or in unbuffered salt solutions, exists in the physical form of CO_2. In the temperature range between 0°C and 50°C, less than 1% of dissolved CO_2 is in the carbonic acid form, including the bicarbonate and carbonate ions (Mitz, 1979). CO_2 can react with amines to give carbamic acid. This carbamate reaction is reversible, and many times faster than CO_2 reacting with water (Ho *et al*., 1987).

11.3.2 Diffusion and absorption/desorption rates of CO_2

The amount of gas that is eventually transferred into the non-solid phase of a food depends on the gas solubility, but the rate of gas transfer into the liquid phase is controlled by the diffusion coefficient of the gas. The flux of a gas diffusing into a food at rest can be expressed by Fick's second law:

$$\frac{dC}{dt} = D\frac{d^2C}{dx^2} \quad [11.5]$$

where C is the concentration of the gas in the product, x is the diffusional position and D is the effective diffusion constant.

The diffusion coefficient of CO_2 into water is reported to be 1.05×10^{-5} cm^2/s at 277.15 K (4°C). A temperature correlation is provided by the Arrhenius equation (Jähne *et al*., 1987):

$$D_{CO_2,H_2O} = 0.05019e^{(-19510/RT)} \quad [11.6]$$

However, chemical reactions facilitate the transport of CO_2, so the effective observed diffusion is often much higher when compared to the above value for passive diffusion alone. Diffusion of bicarbonate ions, metal ion concentration and presence of carbonic anhydrase, have been found to facilitate the transfer of CO_2, increasing the effective diffusion coefficient (Ho *et al*., 1987).

Dissolving CO_2 is a time-consuming process. It takes 3 days for atmospheric and dissolved CO_2 to reach equilibrium in salmon and cod, and 3.5–4.5 h to attain 50% equilibrium (Rotabakk *et al*., 2007; Sivertsvik *et al*., 2004b).

Desorption is driven by the same diffusion as dissolving the CO_2 and hence equally time consuming. Trials done on chicken exposed to 100% CO_2 in 6, 24 and 48 h had respectively lost 37.7%, 35.7% and 27.1% of the dissolved CO_2 after 3 h (Rotabakk *et al*. 2010). The same effect was shown on bulk transported salmon that was repackaged after filleting and portioning. Salmon stored in CO_2 during bulk transport had more CO_2 per portion than salmon stored in atmospheric air (Randell *et al*., 1999).

11.3.3 How to measure solubility of CO_2 in foods

There are several approaches to measuring the solubility of CO_2, including volu-, mano-, gravi- and titrimetric methods, pH changes and others (Dixon and Kell, 1989a). However, most of techniques are either limited to liquid/aqueous products or not capable of producing continuous measurements. Conrad *et al*. (1982) compared a standard Warburg manometric method with a CO_2 electrode for determining the amount of added CO_2 in fish flesh. CO_2 electrodes, operating on the basis of the Severinghaus principle (Conrad *et al*., 1982), have also been used to measure dissolved CO_2 in model food products (Devlieghere *et al*., 1998a, 1998b, 1999, 2000). However these electrodes were designed for aqueous products, are known to drift, have slow response times and produce readings with high variances (Neurauter *et al*., 1999; Zhao and Cai, 1997). Manometric or volumetric approaches have been used in several studies (Fava and Piergiovanni, 1992; Pfeiffer and Menner, 1999; Piergiovanni and Fava, 1992; Vingeault *et al*., 1993; Zhao and Wells, 1995; Zhao *et al*., 1995), but none of these methods were designed for continuous measurement. Other non-continuous methods used for foods or food applications include the trapping of dissolved CO_2 with $Ba(OH)_2$ and titration with HCl for measurement of CO_2 solubility in meat (Gill, 1988; Jakobsen and Bertelsen, 2004), biosensors using carbonic anhydrase (Cammaroto *et al*., 1998) and columetric methods for measurement in an aqueous model system (Löwenadler and Rönner, 1994). Most of these published studies on CO_2 solubility measurements in foods are based on systems that were presumed to be in equilibrium with the surrounding atmosphere, with little or no focus on CO_2 diffusion and the rate of CO_2 absorption.

Methods for obtaining continuous measurements of solubility and absorption rate have been developed for both constant and flexible volumes. Sivertsvik *et al*. (2004a) developed manometric apparatus that was used to determine the solubility and absorption of CO_2 from pressure changes over time. In a closed container, using constant volume and temperature, headspace CO_2 changes from time zero to infinity can be related directly to solubility of CO_2 into a product (Cameron *et al*., 1989; Zhao *et al*., 1995):

$$C_{CO_2}^{t=\infty} = \frac{(g/p)\left(P^{t=0} - P^{t=\infty}\right) Mw_{CO_2}}{RT\rho_p} \qquad [11.7]$$

where $C_{CO_2}^{t=\infty}$ is the total CO_2 (ppm) absorbed into the product including any hydration and dissociation products, P is absolute gas pressure (Pa), g/p is the ratio of gas to product and ρ_p is the density (kg/dm^3) of the product. This method has been used to determine solubility and absorption rate of CO_2 in salmon (*Salmo salar*), cod (*Gadus morhua*), anglerfish (aka monkfish) (*Lophius Piscatorius*), Atlantic wolf-fish (*Anarcichas lupus*) and tuna fillets (*Thunnus thynnus*) (Sivertsvik *et al*., 2004b) and cooked meat products (Sivertsvik and Jensen, 2005).

Rotabakk *et al*. (2007) developed a volumetric approach using the buoyancy force of an MA package to measure the volume of the gas phase inside. Using this

method, it is possible to measure solubility and diffusion rate of CO_2 in a flexible volume without tampering with the package. In a closed package with constant pressure (i.e., flexible packaging materials) and temperature, volume changes in the package from time of packaging to infinity, can be related directly to solubility of CO_2 into the product:

$$C_{CO_2}^{t=\infty} = \frac{1000 \cdot P \cdot \left(V_g^{t=0} - V^{t=\infty}\right) \cdot Mw_{CO_2}}{R \cdot T \cdot W_p} \quad [11.8]$$

where $C_{CO_2}^{t=\infty}$ is the total CO_2 (ppm) absorbed into the product at equilibrium including any hydration and dissociation products, P is the atmospheric pressure (Pa), V_g (m^3) is the volume of the head space gas, Mw_{CO_2} is the mol weight of CO_2, R is the universal gas constant (J mol^{-1} K^{-1}) and W_p is the weight of the product (kg).

The equation (Eq. 11.8) above is deemed to be true if:

- systems follow the ideal gas law – that is, the compressibility factor equals 1;
- there is no permeability of gas through the package material;
- dissolution of CO_2 into the foodstuff is the dominating reaction occurring within the system and the solubility of O_2 and N_2 is ignored (50 and 100 times less soluble than CO_2, respectively).

The solubility of CO_2 was measured on chicken breast fillets and on farmed cod (*Gadus morhua*) using the above method. The results from this and other studies using Henry's constant for various products are summed up in Table 11.3.

11.3.4 Theoretical models of solubility

The amount of dissolved CO_2 in MA-packaged foods is dependent on several factors, including temperature, pressure, packaging material and ratio between gas and product. It is critical to know about these factors when planning on using CO_2 in the packaging of a product. Several models have been made to predict the equilibrium of MA-packaged foods, both in constant volume (Sivertsvik *et al*., 2004a, 2004b; Zhao and Wells, 1995) and in semi-rigid and flexible packages (Devlieghere *et al*., 1998a; Jakobsen and Bertelsen, 2004; Jakobsen and Risbo, 2009; Rotabakk *et al*., 2007, 2008b; Simpson *et al*., 2001, 2004, 2009).

As shown in Eq. 11.2, Henry's constant for CO_2 in water is temperature dependent. Solubility of CO_2 in water decreases with increasing temperature (Fig. 11.1). CO_2 dissolves mainly in the water and fat phase of muscle foods, and studies have observed the same effect on other food to a certain degree (Gill, 1988; Jakobsen and Bertelsen, 2004, 2006; Jakobsen *et al*., 2009; Rotabakk *et al*., 2007; Sivertsvik *et al*. 2004b). For food containing fat, the amount of liquid fat at the storage temperature seems to be relevant to the amount of dissolved CO_2 in the fat phase (Sivertsvik *et al*., 2004b). In muscle food with no fat, a correlation between

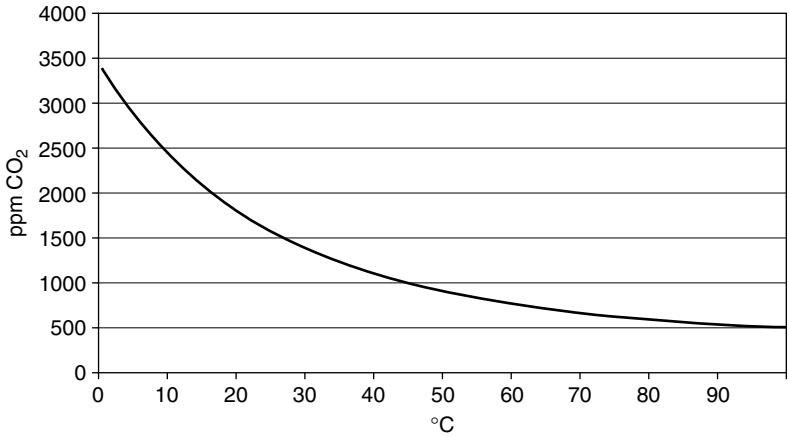

Fig. 11.1 Estimated concentration (ppm) of dissolved CO_2 in water in equilibrium with 100% CO_2 as affected by temperature (°C).

water content and the theoretical solubility of CO_2 in water has been obtained (Rotabakk *et al*., 2007, 2008b, 2010; Sivertsvik *et al*., 2004b). In addition, CO_2 has been shown to have similar solubility in salmon fat as in water (Sivertsvik *et al*., 2004b). Few models take into account the effect of storage temperature. The effect of temperature is indirectly accounted for by different Henry's constants at different temperatures. In any case, without proper control of storage temperature, the benefits of MA packaging may be lost. Higher storage temperature will inevitably lead to loss of dissolved CO_2 in the product and, consequently, loss of inhibitory effect together with higher microbial and enzymatic activity. If temperature is inadequately controlled then the microbial safety of the product is uncertain.

Gas pressure has a vital impact on the amount of dissolved CO_2. Solubility is directly related to the partial pressure of CO_2, as stated in Henry's law (Eq. 11.1) – that is, increased partial pressure gives increased amount of dissolved CO_2. The partial pressure of a gas is the pressure which the gas would have if it alone occupied the volume. The partial pressure of a gas is therefore affected by the total pressure of the gas phase and the relative amount of the specific gas. Increasing the relative amount of CO_2 in the packaging gas will lead to increased partial pressure.

One of the main factors affecting the amount of dissolved CO_2 in an MA product is the ratio between gas and product volume. DF determines the amount of CO_2 available to dissolve in the product together with the partial pressure of CO_2, and by that the inhibitory effect. Studies have shown that DF has a significant impact on the shelf life of MA-packaged products (Gill, 1996; Kennedy *et al*., 2004). Food processors normally want as high a DF as possible without compromising on shelf life. When designing packaging conditions, one must increase the partial pressure of CO_2 if the DF is increased, in order to achieve an equal amount of dissolved CO_2 (Fig. 11.2). However, this will evidently result in increased gas

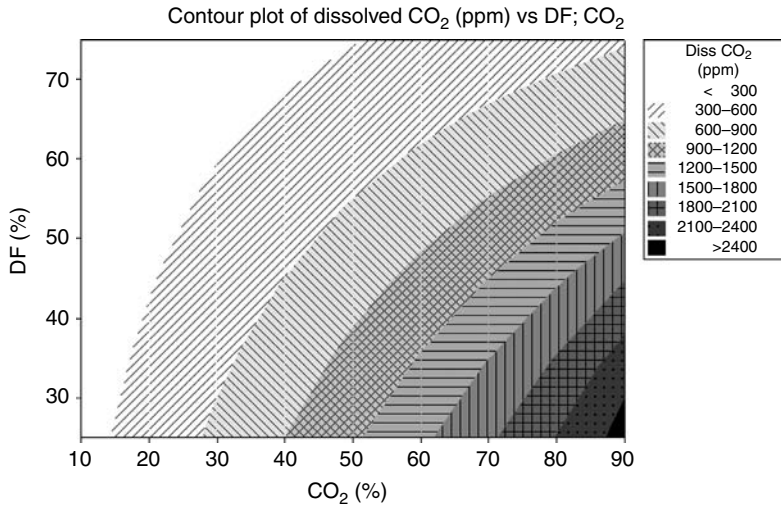

Fig. 11.2 Amount of dissolved CO_2 (ppm) as given by degree of filling (DF (%)) and initial CO_2 concentration in the packaging gas.

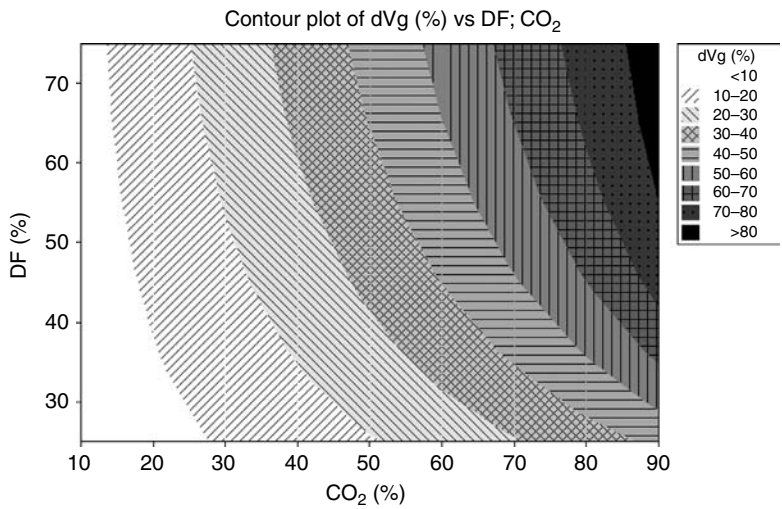

Fig. 11.3 Gas volume reduction (%) of the initial gas volume given by the degree of filling (DF (%)) and the initial CO_2 gas concentration (%).

volume reduction (Fig. 11.3), so the chosen DF and partial pressure of CO_2 is often a compromise to avoid packaging collapse. Theoretical models can be valuable aids for finding the right compromise.

Choice of packaging material is also vital. Packaging materials have various barrier properties against gases, water vapour and odour. This affects the concentration of gases first in the head space, and then in the product.

In high CO_2/low O_2, CO_2 will diffuse out of the package while O_2 diffuses in. Both will have a negative impact on shelf life. Barrier materials are usually more expensive than non-barrier ones, so the choice of packaging materials is a question of storage time and economy.

11.4 Alternatives to MAP

Two other approaches can be used to create a modified atmosphere for a product. One option is to generate the CO_2 and/or remove O_2 inside the package after packaging. Alternatively, the CO_2 can be dissolved into the product prior to packaging. Both methods can result in appropriate packages with higher DF, and thus decrease the package size, which has been a disadvantage of MA packaging from the start.

The first approach involves the most commercialized active packaging technology, namely, oxygen scavengers. These are now available from several manufacturers (Mitsubishi Gas Chemical Co., ATCO, Bioka, Sealed Air/Cryovac, Multisorb and some others), in various forms (sachets, packaging film, closures), with different active ingredients (iron, enzymes, dye). Some of the same companies have also developed CO_2 emitters, using the O_2 in the package headspace to produce CO_2, and developing a CO_2/N_2 atmosphere inside a package without the use of gas flushing. Other resources used to generate CO_2 gas inside packages after closure include dry ice (solid CO_2) (Sivertsvik *et al*., 1999) or carbonate, possibly mixed with weak acids (Bjerkeng *et al*., 1995).

Commercial CO_2 emitters usually contain ferrous carbonate and a metal halide catalyst, although non-ferrous variants are available, absorbing the O_2 and producing an equal volume of CO_2. Carbon dioxide could also be produced after packaging by allowing the exudates from the product to react with a mixture of sodium carbonate and citric acid inside the drip pad, an approach used successfully for cod fillets (Bjerkeng *et al*., 1995). This method increased shelf life as compared to traditional MA packaging, even when using a low-gas head space. The Verifrais package, manufactured by Codimer, which has been used for extending the shelf life of fresh meats and fish, is a similar concept (Day, 1998). This package consists of a standard MA packaging tray, but has a perforated false bottom, under which a porous sachet containing sodium bicarbonate/ascorbate is positioned. When exudate from packed meat or fish drips onto the sachet, CO_2 is emitted, counteracting the package collapse resulting from CO_2 solubility in the food. Lately, CO_2 emitters have been further developed and promising results for fish have been reported (Hansen *et al*., 2009a, 2009b, 2009c). See Chapter 8 for examples of practical use.

The second approach is to dissolve the CO_2 into the product prior to packaging. Since solubility increases at lower temperatures and at higher CO_2 pressures, a sufficient amount of CO_2 can be dissolved into the product during 1–2 h prior to packaging using elevated pressures. This method is called *soluble gas stabilization* (SGS) (Sivertsvik, 2000). This is not an active packaging technology

by definition, but it is a novel alternative to MA, and it has been used successfully alone and/or in combination with O_2 scavengers. SGS has shown promising results for fresh Atlantic salmon (*Salmo salar*) fillets (Sivertsvik, 2000, 2003), cooked peeled shrimp (Sivertsvik and Birkeland, 2006), chicken breast fillets (Rotabakk *et al*., 2006), Atlantic halibut (*Hippoglossus hippoglossus*) (Rotabakk *et al*., 2008a), farmed gilthead sea bream (*Sparus aurata*) and European sea bass (*Dicentrarchus labrax*) (Mendes and Goncalves, 2008). Dissolving CO_2 into the products prior to packaging has also been used successfully for yogurt and milk (Chen and Hotchkiss, 1991; Loss and Hotchkiss, 2002).

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12

Packaging of retort-processed seafood, meat and poultry

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Abstract: Perishable foods like fish, meat and poultry should be quickly processed and packed using an appropriate technique. Retorting is a method of preserving food by heating it in hermetically sealed containers like cans, glass jars, semi-rigid thermoformed containers and retortable pouches. These products have a shelf life of more than a year when held at ambient temperatures during storage. Retort-processed meat, poultry and seafood products offer convenience and are presented in ready to serve formats. This chapter first reviews the types of containers used for retort-processed meat, poultry and seafood products. The retorting process using semi-rigid and flexible containers is also outlined. Then methods to test container suitability for retorting are addressed. A section on the impacts of retorting on the quality of meat, poultry and seafood products completes the chapter.

Key words: packaging, thermal processing, rigid containers, semi-rigid containers, flexible containers.

12.1 Introduction

Food packaging is most important to our current marketing scenario. Proper packaging minimizes deterioration and waste, extends shelf life and also facilitates easy handling down the distribution line. Innovations in processing and packaging technologies and packaging materials have led to the development of a large number of convenience foods in the market today.

Fish, meat and poultry are perishable foods. In order to preserve their quality, these products must be quickly processed and packed using appropriate formats. Retort-processed meat, poultry and seafood, either packaged in metal cans, or newer pack formats such as retort pouches, are popular with customers due to the long shelf life of the products and the fact they are convenient and ready-to-eat.

Retorting is a method of preserving food by heating it in hermetically sealed containers for a specific duration at a particular temperature to eliminate pathogenic microorganisms. The process is also often referred to as canning, even though a variety of container types are used. Nicholas Appert was the first to develop the technique. He published a book in 1810 which described the packing of food into wide-mouth glass bottles, which are then sealed and placed in boiling water. In 1864, Louis Pasteur explained that the heating process killed (or inactivated) the microorganisms which consequently extended the shelf life of food. Shortly after, Peter Durand registered a patent for the use of metal canisters, among other packaging materials, for canning. This initiated the beginning of the canning industry (Holdsworth, 1997). In the early twentieth century, studies on the importance of *Clostridium botulinum* and its role in canned foods were established. Bigelow *et al*. (1920) classified foods based on pH and developed the first scientifically based method for the calculation of minimum sterilization processes for canned foods. This method is known as the graphical or general method of process calculation. Ball (1923) developed the mathematical or theoretical method for process calculations. Schultz and Olson (1940) developed a nomographic method for process determinations. Ball and Olson (1957) published the first comprehensive book on heat processing followed by Stumbo's book on thermo bacteriology (Stumbo, 1973). Mathematical methods which eliminated certain relatively small errors inherent to some of the previous mathematical procedures were developed by Hayakawa and Ball (1968). In recent years, in addition to Hayakawa (1977), Manson *et al*. (1970), Manson (1992), Pflug (1964) and Tung and Garland (1978) and others have further refined mathematical heat process determination concepts and applications.

In thermal processing the penetration of heat into the food is influenced by several factors and a clear understanding is necessary to obtain optimal results in commercial operations. The characteristics of the retort, the packaging container used, heating medium, filling medium, the temperature gradient between the container and retort, ratio of product liquids to solids in the container, arrangement of containers inside the retort and steam distribution within the retort are some of the important factors that need to be considered in order to achieve effective sterilization (Balachandran, 2002). Early research was carried out by Duckwall (1905), who studied the rate of heat penetration in various foods and Zavalla (1916), who studied the effect of filling media, the effect of air in steam retorts and the advantage of jumble stacking of cans in the retort for obtaining better heat penetration. Ingredient-related factors also affect heat penetration in cans – for example, food products containing high levels of fat are poor conductors of heat. Solids with gelling properties also absorb water and change the solid liquid ratio, thereby affecting heat transfer. Liquid and semi-liquid foods are mainly heated by convection while solid foods are heated by conduction. In semi-liquid products, effective heating is achieved by both convection and conduction, implying a longer process time due to the slow rate of heat transfer (Clifcorn *et al*., 1950). Rotation of the retort cage during heating significantly increases the rate of heat penetration (Ali *et al*., 2006; Bindu and Gopal, 2008). Shape and size of the container also affect heat penetration because smaller containers heat more rapidly due to the larger surface area in relation to the volume of the container.

This chapter first reviews the types of containers used for retort-processed meat, poultry and seafood products. The retorting process using semi-rigid and flexible containers is also outlined. Then methods to test container suitability for retorting are addressed. A section on the impacts of retorting on the quality of meat, poultry and seafood products completes the chapter.

12.2 Rigid containers for retort-processed seafood, meat and poultry

The most frequently used containers for retort-processed foods are tin cans. Tin plate containers first made their appearance in the early eighteenth century and in 1810 Peter Durand obtained a patent in England for preservation of foods by a retorting method in containers made of tin, among other materials. After the end of the Napoleonic war can making was continued in England, while in America the first US canning operation opened in 1819. The tin plate metal containers were called 'canisters' from which the term 'can' is believed to have derived.

There are different types of tin plate containers, with each type having certain exclusive uses. The decision to select one container over another is usually dependent on the product, process and cost of production, etc. Today several choices are available (Fig. 12.1) – for example, standard tin plate, light weight tin plate, double reduced tin plate, tin-free steel and vacuum deposited aluminium on steel and aluminium. Before being filled with the product to be processed, the containers are frequently coated internally with lacquer to enhance container performance and stability – for example, enhancement of acid resistance and sulphur resistance, depending on the pH of the food being packed. However, care must be taken to avoid the lacquer tainting the food product. The major tests for determining the physical properties of the rigid containers are given in Table 12.1.

Fig. 12.1 Different types of cans.

Table 12.1 Important tests for determining physical properties of rigid containers

Parameter	Method
Determination of water capacity	IS: 6093-1970
Air pressure test	IS: 9396-1987
Determination of vacuum	IS: 3336-1968
Sulphide blackening test	Anon, 1977
Test for food contact application/overall migration residue	CEC, 1990
Test for seam integrity	Lin *et al*., 1998

12.2.1 Tin cans

The tin can consists of about 98% steel and 2% tin (coated both on the internal and external surfaces of the can). The base steel used for making cans is referred to as can-making-quality (CMQ) steel. Corrosion behaviour, durability and strength of the tin plate depend upon the chemical composition of the steel base. The active elements in the steel plate are principally copper and phosphorous. The more of these elements that are present in the steel the greater will be the corrosiveness of the steel. The degree of workability, strength and corrosion resistance required in the steel plate to be used for can manufacture can be classified by four steel types based on the type of product canned: type L (mostly acidic food such as pickles and juices), type MR (mildly acidic fruit products), type MC (low acid foods) and type MS (mildly acidified vegetable products) (Ellis,1979).

The composition of different types of tin plate is presented in Table 12.2. Steel types L, MR and MC are produced by a cold reduction process, while types M and MC (similar in composition) are produced by a hot reduction process.

It was observed that tinned foods packed in tin-coated cans gradually lost their natural colour during prolonged storage periods. To prevent this, lacquer coatings are usually applied to the inside of the can. This lacquer coating protects the steel and tin and prevents direct contact with the food material. The can body protects the contents against the entry of microorganisms, insects, air, light and moisture. Tin cans are relatively light in weight and can be handled with ease, yet are robust enough to cope with high pressures and temperatures applied to them during retorting.

12.2.2 Aluminium cans

Aluminum containers were used for packing meat and fish products as early as 1918. The poor organoleptic qualities associated with foods packed in tin containers led to the introduction of the aluminium alloy can. Various types of aluminium and its alloys are used for packaging. These cans are now used extensively in European countries because of the availability of the raw material, lower cost of production and availability to sources of economical electricity supply. The aluminium grade '1000' is called pure aluminium (99–99.7% purity). It is this aluminium grade that is used for foil and aluminium slugs used for impact-extruded cans and collapsible tubes. The aluminium alloys of grade 3000 are used mainly as sheet material for deep-drawn cans and craned cans. Manganese is added to the

Table 12.2 Composition of different types of tin plate

Composition	Type of steel plate		
	L	MR	MC
Carbon	0.12 max	0.12 max	0.12 max
Sulphur	0.05 max	0.05 max	0.05 max
Silicon	0.01 max	0.01 max	0.01 max
Phosphorus	0.015 max	0.02 max	0.03–0.05
Copper	0.06 max	0.20 max	0.2 max
Manganese	0.2–0.6	0.2–0.6	0.2–0.6
Nickel	0.04 max	0.04 max	Not specified
Chromium	0.04 max	0.04 max	Not specified

Source: Gopal (2007).

aluminium to increase its strength for such applications. The best and most promising alternative to tin plate considered to date is aluminium modified by alloying with manganese and magnesium.

Aluminium has many important characteristics as a can-making material. Aluminum possesses good resistance to external atmospheric corrosion, thereby preventing rusting. Aluminum containers are easy to fabricate and are recyclable. Cans may also be produced in a wide variety of sizes and shapes, possessing attractive appearances. They are light in weight and have excellent scrap value. Aluminium cans, being light in weight, require special attention during heat processing. After heat processing, container cooling must be conducted under pressure. Aluminium cans, with ring pulls as for easy opening closures are becoming more and more popular with consumers on account of the added convenience associated with them.

12.2.3 Tin-free steel (TFS) cans

Tin-free steel cans, an important alternative to the tin can, are produced under different commercial names. They were originally developed in Japan and are prepared by electroplating cold-roller steel sheets with chromium in chromic acid. TFS cans have a steel base with a chromium/chromium oxide coating on the surface, thereby replacing tin in conventional cans. The appearance of the can is bright or semi-bright as compared to tin plate. Because of the low abrasion resistance of TFS, it needs to be protected by a lacquer film. However, the surface of the TFS provides an excellent substrate for lacquer adhesion, which ensures superior performance in terms of product compatibility for many food products. TFS cans have been shown to be suitable for storage of shrimp curry (Sreenath *et al*., 2008), squid masala (Sreenath *et al*., 2007) and Rohu curry (Mallick *et al*., 2006).

12.2.4 Polymer-coated TFS can

The polymer-coated TFS can is made of electrochemically chromium coated steel (ECCS) plate. A clear polyethylene terephthalate (PET) coating is applied

on either side of the two-piece cans which usually have a 6-ounce capacity (307 × 109), where the first number of the three digits represents the inches and the next two digits sixteenth of an inch (hence a 307 × 109 can is 3 and 7/16 inches in diameter and 1 and 9/16 inches in height). PET is a universal polymer coating that can be widely used for a variety of products. The finished plate has a thickness of 0.19 mm (0.15 mm of base steel + 20 μm PET coating on either side of the can). The cans are manufactured from steel plate by a draw and redraw (DRD) process. The chromium coating, along with the PET coating, provides the can with a smooth, greyish, glistening appearance. In addition to this, it also acts as a barrier between the product and the base steel. The bottom of the can is designed for better stackability so that it can be stacked vertically without risk of toppling over on the shelf. This also helps to reduce the storage space requirement for the cans. TFS cans are suitable for thermal processing of fish and meat products. The cans have easy-open ends and the materials used for these closures are the same as for the can bodies, possessing a thickness of 0.28 mm (including PET coatings on either side of the closure). The edges of the lid are provided with a Neoprene rubber sealing compound that forms a hermetic seal with the body wall when double seamed. The lids are supplied scored to the canner and this scoring appears as concentric rings towards the periphery on the outer side of the closure, thereby facilitating easy and complete opening of the can by just pulling the tab. The tab is attached to the lid by means of a rivet that prevents any possible leakage through the lid-rivet joint. A unique feature of the easy-open end (EOE) is the triple-fold technology that helps to avoid the potential injury to fingers on opening the can and eliminates the requirement for can-opening devices. This is a particular advantage over the conventional tin cans, where the cut surface is potentially a source of injury.

Metal cans are advantageous as packaging materials because of their superior strength, high-speed manufacture and for their ease of filling and dosing. Disadvantages of metal cans are weight during transportation and the volume they occupy. Even empty cans require space since they can only be stacked one over the other. Different sizes of cans are in use commercially. Various sizes of cans, often designated by different trade names, are usually employed commercially for different varieties of foods. Details of common sizes of cans used in the food industry are provided in Table 12.3.

12.2.5 Glass

Glass containers have been used for many centuries and still are one of the most important materials used in food packaging today. Glass is a mixture of silicates formed by heat and fusion with cooling to prevent crystallization. It is an amorphous, transparent or translucent super-cooled liquid. Glass usually consists of the following three types of oxides: (i) glass-forming oxide of silica, (ii) the fluxing oxide, sodium potassium or lithium oxides and (iii) stabilizing oxides, which are generally calcium and magnesium. Due to its associated properties, glass has a unique place in both food and beverage packaging. Glass is strong, rigid and

Table 12.3 Common name and dimension of cans employed in the industry

Common name	Dimension (the first number indicates inches and the next two numbers 16th of an inch)
8 ounce	301 × 206
8 ounce tall	211 × 304
½ Tuna	307 × 113
No.1. Picnic	211 × 400
No.1. Tall	301 × 411
No.1. Flat	404 × 206
No.1. Tuna	401 × 206
No.2	307 × 409
No.2. Cylinder	307 × 512
No.2. Vaccum	307 × 306
No.2 ½	401 × 411
No.3	404 × 414
No.3. Cylinder	404 × 700
No.10	603 × 700
No. Squat	603 × 408
	(Length × Breadth × Height)
¼ Dingley	404 × 302 × 014
½ Oval	309 × 515 × 103
½ Oblong	508 × 204 × 103

Source: Gopal (2007), with permission.

chemically inert. It does not appreciably deteriorate with age, is an excellent barrier to solids, liquids and gases and provides excellent protection against odour and flavour contamination. The transparency of glass provides product visibility. Glass can also be moulded in a variety of shapes and sizes.

The major difficulty in using glass is its fragility, which can be categorized into three distinct forms of breakage: (i) impact breakage, (ii) internal pressure breakage and (iii) thermal shock breakage. Another problem associated with glass is limitations in its usage for high-temperature sterilization applications. Sterilization of glass bottles in boiling water and temperatures above 70°C presents risks. Heat processing of vacuum-sealed glass containers requires superimposed pressure during cooling to hold lids or closures in place. Glass can also enhance photo oxidation which may cause problems for photo-sensitive food products and is the heaviest of all food-packaging materials. It is also a very noisy material when used on filling lines. These shortcomings limit the use of glass containers to certain semi-preserved items like salted fish, pickled products, jams, jellies, fruit juices, beverages, etc.

12.3 Semi-rigid and flexible containers

The following sections consider the applications of different types of semi-rigid and flexible containers, looking at implications in the production processes,

Table 12.4 Important tests for determining physical parameters of semi-rigid and flexible containers

Parameter	Method
Thicknesses	IS: 2508-1984
Tensile strength and elongation at break	IS: 2508-1984 ASTM D 882
Heat seal strength	ASTM F88M-09
Bond strength	ASTM D903–98
Water vapour transmission rate	ASTM -E96-66
Oxygen transmission rate	ASTM- D 1434-82
Bursting strength	Duxbury *et al*., 1970
Residual air test	Shappee *et al*., 1972
Overall migration test	CEC, 1990

suitability for varying sorts of food and eventual ease of use for the consumer. The important tests for determining physical parameters of semi-rigid and flexible containers are given in Table 12.4.

12.3.1 Semi-rigid containers

Semi-rigid plastic containers are thermoformed containers which are economical and offer convenience to the user. The containers are thin in profile. The filling volume varies depending on the size and use of the container (see Fig. 12.2). The containers are produced by cold-forming using a vacuum forming die and compressed air (Conley and Cornmann, 1975). The heat setting fixes the shapes and can also facilitate sealing of the filled containers. The original semi-rigid containers were manufactured from aluminium which was coated on the interior with polypropylene (PP). The disadvantage of using aluminium in trays or other container forms was that it was easily prone to denting. The plastic containers developed were laminates comprised of PP/EVOH or PVDC/PP. The widely used high-barrier retortable plastic containers consist of EVOH/PP or crystallized PET (CPET) which is stable at a temperature up to 230°C, unlike amorphous PET which softens at 63°C (Robertson, 2006). The thickness or gauge values associated with these containers may be about 2 mm.

Thermoformed containers can be fashioned into various shapes and sizes and can be handled without the fear of breakage as in the case of glass. The plastics are light in weight, stable, can be combined with other materials and offers resistance to chemical attack. High-barrier lidding materials are also available for top-sealing of the trays. The lids are frequently laminate constructions and consist of PET or PS as the outer layer material, PVDC or EVOH as a middle layer to reduce the gas transmission rate and EVA (ethylene vinyl acetate) or PP as the inner layer. They can be used for single or multi-compartment plastic trays and can be used for protection of ready-to-eat, thermally processed meals. The outer polyester layer and the metallic layer can be used for printing. Easy to open lids and closures are widely available in the market (Gerald, 1978). The thermoform trays have major advantages over other rigid and flexible containers. According to Hoddinott (1975)

Fig. 12.2 Semi-rigid containers.

they are easy to open, can be made into different shapes and sizes, are cheap and economical, can be filled easily, vacuum sealed or gas flushed and have a higher heat penetration rate due to the material possessing low gauges. The trays can also be transported stacked in 'nest format' and are microwaveable.

12.3.2 Process operations for the retorting of foods in semi-rigid trays

The retorting operation involving retortable trays consists of a number of steps from tray manufacturing to sealing. Alternately, readymade trays can also be used along the process line for packing the products. Retortable plastic trays are manufactured using a thermosetting machine. Thermoforming is the process by which the sheets are moulded into different shapes and sizes by the application of vacuum, pressure, heat or a combination of the three, with the help of a die to form a suitable container. Initially, there is a multilayer sheet-forming process which is fed with the help of a feed block which may have a standard single or a multi-manifolded die (Schrenk and Alfrey, 1978). The sheets are coextruded together to prevent delamination. This co-extrusion is cost effective and produces a thinner and finer layer with excellent barrier properties.

Once the trays are formed, the food material to be processed is filled inside the trays. Both volumetric and gravimetric filling can be achieved using filling nozzles and by controlling the dosage automatically. The filling operations are very carefully performed to avoid contamination of the heat-sealing area on the tray. Correct positioning of the trays on the filling line and sealing station is maintained to avoid any containment difficulties later. Air and gases present inside the container are removed by applying a vacuum. This is made effective by using a vacuum-sealing machine, wherein the air is removed and, simultaneously, the lid is sealed on to the container. The removal of air is necessary to counter the internal pressure developed in the container due to the heating and expansion of the gases

and to ensure a uniform heat transfer during retorting. In addition to application of vacuum, steam flushing can also be carried out to remove the air, where hot steam displaces the air inside the container.

Effective sealing of the pack is a critical step in the retorting process as effective containment is necessary in order to deliver a safe and shelf-stable product, for years typically. The two sealing methods used to seal retortable trays are hot-bar sealing and thermal-impulse sealing. In hot-bar sealing, a constant temperature, resistance-heated metal bar seals the container against a rubber fixture, thereby facilitating effective sealing. In thermal-impulse sealing, the seal areas are held together by a pair of jaws, heated to fusion temperature by short electrical impulses and followed by simultaneous cooling under pressure, following achievement of the seal. The main problems associated with imperfect sealing are the development of wrinkles in the top film, seal contamination and subsequent spoilage of the material inside.

Retorting is carried out in an over-pressure retort, which may be a steam-air or steam-water mixture retort. Counter air or pressure is used to counterbalance the internal package pressure and seal integrity (Yamaguchi *et al*., 1972) during retorting and during the cooling process. Cooling is achieved within the retort itself by continuously pumping chilled water into the retort while maintaining the pressure simultaneously. Once the product is sufficiently cooled to below the required temperature, the retorted products are removed from the retort.

The processed product containers are wiped clean and checked for any deformation or seal integrity defects. Each day's production is coded separately and the containers are then labelled and packed in required master cartons. The master cartons used for packing and storing are mainly corrugated fibre board (CFB) boxes.

The CFB boxes consist of one or more layers of fluted kraft paper stuck to flat sheets of kraft paper. Depending on the number of flutes and liners they are classified into different ply cartons. Grading of fibre boards is usually done by testing their bursting strength; the strength is expressed in kg/cm^2. Another test is puncture resistance, which is indicative of the performance of the board and is a function of materials that go into the manufacture of the board or box. Flat crush resistance, which is measured in pressure units, represents the resistance when squeezing a board between the thumb and fingers, whereas the edge-crush test gives the force required to crush vertically the columns of fluting media. Box compression tests are mainly done to determine the stackability of the CFB in a warehouse. These tests calculate the load required for the stack to collapse or deform. The containers are then stacked and stored under controlled conditions before final inspection and distribution.

12.3.3 Retort pouches

The concept of using a pouch as a container was developed by the US Army Natick Laboratories and a consortium of food-packaging companies in the early 1960s. The feasibility of using retort pouches for the manufacture of a wide range of food products was reported by Tripp (1961). In time, heat-sterilized, low-acid

Fig. 12.3 Different types of opaque and see-through retortable pouches.

solid foods in pouches created a new segment within the canned foods category (Brody, 2003). In 1967, Chinese dumplings and curry were packed in aluminum foil containing retortable pouches and marketed. In the years 1968–1969, commercialization of curry in both foil-free and aluminum foil containing pouches were undertaken and this initiated the era of retort pouch usage in Japan (Tsutsumi, 1972). The most comprehensive work in that period on flexible packaging for thermally processed foods was prepared by Lampi (1977). Sara *et al*. (1989) also studied the effect of increased over-pressure levels, entrapped air and temperature on the heat penetration rates in flexible packages. Retort-pouched products are shelf-stable, ready-to-eat and offer convenience to the consumer (Rangarao, 2002). Sacharow (2003) carried out market studies in the United States and Europe and reported a bright future for retortable pouches. The different types of pouches available in the global market are provided in Fig. 12.3.

Current retort pouch formats are typically comprised of three or four layers of materials, namely, an outer polyester layer, a middle aluminum layer and an inner cast PP layer (Griffin, 1987) and laminated together using thermostable adhesives. Of these materials, aluminium foil plays a pivotal role as the barrier layer which ultimately provides the product with a longer shelf life (Rangarao, 2002). PP has a high melting point of about 138°C and is used as the inner laminate layer to provide critical seal integrity, flexibility, strength and taste and odour compatibility with a wide range of food products (Shorten, 1982). The adhesives used to hold the layers together are usually modified polyolefins such as EVA. Taylor (2004) has reported the possible use of liquid crystal polymers – that is, Vectran™ by Ticona (Summit, NJ, USA) which have superior oxygen and water vapour barrier properties and heat resistance when compared to other polymeric films.

Table 12.5 Properties of material used for retort pouch manufacture

	Materials	Advantages	Disadvantages
Outer layer	Polyester	Good heat resistance and reverse colour printing	Probability of having pin holes high
	Biaxially oriented nylon	High strength	Shrinks during heat processing
	Non-oriented nylon	High strength and very good heat resistance	Difficulty in quality printing
	Biaxially oriented polypropylene	High strength and sealing	Develops shrinkage during heat processing; has the tendency to curl
Middle layer	Aluminium foil	High barrier	Pin holes, has opaque colour, poor heat sealing quality
	Polyester	High strength, heat resistant	Pin holes
	Nylon	High strength and impact resistance	Expensive
Inner layer	High density polythene	Good impact resistance, high sealing property, cheap	Poor heat resistance, translucent
	Non-oriented polypropylene	Transparent, good heat resistance	Poor impact resistance

Source: Gopal (2007).

Some pouches contain polyvinylidiene chloride (PVdC), ethylene vinyl alcohol (EVOH) or nylon (usually PET) instead of the aluminium layer to permit visual assessment of the food product. These are termed foil-free laminated materials. These plastics are good barriers to oxygen molecules, but are not complete barriers, and therefore product shelf life is reduced (Jun *et al*., 2006). Nowadays retort pouches containing coatings of silicon dioxide or aluminium oxide, in addition to the other aforementioned layers, are commercially available on the market. These pouches have good barrier properties and are comparable to aluminium foil pouches. The different types of retort pouches and the material layers which constitute these packaging materials are provided in Table 12.5.

12.3.4 Heat sterilization process in retort pouches

The retort pouch filling process is similar to can filling and requires the same care and attention. The major steps involved in retort pouch packaging are filling, air removal, sealing, traying, autoclaving and cooling (Madhwaraj *et al*., 1992). Once the product is filled into the pouch and the pouch is sealed it is then subjected to temperatures of 121.1°C with counter-pressure so that the cold point or slowest heating point within the food reaches the predetermined time-temperature integral (Brody, 2003). Once this temperature is reached, the product is cooled, labelled and stored (Balachandran, 2002; Madhwaraj *et al*., 1992; Venugopal and Shahidi, 1998).

Pouches used for retorting can either be preformed or produced from laminates on the process line. Preformed retort pouches are more commonly used and they are filled manually or by using automatic filling machines. Sauces and curry products, however, can be packed instantaneously in pouches that are produced from laminated rolls which are simultaneously formed, filled and sealed (Yamaguchi, 1990). In the case of products consisting of solid contents, pouches are filled with solids, together with some liquid, and sealed using a vacuum-sealing machine. Extreme care should be taken during filling of pouches to avoid contamination of the seal area, since this would result in improper sealing. Duxbury *et al*. (1970) reported that the final fill level should not be within 1.5 inches of the open top of the package so as to minimize product contamination of the seal area. Nughes (1971) suggests leaving as much as one third of the pouch volume free for the same reason. Lampi and Rubinate (1973) reported that a significant percentage of process-related failures was due to the contamination of seal areas during the filling operation. Schulz and Mansur (1969) indicated that steam flushing not only cleaned seal surfaces, but also removed residual air from the pouch.

Residual air trapped inside the pouch will affect heat transfer, product quality and seal integrity of the pouch. Its presence has been shown to affect physico-chemical, sensory properties and product shelf life (Olives, 2002). The residual air in the pack should be less than 2% of the volume of the pouch contents (Venugopal, 2006) and higher levels of air in the pouch may result in deflating of the pouches during thermal processing. The most commonly used methods for removal of residual air are vacuumization and steam flushing. Vacuum chamber (Goglio, 1968), counter-pressure (Tsutsumi, 1972), steam flush (Schulz and Mansur, 1969) and water head pressure (Heid, 1970) are some of the methods employed to remove air from retort packs prior to sealing. Air from the solid-pack pouch can be removed with the help of a vacuum machine and in semi-solid pack types through the use of steam injection. Super-heated steam is generally used because it causes less moisture condensation in the pack seal area. The stretch method is applied effectively for types of products such as curry (Tsutsumi, 1972). In this method the air within the pouch is reduced to a minimum by stretching both sides of the pouch prior to sealing. For large pouches, vacuum-sealing machines are very effective in removing residual air from the pack (Yamaguchi *et al*., 1972).

Sealing is an important operation in the processing and packaging of retort pouches. Methods of sealing flexible polymeric film pouches have been reviewed thoroughly (Brown and Keegan, 1973; Young, 1975), as has the type of sealing equipment used (McGillan and Neacy, 1964). A seal width of 5–10 mm is desirable for good seal strength. Hot-bar sealers and impulse sealers are commonly used for retort pouches (Tsutsumi, 1974, 1975). The use of the hot-bar sealing method is more preferable than impulse sealing, since, in the latter case, the resulting seals are narrower. Hence, the pouches should be double-sealed to reduce the risk of seal defect (Nieboer, 1973). It has been reported that the over-seal of retort pouches should be extended over the mouth of the pouches to prevent mould growth in any package above the first seal, which is closest to the product (Venugopal, 2006).

Sterilization is usually carried out in a batch or continuous retort system. The filled pouches are laid on trays or racks to maximize uniform heat transfer. An additional mesh restraint is placed over the trays to restrict pouch inflation and distortion during the retort process (Jeffs, 1984). The temperature and duration of the retort process depends on a variety of factors like type and size of the product and container, type of retort and process used, types of heating medium, etc. (Ramaswamy and Sablani, 1997). Usually the product is retorted at 121.1°C for a predetermined time. Retort pouches have the tendency to burst open due to the development of internal pressure developed by expansion of headspace gases during retorting. Over-pressure is supplied to the retort to counter the steam developed during heating and cooling (Bhowmik and Tandon, 1987; Tung *et al*., 1990).

Different types of retort systems and their operations are thoroughly described by different authors (Lampi, 1977; Venugopal, 2006; Yamaguchi, 1990). Steam-air mixture and water-immersion over-pressure retorts are commonly used for thermal processing of food in retort pouches. Pflug (1964) and Pflug and Borrero (1967), using both laboratory and commercial batch retorts, made a comparative study of steam, steam-air mixtures and water as the processing media. In continuous retorts, a hydro lock sterilizer was used for processing pouches (Goldfarb, 1970; Lawler, 1967). After retorting, the pouches are removed carefully from the retort, washed in chlorinated water to avoid post-process contamination, dried using air knives to remove the water and packed in suitable cartons to facilitate transportation and display on the shelves of supermarkets.

12.4 Methods to test the suitability of packaging materials for retorting

The following sections look into various ways of checking whether a certain packaging solution is suitable for particular sorts of food. The different considerations presented by the use of rigid as well as semi-rigid and flexible packaging are studied.

12.4.1 Testing rigid containers

Measurement of seam integrity

Testing for seam integrity is performed as per the method described by Lin *et al*. (1998) Using a micrometer, the seam length (L), seam thickness (ts), body hook, cover hook, body plate thickness (tb) and cover plate thickness (tc) of the double seamed can are measured. From these parameters, the percentage overlap is calculated using the formula. The minimum percentage of overlap is 45%.

$$\%\text{Overlap} = \frac{\text{BH} + \text{CH} + 1.1\text{tc} - \text{L}}{\text{L} - (2.2\text{tc} + 1.1\text{tb})} \quad [12.1]$$

where

BH = body hook length
CH = cover hook length
tc = cover plate thickness
tb = body plate thickness
L = seam length.

Seam integrity parameters can also be analysed using a semi-automatic double-seam analyser. Double-seamed cans are selected at random and three cut sections are made on the double seam, one after the other, using a twin-bladed seam saw which rotates at a speed of about 500 rpm. The cut width is 12.9 mm, which accurately fits the seam-analyser camera. The following parameters are then analysed: seam length, seam thickness, body plate thickness, end plate thickness, body hook length, end hook length and percentage overlap.

Air pressure test

For the purpose of determining the pressure-holding capacity of the cans, and to check for any leakage through the double seam, the cans are subjected to air pressure tests (IS: 2471, 1963; IS: 9396, 1979). The cans are pierced with a piercing pressure gauge and then air is pumped inside using a foot-operated pump until any distortion of the can or any leakage through the double-seam area is noticed. The double-seamed cans are immersed in boiling water for 5 min prior to the test. The cans are also processed at different temperatures and pressures of 115°C (68.94 kPa), 121.1°C (103.42 kPa) and 126°C (137.89 kPa) in a pilot-scale retort to determine their ability to withstand different processing conditions.

Determination of vacuum

The vacuum inside the processed cans can be determined by using a piercing vacuum gauge (IS: 3336-1968). Alternately a 'tap tone' can also be used, to check the vacuum and pressure levels inside glass, metal or plastic containers. The tap tone uses acoustic technology to measure pressure or vacuum in containers with metal closures that do not have a measurable lid deflection. A 'tap' is applied to the top of the container lid using an electromagnetic pulse which is transmitted through the can. The lid then vibrates at a natural resonant frequency or 'tone' depending on the internal pressure or vacuum. The 'tone' signal is then sensed and recorded. The digital signal processor (DSP) produces a real-time signal spectrum and calculates the resultant frequency of the 'tone' for that lid. The frequency is then compared to user set limits. Containers with a frequency outside these limits are rejected (www.taptone.com).

Overall migration

The tests for food contact applications are typically carried out in line with the test methods described by the CEC (1990) by determining the water extractives at 121.1°C for 2 h and soluble chloroform extractives. The cans are filled with

200 mL of hot glass distilled water and immediately heat sealed. The sealed cans are heat processed at 121.1°C for 2 h. After processing, the processed water is transferred into clean beakers and evaporated up to 50 mL. The contents of the beaker are then transferred into another clean pre-weighed, tarred platinum dish and evaporated to dryness. After cooling the weight of the dish is again taken to the nearest 0.1 mg to find out the amount of water extractives. To those dishes containing water extractives 50 mL of chloroform is added to dissolve all the chloroform extractives. The contents are filtered and evaporated to dryness and weighted to the nearest 0.1 mg to determine the amount of chloroform extractives.

Water-holding capacity

The water-holding capacity of cans can be determined as per IS: 6093 (1970). Two holes of 3–4 mm diameter are drilled about 5 cm apart as close as possible to the countersink, from the inside surface outwards on a can end. This is attached by double seaming on the other end of the can body. The can is then weighed to the nearest 1 g and filled with water at 27°C, employing a narrow water jet through one of the holes. Surplus water on the outside of the can is removed using a blotting paper and the filled can is weighed to the nearest 1 g. The difference between the weights is noted and to this 0.45% of the value is added. This represents the capacity in millilitres.

Lacquer delamination test

The polymeric coating of TFS cans is subjected to a delamination test using various organic solvents like acetone, carbon tetra chloride, chloroform, diethyl ether, ethyl acetate, n-heptane, methanol and petroleum ether. Can panels of 1 × 1 cm size are taken and immersed in organic solvents. They are taken out of these solutions after 24 h and examined for any delamination of the PET coating; if no peeling is evident, the panels are immersed for another 12 h. The panels are taken out and heated in a water bath for few minutes and examined for delamination of the coating.

Sulphide blackening test

Resistance of cans to sulphide blackening is analysed following the Cysteine test (Anon, 1977). For this test, cans are filled with the test solution consisting of 0.5 g of cysteine chloride in 1 L of buffer solution (3.56 g KH_2PO_4 and Na_2HPO_4. $2H_2O$ in 1 L of distilled water). Filled cans are double seamed and retorted for 30 min at 125°C. They are then left to cool down at room temperature for 24 h, following which they are opened and evaluated for any blackening.

12.4.2 Retort pouches and semi-rigid thermoform containers

Different parameters are tested to determine the physiochemical properties of thermoformed containers and retort pouches. The majority of the tests performed are similar, though, due to the flexible nature of both types of containers.

Thickness

The total thickness of retortable pouches and semi-rigid containers is determined as per ASTM (1964) guidelines. The thickness of the pouch has a direct influence on heat penetration characteristics and, ultimately, on product quality. Non-uniform thickness can affect machine performance, product protection and integrity of the packages (Hemavathi *et al.*, 2002). The acceptable limit for variation in thickness of individual layers is ± 2 μm (inner ply) or 10% of the total gauge value (Lampi, 1977, 1980). A narrow profile helps in the rapid transfer of heat to the inner regions of the pack, quite unlike thicker-walled containers like cans and glass bottles. About 20–30% reduction in process time was observed by Simpson *et al*. (2004) for vacuum-packed mackerel held in retortable pouches and processed in a steam-air mixture at 116.8°C when compared to cans.

Barrier properties

Pin holes in plastic films and aluminium foil are unlikely to be created when the materials are laminated (Yamaguchi, 1990). It has also been confirmed that microorganisms do not pass through even a single-layered plastic film (Lampi, 1977). Hence, problematic issues such as pin holes, which affect the barrier properties of retort pouches probably arise in the handling of packaging materials during and after their use in food manufacture.

Oxygen transmission rate (OTR)

OTR is of great importance in the packaging of processed foods and it is critical that headspace oxygen is excluded (Kumar, 1994). The OTR is determined as per the method described in ASTM, D1434 (1982a). The gas transmission rate for a packaging material is expressed as mL m^{-2} 24 h^{-1} at 1 atm. pressure maintained at 21°C. The OTR in imported and indigenous retort pouches was found to be practically nil for three-layered laminated pouches tested by Vijayalakshmi *et al*. (2003).

Water vapour transmission rate (WVTR)

The amount of moisture or water which is transmitted through a film or packaging material helps in maintaining the shelf life of the product. The determination of WVTR is usually done by gravimetric analysis which measures the loss or gain of moisture under controlled conditions. The WVTR is determined as per the ASTM E96-80 (1982b) and expressed as g/m^2 /24 h at 90 ± 2% RH and 37°C. For multilayer laminates like retortable pouches, which have a combination of three or more layers, the WVTR rates are usually very low when compared to monolayer films.

Tensile strength and elongation at break point

These tests are applicable for flexible films only. Strength is required to protect the product during processing, distribution and retail handling. Polyester and nylon are commonly used as oriented films since they are generally stronger than

unoriented films (Ghazala, 1994). The tensile strength is determined using ASTM D 882-02 and the tensile strength at break point is calculated in kg/cm^2 from the original area of cross section. Elongation at break point is expressed as percentage of the original length between the reference lines.

Heat-seal strength

Heat-seal strength is an important property in order to achieve packaging integrity and to provide shelf life. PP can tolerate temperatures up to 130°C and consequently, is suitable for reheating in the microwave (Forshaw, 1990). PP possesses good heat-seal strength and, as such, is used as the inner laminate layer for heat sealing. The breaking strength of the heat-sealed seams is instrumentally determined in a universal testing machine using ASTM 88-68 (1973). The maximum stress applied to the specimen at yield or breakage is recorded.

Bursting strength

Bursting strength is determined as per the method described by Duxbury *et al*. (1970). Low bursting strength values indicate easy delamination of the layers during thermal processing which results in physical destruction of the pouch and reduction in barrier properties (Vijayalakshmi *et al*., 2003). This test determines the capability of the pouch to hold air at 25 psi pressure for 30 s without bursting, after which time it passes the test.

Residual air

Residual air in packs is determined as per the method described by Shappee *et al*. (1972).

Bond strength

This is determined as per the ASTM, D 90349 method (1972). The test is carried out by initiating the separation of the laminate layers using diethyl ether or chloroform or toluene and then measuring the tensile strength.

Overall migration test

Plastics, in finished forms, contain non-polymeric components (mainly additives) which may leach out into the packed food when it comes into direct contact (Vijayalakshmi *et al*., 2003). The selection of suitable packaging material for food contact application is decided upon on the basis of the physical, mechanical, barrier and performance properties required for the films (Iyer, 1992). These may contaminate the food and present toxicity risks if consumed. Since the migration of certain compounds is inevitable, different countries have prescribed limits for these extractible substances. As per Indian standards, the limit for finished materials is 10 mg/dm^2 or 60 ppm. According to Directive 90/128/EEC the overall migration limit is 10 mg for all transferred substances per dm^2 of the food contact material surface. Vijayalakshmi *et al*. (1992) reported higher migration of components from retort pouches into n-heptane, rather than into water. This may be due to the structural similarities of n-heptane with the casted contact PP layer.

12.5 Changes in the quality of seafood, meat and poultry due to retort processing

Retorted foods are heated at high temperatures to eliminate microorganisms, particularly pathogenic entities. However, the use of prolonged heating at high temperatures can result in unwanted chemical reactions, leading to reduced nutritional and sensory quality. For example, Aitken and Connell (1979) highlighted the fact that the flesh of certain fish cannot be canned because it disintegrates after heating (the following fish species do not suffer from this problem, so are commonly canned: tuna and bonito, sardine, herring, shrimp, prawn and salmon). Flavour changes during heating include those due to the Maillard reaction, fatty acid oxidation and the formation of low molecular weight volatile compounds like ammonia and hydrogen sulphide. The colour of processed food is an important factor from a consumer acceptability perspective. Naturally occurring pigments and components may be degraded or destroyed during heat processing and Maillard browning may occur.

The sections below outline some significant changes in muscle biochemistry, microbiological and nutritional quality caused by retort processing. In general, high-temperature, short-time processing of muscle-based foods minimizes the negative thermal changes considerably and hence has advantages over conventional retorting where the changes are of a larger magnitude (Awuah *et al.,* 2007).

12.5.1 Biochemical changes in retort-processed muscle foods

Changes in proteins

Thermal processes, such as those used in retort processing, affect proteins in two ways. First, they result in changes to the secondary, tertiary and quaternary structure of proteins. Bonds are broken, which causes unfolding of protein structures. This improves protein bioavailability, since the amino acids which are formed can more easily be absorbed by the human body. Second, though, the alterations in the primary protein structure may also lower digestibility and produce proteins that are not biologically available (Swaisgood, 1985). The phenomena resulting in improvement in or loss of both nutritional quality and physiological properties of food proteins are the results of protein denaturation and chemical modification of amino acids (Finot, 1997). In the canning processes, changes in proteins occur mainly at three different stages, namely pre-cooking, thermal processing and diffusion into the filling media. Bender (1972) and Broek (1965) have reported the effects of thermal processing on fish proteins. Fellows (1990) reported an approximately 10–20% reduction in amino acids in canned foods. In meat, protein denaturation also takes place during retorting and the collagen present in the connective tissue is converted to gelatin. At the temperatures encountered in retorting, thermally induced changes in intramuscular connective tissue tenderize the resulting product while changes in myofibrillar protein promote toughening of the muscle tissues (Palka, 2004).

Browning reactions

Heat treatment triggers browning or Maillard reactions in foods which are a complex series of reactions between amino acids and sugars present in the product. Compounds involved in Maillard reactions include amino compounds such as free amino acid and volatile amino compounds associated with microbial spoilage (Nakamura *et al*., 1973), carbonyl compounds such as reducing sugars and aldehydes and ketones derived from lipid oxidation (Pokorny *et al*., 1973). During the initial stages of the reaction, colourless compounds are formed, while during the later stages brown-coloured pigments called melanoides are formed (Whistler and Daniel, 1985). Even though the characteristic cooked flavour is desirable, there will be a resulting loss in quality. Maillard reactions can be inhibited by reducing pH or temperature if the product is in liquid form or by decreasing moisture to very low levels within the product. The removal of one of the substrates responsible for browning, mainly sugar, may also reduce the reaction (Yamaguchi and Kishimoto, 1976). These authors also studied the relationship between process temperature and retort pouch thickness on browning and concluded that minimum browning was achieved at 130°C using 20 mm, 135°C using 15 mm and 140°C using 8 mm. During thermal processing, carbonyl compounds from oxidized lipids may be solubilized and react with the nitrogenous compounds in fish flesh to form browning compounds (Fujimoto and Kaneda, 1973). During thermal processing of foods, high molecular weight melanoidins are generated by a cross-linking reaction which occurs between low molecular weight Maillard reaction products and high molecular weight non-pigmented proteins. Since meat contains a high amount of protein, carbohydrate oligomerization and browning of proteins may be involved in the formation of melanoidins during thermal processing (Shahidi *et al*., 2004). These authors also reported that model experiments showed that high and low molecular weight pigmented compounds were generated during thermal processing and contributed to product browning.

Other colour changes

Carotenoids present in both fish and meat products are isomerized from 5, 6-epoxides to 5, 8-epoxides which, consequently, possess a less desirable colour. Heating also denatures myoglobin and oxidizes carotenoid pigments (Haard, 1992). Trout, pollack and shrimp processed to an equal lethality developed a darker colour when processed in cans rather than retortable pouches; authors attributed this observation to the longer processing times required to complete the processing cycle for products heated in cans (Chia *et al*., 1983). Colour changes are more pronounced when the raw material is of poor quality – for example, green discolouration development in canned tuna is attributed to a number of factors, some pertaining to quality status, trimethylamine oxide (TMAO), myoglobin and cysteine concentration and the cooking operation itself (Khayat, 1978). Determination of the combined TMAO and trimethylamine (TMA) content of the raw fish can be used to indicate the probability of greening occurring in the can during the heat process (Yamagata *et al*., 1971).

Changes in fats

The severe heat treatment applied during retorting and the presence of certain catalysts in the fish muscle favours lipid oxidation and hydrolysis resulting in off-flavours and loss of nutrients (Hsieh and Kinsella, 1989; Harris and Tall, 1994). The influence of the physical state of the muscle affects the rate of oil oxidation (Frankel *et al*., 1996). Oxidation can increase depending on the partitioning of the polyunsaturated fatty acids (PUFA) in the oil–water emulsion (Coupland *et al.*, 1996). Unsaturated fatty acids have high surfactant activities and tend to accumulate at the oil–water interface, thereby rendering them more susceptible to lipid oxidation (Coupland *et al.*, 1996). Damage to the unsaturated fatty acids can lead to primary and secondary lipid oxidation products, which can result in browning (Aubourg, 1999). Thiobarbaturic acid reactive substances (TBARS), produced during lipid oxidation, are highly reactive and react with other food components like amino groups to produce interaction compounds possessing fluorescent properties (Pokorny*,* 1981). Lipid oxidation typically occurs more rapidly in seafood, owing to its higher unsaturated fat content, followed by poultry (particularly dark poultry meat like that found in thigh and wing muscle as well as that derived from waterfowl) and meat.

Free fatty acid (FFA) content was shown to increase during the sterilization of different albacore muscle zones (Aubourg *et al*., 1990). Time-temperature data for canned tuna processed to an F_0 value for 7 min indicated that treatments utilizing higher temperatures led to a greater degree of hydrolysis, even if the processing time was of a short duration (Medina *et al.*, 1997). The filling medium employed, whether oil or brine, has been shown to be independent of the extent of FFA formation (Medina *et al*., 1994). Tanaka *et al*. (1985) observed a remarkable decrease in the FFA values of canned mackerel in dry packs (those without any filling medium) processed to equal lethalities at different temperatures. Precooking and subsequent removal of exuded liquid greatly increased the level of FFA in the muscle (Medina *et al*., 1995). Tanaka *et al*. (1985) also found that, at lower processing temperatures, increased levels of FFA formation occurred when processing times were longer.

When meat is heat processed it undergoes a series of chemical reactions which generates numerous quantities of volatile compounds responsible for the particular flavour associated with the cooked meat in question. The major constituents which contribute to meat flavour are amino acids, peptides, organic acids, nucleotides and other components (Shahidi, 1989). The lipid components of meat within the intramuscular region also affect the flavour and quality of thermally processed products. Phospholipids present within the intramuscular lipids of meat are primarily responsible for the development of meat aromas (Mottram, 1983).

12.5.2 Nutritional quality of retort-processed muscle foods

A retort process should be designed in such a manner that nutritional constituents present in the initial material are maximally retained for human nutritional benefit

(Aubourg, 2001). Severe heat treatment permanently destroys spoilage bacteria, deactivates enzymes, denatures proteins, but also reduces vitamin and mineral content. Vitamin degradation is dependent on numerous factors such as the presence of certain chemicals and high temperature. Fat soluble vitamins like A, D, E, β-carotene, water-soluble vitamin C (ascorbic acid), vitamin B_1 (thiamine) and vitamin B_2 (riboflavin) are heat-sensitive (Ryley and Kajda, 1994). Vitamins like, thiamine, riboflavin, niacin, pyridoxine and panthonic acid are considered heat labile and undergo drastic changes during thermal processing (Banga *et al*., 1993). The vitamin thiamine is extremely sensitive to thermal processing (Chia *et al.,* 1983), while vitamins like A and D, which are found in abundance, are retained to a much greater extent (Bender, 1987). Water-soluble nutrients leach into the liquid medium, but in general, nutrient retention in canned seafood products is acceptable (Pigott and Tucker, 1990). Braeckan (1962) found vitamin B_1 levels to be similar for both fresh and canned fish.

Some loss in minerals like sodium, potassium, magnesium, phosphorous, copper, iron and calcium has been shown to occur in canned tuna through leaching into the dipping medium (Seet and Brown, 1983). Fish with higher fat contents generally have reduced losses in minerals. Major advantages of thermally processing fish are that the bones become soft and can be consumed safely, thereby providing valuable dietary calcium. Metal ions and salt in processed meats serve as pro-oxidants, which function to promote oxidation of unsaturated fats. During the heating process, haemoglobin breaks down. Haeme compounds, in untreated meat become rapidly oxidized and produce ferric and ferrous ions (Shahidi, 2002). Shahidi *et al*. (2004) reported that during thermal processing phospholipids and their degradation products inhibit the generation of sulphur-containing heterocyclics, thereby maintaining key sulphur compounds at optimum level within the product.

12.5.3 Microbiological changes in retort-processed muscle foods

Heat processing or sterilization is the most dramatic procedure carried out during the manufacture of canned products and by definition guarantees the sterility of the final product (Aubourg, 2001). To maintain the quality of, for example, canned fish, three conditions must be maintained. First, the container should be hermetically sealed and the seal integrity should be guaranteed so that the can is sterile at all times (Lopez, 1987). Second, the adequate thermal process lethality required to kill target organisms should be achieved. The temperature at the cold spot, which is the most inaccessible part of the containerized food product, should be recorded by heat penetration studies (Banga *et al*., 1991). Time and temperature studies depend on the characteristics of the product and container, geometry of the package and the type of heating medium used during the retorting process (Lund, 1975; Oliviera *et al*., 1986). Third, a scrupulous and hygienic post-process treatment should be carried out and the products stored adequately. The water used for cooling should always be chlorinated so that it is not a source of contamination. Kramer (1982) and Ruiz-Roso *et al.* (1998) suggested a three- to four-month storage period to

obtain advantageous textural changes and optimal palatability in most canned fish products. Thermally processed products should be stored at ambient temperature, below 30°C, in order to prevent the outgrowth of thermophilic spores which may have survived processing. The effect of storage temperature and duration of canned storage is also very important for retorted fish products, especially those preserved in sauces and which are acidic in nature, primarily due to the corrosive action of the food acids on the containers used (Lopez, 1987).

12.6 Future trends in processing and packaging

The market for muscle foods is estimated to double between 2000 and 2050 (Troy and Kerry, 2010). As production volumes increase, the current high standards in production, packaging and marketing of meat, poultry and seafood will need to be maintained. Safety issues like HACCP implementation and traceability will be top priorities for the market. Consumer demand for high-quality convenience products will also continue to evolve and grow. The sustainability of production and climate change will play a role in the future and new products developed and marketed should be nutritionally superior (Troy and Kerry, 2010).

Techniques like high-temperature, short-time (HTST) processing, which have less of an impact on product sensory and nutritional quality, could be used more frequently in the future. Rotary retorts which facilitate agitation are advocated for the processing of liquid and semi-solid products, as opposed to stationary retorts. Agitation helps in reducing processing time and permitting faster product heat penetration, thereby enhancing product quality and enabling the production process to become more efficient. Currently both steam-air retorts and water-immersion retorts are used for thermal processing. Both have their own advantages and disadvantages. Steam-air retorts have a lower capital investment, are easy to manage and can be operated manually. Most types of containers can be processed in them and they are energy efficient. The main disadvantages are that rotary processes can only be carried out at lower rpms. Water-immersion retorts require higher initial capital investment and have a slower processing time. The maintenance and running costs are also high. However, in water-immersion retorts it is possible to process just about any product in any containers and the heat distribution can be maintained uniformly. Higher rpms are also possible in this type of retort.

The packaging industry needs to develop materials that can be retorted and reheated in a microwave or in the conventional domestic oven. The lidding material for thermoform trays should be capable of maintaining sterility and should be self-venting when heated in a microwave. The concept of self-heating products in pouches or trays, which are available in the United States, is still to be adopted worldwide. Standing retort pouches which are gusseted to provide this function, have an additional advantage over the ordinary retort pouch, again from the point of usage, but also in terms of retail stacking and product promotion and advertising. Additionally, these packs could be resealed so that the pack contents need not be consumed at any one time only. Thermally processed products facilitating

product tracking and traceability will also be in demand. The increasing trend in consumption of such retort products and the wide range of products available indicates a bright future for the market. These products will cater to the different markets and offer convenience of use to consumers in the most remote parts of the globe, thereby making their presence a universal one.

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13

Packaging for frozen meat, seafood and poultry products

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Abstract: Packaging of muscle-based foods has been a common practice since frozen foods were first exploited commercially. Like any other preservative technique, freezing muscle foods retards microbial growth and enzymatic activity, but also has implications for food quality. Ice crystal size represents a major concern because tissue damage can result in dripping losses during thawing. Recent advances in freezing techniques include methods for developing small ice crystals and modifying crystallization rates, such as high-pressure assisted freezing. Selecting appropriate freezing temperatures can contribute to extending shelf life, as can using packaging materials with selective permeability. The development of new synthetic packing materials has made it possible to package meat, poultry and seafood products more safely and attractively.

Key words: spoilage microorganisms, microflora, semicrystalline plastics, sealant layer, atmosphere modification, cryoconcentration.

13.1 Introduction

In the introduction, we look at the developments that have led us to where we are today in terms of packaging methods for frozen meat, seafood and poultry.

13.1.1 From Birdseye to freezing today

Many years ago, freezing foods was common practice in regions of the world that experience cold winters, so that important muscle food reserves like meat and fish could be preserved. Trading of frozen meat began with shipments from Australia to England in 1882. However, commercial domestic refrigerators were not available until the development of mechanical ammonia freezing systems,

which would later allow more consumers to access frozen foods. This reduced shopping trips, saved time and money, and allowed a large amount and a wide variety of foods to be stored for convenience and future consumption.

The commercial development of frozen foods is attributed to an American explorer by the name of Clarence Birdseye. He observed, circa 1915, how the native people of the Canadian Labrador province preserved fish by freezing, using a combination of chilling wind and cold water. He observed that when the fish were thawed, no difference in texture or flavor was noticeable in the products. After some experimentation, Birdseye patented the packaging-freezing machine, which used a refrigerated plates system. This system quickly froze foods into a solid congealed block comprised of numerous tiny ice crystals, limiting damage caused by freezing (Birdseye, 1931). He realized that the key to successful freezing was the rapidity at which one froze water in foods, fast freezing being desirable because it prevented the growth of large ice crystals. Quick-freezing of packaged muscle foods thus became a commercial alternative, having the advantages of reducing humidity loss and freezer burn, and the associated negative changes in color and odor (Birdseye, 1929a, 1929b). Many other companies have since developed quick-freezing systems, taking advantage of novel packaging materials and formats that have optimized and economized the process. The development of synthetic packaging materials greatly improved the quality and safety of frozen foods.

13.1.2 Packaging and freezing

Packaging provides food protection against chemical, biological and physical contaminants. Chemical protection acts like a barrier to minimize compositional changes triggered by environmental factors like oxygen, moisture or light. Biological protection prevents the growth of pathogens or spoilage microorganisms, yet maintains a suitable environment within the pack to allow for the development of desirable microflora. Physical protection secures products from mechanical or physical damage during distribution (Marsh and Bugusu, 2007). On the other hand, freezing involves lowering the product's thermal center to 0°C, resulting in crystallization of most of the water and some solutes. The freezing rate, heat transfer coefficient and amount of heat removed directly impact on ice crystallization, loss of moisture and microbial growth, and thus determine the final quality of the frozen product (Bejarano Wallens and Venetucci, 1995). For any specific frozen product, shelf life depends on its specific characteristics (raw materials, ingredients, formulation, etc.), pre-freezing treatment, freezing process, packaging film and storage temperature (Zaritzky, 2008).

Meat and muscle-based products are perishable. Even after processing, the packaging and freezing of these foodstuffs must operate together to ensure the microbiological safety of the product, and avoid biochemical deterioration during transportation and storage. Packaging plays a key role in maintaining the quality of frozen foods. Poorly packaged frozen foods undergo weight losses due to the sublimation of surface ice (Campañone *et al*., 2001). Lower weight losses occur at lower storage temperatures, due to the difference between vapor pressure at the

meat surface and the surrounding air (Mendez Bustabad, 1999). The dry porous layer that forms in badly packaged frozen foods alters the sensory characteristics of the product, leading to quality loss due to spoilage, changes in color, taste and texture (Campañone *et al*., 2002). An effective packaging system is essential to offset the detrimental quality changes that occur during the frozen storage, with packaging materials and methods that protect the product from microbial and chemical contamination, dehydration and physical damage, and also protect the environment of the packaged product (Jiang and Lee, 2006, 2007).

13.1.3 Packaging material

Packaging materials employed in frozen meat, seafood and poultry products comprise a wide range of materials, from paperboard in secondary packaging (Santos *et al*., 2008) to laminated plastics in contact with the muscle-based food. Modern synthetic packaging materials, or polymer technologies, allow manufacturers to offer a wide range of packaging materials for the frozen food industry, such as flexible multilayer packaging. Production of flexible packaging suitable for frozen meat, poultry and seafood is predicted to increase in coming years as a result of ongoing demand for these foodstuffs, which are perceived as economical protein sources (Harrington, 2010).

Flexible packaging must fulfil a protective function, as well as being economically viable and environmentally friendly. In order to ensure product protection, it must have excellent barrier properties against gases (water vapor, oxygen and others) and good-quality seals. The use of environmentally friendly materials is also important, as is efficiency (Breil, 2010). Selecting the right type of film depends on the requirements of the product. Table 13.1 shows the properties of some polymers used for flexible packaging. Key requirements for flexible packaging for frozen foods include: toughness at low temperatures, adequate modulus or stiffness, high hot-tack strength and high seal strength. Linear low-density polyethylene (LLDPE), ultra low-density polyethylene (ULDPE), ethylene vinyl acetate (EVA) and polyolefin plastomer (POP) resins, are all commonly used, because they have the necessary stiffness for high-speed packaging and the tear and puncture strength to prevent damage during transportation and storage (Butler and Morris, 2010).

In flexible packaging, different types of polymeric materials are used to create multilayer films, which enhance mechanical and barrier properties. The orientation of the films changes their physical properties due to the alienation of structural components. Generic packaging materials comprise an outside printable skin layer, one or more core layers and an inside skin layer in contact with the foodstuff. These layers can be glued by their own thermo-sealant properties or an adhesive resin can be employed (Fig. 13.1a). The orientation of plastic films is manipulated by stretching the materials while they are still hot and flexible, to create stretchable films, heat-shrinkable films, or stiff laminates. Films can be either monoaxially or biaxially stretched (Fig. 13.1b). The properties of oriented or un-oriented polymer films are essentially the same, and permeability is

Table 13.1 Properties of common polymers films employed in flexible packaging

Polymer name	Abbreviation	Density (g/cc)	Moisture vapor transmission rates[a]	Gases permeability[b]			Elongation (%)	Heat seal (°C)	Printability
				O_2	N_2	CO_2			
High density polyethylene	HDPE	0.940–0.965	150	600	70	4500	100	135	Fair
Low density polyethylene	LDPE	0.915–0.925	420	550	180	2900	400	121	Fair
Polypropylene	PP	0.89–0.902	150	240	60	800	300	177	Good
Polyvinyl chloride	PVC	1.16	5–20	150	65	970	20	107	Excellent
Polyvinylidene chloride	PVDC	1.7	0.15	14	12	4	60	138	Poor

Source: Adapted from Butler and Morris, 2009; Hanlon, 1992.
[a] MVTR: g/24 h/100 in^2 /mL @ 38°C, 90% RH.
[b] Gases permeability: cc/24 h/100 in^2 /mLil @ 25°C, 50% RH.

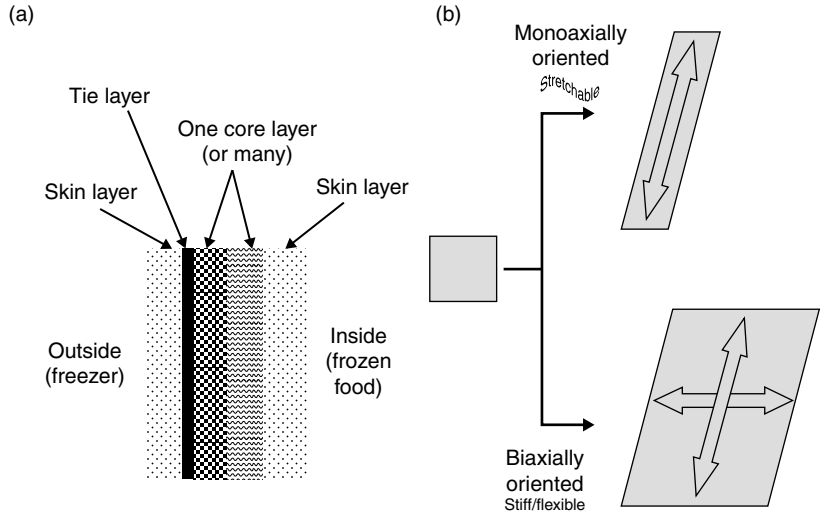

Fig. 13.1 (a) Generic multilayer film design with different film compositions depending on packaging necessities and material properties. (b) Sheet axial orientation of polymers to change film properties.

not usually affected (Hanlon, 1992). Monoaxial stretching is designed to form a wide, thin sheet of plastic film (greater than 80 times its original length), orienting the molecular structure of monomers, resulting in an easily stretchable film (in the direction of the stretching). Biaxial stretching, or tender process pouring, pulls the film in both directions at once, resulting in a firm plastic with different properties than the non-stretched plastic sheet (Driscoll and Rahman, 2007). Oriented plastic films improve the characteristics of the original polymer, resulting in exceptional mechanical properties in combination with barrier and optical properties. Biaxially oriented polypropylene (BOPP) and biaxially oriented polyethylene terephthalate (BOPET) films are both low-cost packaging materials, in comparison with non-oriented films. The overall improved barrier properties attained result from the orientation of the molecule chains. In non-oriented polymers these are random, whereas the stretching process results in a clear molecule chain orientation. Biaxial orientation of plastic films represents a refinement process that is applicable to almost all plastics. The crystallinity of polypropylene (PP) and polyester (semicrystalline plastics) is augmented by the stretching process, which considerably improves their mechanical properties (Breil, 2010).

In practice, these two kinds of packaging films can be applied in the following ways:

- Multilayer packaging films can be used as shrink films in primal packaging. The film used in this application is a polyvinylidene chloride (PVDC) barrier film, with the sealant layer designed to provide toughness and puncture resistance, and oriented to provide acceptable shrink properties. Packages are

Fig. 13.2 Raw meat, poultry or seafood products packaging in stretchable materials.

normally vacuum packaging with a good sealant polymer such as EVA, ionomer, or LLDPE, where moisture barrier properties are more critical (Butler and Morris, 2010). Poultry breast, legs, nuggets and breakfast sausages, among others, are packaged in stretchable films either with or without trays (Fig. 13.2).

- Multilayer packaging films can be used as barrier films, designed to keep oxygen from entering the package in order to extend shelf life and give the retailer extended product display time. This packaging also allows the consumer to keep the product in their refrigerator, unopened, for some time after purchase. These packages can be printed with attractive graphics to increase sales. The films may contain a barrier polymer, printing surface (such as polyethylene terephthalate (PET) or nylon, which also provides thermal resistance during sealing and helps provide abuse resistance during distribution, LLDPE or ULDPE toughness layers, a sealant layer that could be LLDPE, and a polyolefin plastomer or an ionomer (Butler and Morris, 2010). Frozen meat, poultry or fish products, like hamburgers, nuggets and marinated poultry breast, among others, are packed in laminated printed bags (Fig. 13.3).

13.1.4 Atmosphere modification in frozen meat products

Vacuum and atmosphere modification are the most commonly employed techniques in packaging frozen meat and meat products. The display life, color and appearance of meat and meat products is also influenced by the degree of vacuum

Fig. 13.3 Printed flexible packaging application to processed meat, poultry or seafood products.

or atmosphere modification, though film shrinkage, the duration of freezing prior to packaging and display conditions are also contributing factors (Jeremiah, 2001). Low temperatures can be used to achieve special effects in modified atmosphere-packaged (MAP) seafood products. For example, in frozen cod fillets it not only provides a more stable MAP product, but also allows much greater flexibility for production and distribution (Rosnes *et al*., 2003). Although chemical reactions proceed very slowly under freezing temperatures, they may still cause significant quality changes over time. The oxidation of unsaturated fats, vitamins and pigments continues to take place during frozen storage, resulting in loss of color, flavor and nutritive value, but these problems can be significantly reduced or completely eliminated by vacuum packaging (Floros and Matsos, 2005).

It has been reported that combining freezing and vacuum packaging reduces moisture losses and rancidity, presenting less pigment oxidation, and a tender texture in beef, venison and poultry (Farouk and Freke, 2008; Kenawi, 1994; Lee *et al*., 2008). In the same way, modified atmosphere packaging of mackerel, salmon (60% N_2–40% CO_2) and whiting (30% N_2–40% CO_2–30% O_2) presented lower total viable counts, with no influence on odor or acceptability scores (Fagan *et al*., 2004). Although low-oxygen permeability packing of Atlantic hake was more effective in retarding lipid oxidation than high-oxygen permeability packing, cholesterol degradation and oxidation were not hindered (Saldanha and Bragagnolo, 2008). Exclusion of oxygen from such packs extended shelf life of shrimp for at least 9 months when packed in a full N_2 atmosphere, and gave better overall quality in terms of color stability, lipid oxidation and toughness (Bak *et al*., 1999).

13.2 Quality improvement through frozen packaging

If the changes that a specific frozen food undergoes are known, it is possible to select appropriate packaging material and package format options, and so minimize quality loss (Krotcha, 2006). The quality improvement of meat and muscle-based products can be related to their microbiological and physicochemical quality characteristics.

13.2.1 Microbiological quality

Meat presents a nutritious substrate for microorganisms and so, depending on the packaging used, either aerobic or anaerobic bacteria can dominate the microflora (Moorhead, 2006). The rate at which a microorganism is frozen relates to its ability to survive, and its growth phase may affect its susceptibility or resistance to freezing, since Gram-positive bacteria are generally more resistant to freezing and thawing than Gram-negative species (Archer, 2004).

Packaging must therefore ensure the inhibition of freeze-resistant microorganisms. For example, in poultry, *Campylobacter* spp. may lead to infection through improper handling and insufficient cooking, and although freezing cannot replace sanitary production and handling, it could reduce the chance of high initial contamination during the production process (Sampers *et al*., 2010). Antimicrobial compounds can be added to packaging materials; for example, allyl-isothiocyanate incorporated into the packaging (Nylon/EVOH/PE with N_2) surrounding frozen beef patties was shown to be capable of eliminating 3 $\log_{10}$ CFU/g of *E. coli* O157:H7 from frozen (−18°C) patties within 10 days of storage (Nadarajah *et al*., 2005).

13.2.2 Physicochemical quality

The physicochemical quality changes that take place in meat during frozen storage are: (i) denaturizing of proteins, (ii) ice recrystallization, (iii) oxidation of lipids, (iv) sublimation (freezer burn), (v) enzymatic reactions, (vi) degradation of pigments and vitamins and (vii) flavor deterioration (Calvelo, 1981; Zaritzky, 2008). These occurrences can be partially controlled by the correct selection of suitable packaging and through reduced oxygen concentrations in meat packs, especially processed meats, in order to reduce lipid oxidation (Moorhead, 2006). It should be stated, however, that the latter is affected by numerous factors, including the product's lipid content and its type and degree of pigmentation. For example, lipid and protein oxidation appeared to occur simultaneously in chicken meat during frozen storage, and was more intense in leg than in breast meat, probably as a result of pro-oxidative and anti-oxidative factors in chicken leg and breast meat (Soyer *et al*., 2010). Fish and pork, which contain higher proportions of more reactive polyunsaturated fatty acids, are more susceptible to the development of rancidity (Zaritzky, 2008). Packaging must provide a good barrier to oxygen in order to prevent the development of off-flavor, dehydration and consequent freezer burn (Kotrola, 2006).

The size of ice crystals is an important issue in the packaging of frozen muscle-based products, since a lack of control over the freezing process will lead to large ice crystal formation, causing tissue damage. In the course of converting muscle to meat (ageing), a part of the intracellular liquid migrates to the extracellular spaces. During freezing, water outside the muscle fibers freezes first, thus intracellular water tends to be drawn out of the fibers by osmotic effects, increasing the intracellular concentration of solutes, or cryoconcentration. This cryoconcentration of the intracellular media and the growth of crystals result in protein denaturation and cell membrane breakdown, as a consequence of changes in osmotic pressure, pH, ionic force, viscosity and water activity. These factors in turn produce alterations in the water-holding capacity of muscle on thawing, changes in texture and changes in surface color (Calvelo, 1981; Genot, 2000; Pérez-Chabela and Mateo-Oyagüe, 2006).

Different freezing rates result in different-sized ice crystals, directly affecting the meat and meat product's properties during thawing. If the zone of maximum ice crystal formation (around −4°C) is reached rapidly, tiny ice crystals will form, causing no damage to meat tissue. Larger individual crystals are formed at lower freezing rates (Birdseye, 1929a, 1933). When the temperature falls, ice is reformed on the remaining crystals, causing them to grow (Ranken, 2000). Irregular needle-shaped ice crystals form a fibrous microstructure during slow freezing, resulting in a lot of physical damage. Meat fiber damage and drip loss can be reduced in meat using a faster freezing rate and by employing inhibitors of ice crystal growth (Mousavi *et al*., 2007).

When low freezing rates are employed, ice crystals are formed in the less concentrated extracellular liquid, and grow progressively. Juiciness and tenderness are negatively affected on thawing, due to exudation from the meat. During middle or average freezing rates (2–5 cm/h), ice is formed in both the interior and exterior of muscle cells. Tissue damage during middle freezing is significant, and the amount of exudates lost from the meat product is directly proportional to the freezing rate used. Quick-freezing rates allow for crystallization to occur in the cell interior. Numerous small-size crystals are formed, and protein denaturation is limited, together with tissue structure damage, thereby provoking less liquid exudation. Ice crystals grow progressively over storage time and fluctuations in temperature provoke a diminution in meat water-holding capacity due to protein dehydration (Genot, 2000). During frozen storage, changes in protein conformation occur. Protein–protein aggregation can result from the dehydration of protein molecules, due to water molecule displacement from hydrated protein side-chains and surrounding areas, to a lower vapor pressure zone, resulting in the formation of ice crystals. This water displacement provokes the protein molecules to come into closer contact with each other, probably causing intermolecular cross-linkages. As the product thaws, liquid water returns to the vicinity of the protein aggregates. However, since protein–protein interactions are stronger than protein–water interactions, rehydration of the protein molecules is incomplete (Matsumoto, 1980) (Fig. 13.4). There are three accepted theories to explain denaturation of structural proteins during freezing: (i) an increase in solute concentration, (ii) dehydration

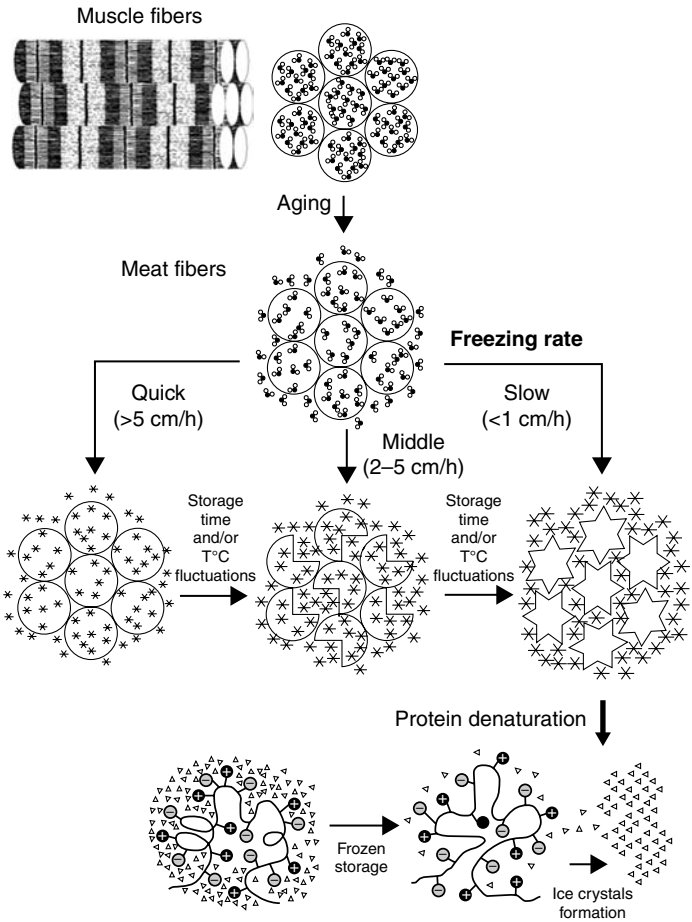

Fig. 13.4 Schematic representation of the freezing rate effect on ice crystal's size and localization in meat fibers and the consequences in exudation loss and protein denaturation caused during frozen storage (adapted from Genot, 2000; Matsumoto, 1980).

of the cell and (iii) auto-oxidative changes that alter the balance of protein–protein and protein–water interactions (Venugopal, 2006).

Under the same freezing conditions, heat transferred parallel to the muscle fibril direction takes place quicker than that perpendicular to the muscle direction, since heat resistance is offered by meat components such as epimysium, perimysium, endomysium and ligament sheaths (Su and Zhu, 1999). The fibrous nature of meat and the entangled fiber network arising from ice crystal compression, where a more entangled network of fiber strands in frozen meat is accompanied with fiber shrinkage caused by compression forces from intra- and extracellular ice crystals, governs the direction of freezing as ice crystals, which orient themselves in the direction of the fibers (Mousavi *et al*., 2007).

13.3 Recent advances in frozen packaging

Among traditional freezing techniques, air is by far the most widely used method of freezing food, due to the economical, hygienic and relatively non-corrosive nature of the equipment used. However, air provides relatively low rates of heat transfer. Batch or continuous freezing systems are also commonly employed, achieving much more efficient heat transfer with significant energy savings, when contact freezing is used. Contact freezing involves heat transfer by contact between the product and metal surfaces, which in turn are cooled by either primary or secondary refrigerants, or direct immersion in a refrigerated liquid. In cryogenic freezing, normal refrigerants, such as liquid nitrogen or solid carbon dioxide, are employed directly to freeze the food product (James, 2008).

New technologies have now been proposed that promise significant improvements in accelerating the freezing process, and thus product quality (Sun and Zheng, 2006), including:

- high-pressure shift freezing,
- ultrasound-assisted freezing, and
- antifreeze or ice nucleation proteins.

13.3.1 High-pressure shift freezing

At atmospheric pressure, when water is frozen, its volume increases due to ice formation causing tissue damage. Since the density of high-pressure ice is greater than water density, during phase transition, high-pressure ice does not expand in volume, reducing tissue damage. There is a commercially available application of this technology in the high-pressure freezing machine HPM 010 from ABRA Fluid AG (Widnau, Switzerland, www.abra-fluid.ch).

Product safety could be enhanced by assumed synergetic inactivation effects on enzymes and microorganisms during high-pressure processes at subzero temperatures (Sanz and Otero, 2005). In pork frozen with high-pressure assisted freezing (200 MPa, −20°C), small ice crystals formed at the product's surface and central zones (Martino *et al*., 1998).

13.3.2 Ultrasonic-assisted freezing

The application of ultrasound or acoustic energy causes the compression and refraction of sound waves in the freezing system's aqueous phase, and the resulting cavitation produces gas bubbles, which act like nucleating agents.

If it is applied to the process of freezing fresh foodstuffs, ultrasound cannot only increase the freezing rate; it can also improve the quality of the frozen products (Zheng and Sun, 2006). To achieve this, it is essential to form ice crystals as small as possible, with as similar an ice crystal distribution across the product as possible to that of the water in the unfrozen product. This requires that freezing takes place simultaneously in both intracellular and extracellular regions (Zheng and Sun, 2005).

13.3.3 Antifreeze or ice nucleation proteins

The function of these proteins is to influence ice crystal development; they interact directly with ice thereby inhibiting ice recrystallization. These proteins are useful in maintaining the high quality of chilled and frozen meats, as in slow freezing large ice crystals may form within cells, resulting in drip loss during thawing. Meat quality can be maintained by employing antifreeze proteins (soaking in PSB or injecting before slaughter, prior to freezing), reducing crystal size when frozen at −20°C (Payne and Young, 1995; Payne *et al*., 1994).

13.4 Future trends

Muscle foods are economically and nutritionally important in the food industry, so the importance of quality conservation during transport and handling is very important. Frozen packaging is an excellent way to extend shelf life. The simultaneous effect of low temperatures and selective permeability of the package delay most of the enzymatic and microbiological activity that causes quality deterioration in muscle-based foods, if good manufacture practices and cold chain are adequately maintained. The development of new combinations of packing materials allows manufacturers to offer safer and more attractive ways of packaging meat, poultry and seafood products. Novel techniques such as high-pressure freezing could offer better quality products since small ice crystals would improve thawing quality.

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14

Advances in the manufacture of sausage casings

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Abstract: This chapter treats most recently developed casing types for the production of meat products which appeared on the market or will consequently be offered by most known casing producers. These novel casing types are mainly man-made (synthetic) polymer casings or a combination of these with other already-known materials. Latest developments include barrier and smoke permeable man-made polymer casings, as well as additive release transfer casings, rationalizing sausage and ham production to a high extent. Further advances in polymer science and technology will lead to further casing types and cost-effective production methods for the meat-processing industry.

Key words: man-made (synthetic) polymer casings, functional casings, smokeable thermoplastic casings, additive release transfer casings (ART casings).

14.1 Introduction

The usage of natural casings is as old as the butchering of animals and goes as far back as prehistoric times. Recorded history has described comprehensively the organized slaughtering practices and processing procedures employed by the meat trade in the production of sausages in natural casings. However, the use of artificial casings for similar applications only developed in the twentieth century, when modern chemistry and advanced technology allowed for the massive production of various polymers like cellulose, viscose, collagen, nylon, polyolefin, etc. From the 1930s through to the 1950s, cellulose and fibrous casings (which were inedible) were developed. Inedible collagen emerged in the late 1930s but its usage only became popularized in the 1950s and 1960s. This was quickly followed by the introduction of edible collagen casings (first replacement of natural casings for frankfurter production). In the same period, peelable cellulose casings

became the standard in the United States for skinless hot dog production. Synthetic polymer casings or plastic casings emerged in the 1960s with the introduction of polyvinylidene chloride (PVDC) casings, followed by monolayer nylon casings in the 1970s and 1980s, and, finally, the appearance of synthetic, polymer-based, high-barrier multilayer casings containing nylon, which revolutionized meat-processing in the 1990s and, by 2000, was adopted globally by meat processors.

14.2 Definition and types of sausage casings

From a packaging perspective, sausage casings can be divided in two distinct groups: *permeable casings*, in which meat products are not shelf-stable (need to be packed for further distribution), and *impermeable casings*, in which meat products are distributed and, therefore, provide all of the necessary packaging functions. In order to understand the variety of casings available on the market today, their basic functionality and global importance, the following classification, according to their origin, can be considered (Table 14.1).

The main focus of this chapter is targeted towards the use of synthetic casings by the processed meats industry and, in particular, towards synthetic multilayer polymer casings which possess a wide range of functionalities and have been the subject of tremendous commercial development over the past two decades.

The word *casing* suggests a container or covering which encompasses the foodstuff (meat, meat batter, ham mass, etc.) in order to hold or protect the product. The function of holding requires that the casing provides a defined product form (a round or shaped product when moulded) and protects and preserves the product content from contamination and deterioration, thereby extending shelf life throughout the cold food chain.

The *primary* roles associated with any casing are shaping or forming of the meat product, capacity to withstand pressure at filling and upon applying the closure (twisting or clipping) and providing selective permeability which is the primary feature defining the shelf life of the end product. The *secondary* roles of casings

Table 14.1 Classification of artificial casings according to origin

Animal origin	Non-edible collagen casings Edible collagen casings
Plant origin (synthesized)	Cellulose casings Fibrous cellulose casings
Combined origin	Textile casings Linen casings
Synthetic	PVDC casings Polyesther casings Polyamide (nylon) casings Multilayer casings (nylon + polyolefin + other polymers)

consist of features like thermal resistance, special surface effects, printability, curving or ringing capability and other more supplementary characteristics.

The term *functional casings* is used to describe casings that possess additional functions other than those described in their primary or secondary roles, which might be, for example, additive absorption on the inner surface of the casing and subsequent release during thermal processing and transfer onto the meat mass. Even though in theory the nomination 'functional casings' could encompass any highly sophisticated 'tertiary' function, it seems that most casing manufacturers use this term in their commercial literature only for casings which may confer an additive transfer role to the product.

14.3 Advances in sausage casings

The following sections give details as to the wide variety of casings available to meat producers and how these have been developed as manufacturers strive to improve on available choices.

14.3.1 Synthetic polymer casings

The advent of synthetic polymer casings enabled serious developments in the manufacture of more sophisticated packaging structures with multifunctional properties, generally being used for high-volume sausage items. New, alternative, sausage casing production methods, novel applications and permanent innovations of existing casing types, as well as procedures associated with their uses, are continually being investigated and verified by the meat-processing industry (Savic and Savic, 2002). The overall objective is to produce better-quality sausages using highly rational methodologies which need to be ever more cost-effective, yet produce a product with higher safety specifications.

High-barrier multilayer shrinkable casings

With the appearance of multilayer shrinkable casings in the 1980s, the production of cooked meat products increased globally, enabling an efficient massive production of shelf-stable cooked sausages to occur. Although developed in the 1980s, multilayer shrinkable casings appeared on the market in large volumes only in the 1990s and achieved their highest sales as recently as 2000 onwards. Over the course of these years, the development of casings continued at a rapid pace and this was supported by general development in the area of film production, enhanced resin versatility, developments in equipment manufacture and performance and general developments in electronic technologies.

Currently and globally, there are a few dozen manufacturers with plastic casings (including barrier multilayer casings) in their portfolios (Table 14.2). Most of the barrier structures are patented by a handful of casing producers who originally planned to reserve their right in exploiting the advantages of these inventions within their economic spheres. However, in many countries with lower gross

domestic income (GDI) these patents were not valid or not properly pursued, whereas the demand for low-cost high-performance plastic casings was pronounced. Additionally, a number of casing manufacturers are also located in parts of the world where imitating patent structures is well recognized and where interpretation and copying occurs. Consequently, a three- or five-layer biaxially oriented multilayer casing structure is a standard used in many countries around the world and the most common forms are: PA/PE/PA or PA/Adhesive/PE/Adhesive/PA layers (Stenger, 1993). Following this basic structure, many other forms appeared on the market; the most commonly known are shown in Table 14.3.

Table 14.2 Overview of main (top 10) globally active man-made polymer casing manufacturers listed in alphabetical order

	Manufacturer	Country	Brand name(s)	Casing types	Website
1	Atlantis-Pak	Russia	Amiflex	P	www.atlantis-pak.ru
2	Case-Tech	Germany	K-series	P, Ce, F	www.walsroder.com
3	Gunze	Japan, Belgium, USA	Vector series	P	www.gunze.co.jp
4	Kalle	Germany	Nalo series	P, Ce, F	www.kalle.de
5	Podanfol	Poland	Pecta series	P	www.podanfol.com
6	Supravis	Poland	Supravis GL	P	www.supravis.pl
7	Unipac	Brazil	Darlon	P	www.unipacnet.com.br
8	Viscofan	EU, Brazil	F2, F9	P, Ce, F, Co	www.viscofan.com
9	Viskase	USA, France	Visflex, Vismax	P, Ce, F	www.viskase.com
10	ViskoTeepak	Mexico	Nova Series	P, Ce, F	www.viskoteepak.com

P = plastic casings, Ce = cellulose casings, F = fibrous casings, Co = collagen casings.

Table 14.3 Overview of structures and permeabilities of common commercially available barrier multilayer shrinkable casings (tubular films)

	Structure	Layers	Oxygen perm.	WV perm.	Reference
1	PA/Adh/PO/Adh/PA	3 and 5	10 –20	3–5	Stenger, 1993
2	PA/Adh/PO	3, 4 and 5	20–35	1.8–3	Sugimoto *et al*,, 1989
3	PA/EVOH/Adh/PA	5	4 –5	2–4	von Widdern and Weber, 1995
4	PA/Co-PO/PA	3	10–25	8–10	Vicik, 1996
5	PA/EVOH/PA/Adh/PO	5	4.3–4.6	1.7–1.8	Schröder *et al*., 2003
6	PA/Adh/PO/Adh/PA/EVOH/PA	7	2–3	2	Pophusen and Schröder, 2000
7	PA/Adh/EVOH/Adh/PA	5	2–4	4–6	Vicik, 2003
8	PA/PA/Adh/PO/Adh/EVOH/PA	7	2–3	2	Siddiqui, 2009
9	PA/Adh/PA/PVA/PA/Adh/PA	7	1–2	7–10	Schiffmann, 2008

Today, most multilayer casings are composed of a five-layer structure; however, seven layer structures are growing in popularity as such materials should provide better barrier performances using fewer materials. However, high-barrier casings possessing extremely low permeability values are not necessary when producing cooked products with a shelf life of up to 2 or 3 months, as most of the products in central and Western Europe have shelf stabilities within that range. However, in warmer regions of Europe, United States and South America, quite often, higher barrier values are required by the market in order to cope with possible irregularities in the chill chain and to prevent eventual weight losses during longer periods of storage. In the aforementioned regions of the world, food regulations allow for the usage of many preserving agents in sausage recipes and consequently, shelf lives of 6 months or more can regularly be encountered (Table 14.4).

Important processing influences on barrier properties of casings

The effect of thermal or pressure processing on multilayer high-barrier casings is an area of future research, but principles on how pasteurization (69–75°C), high pasteurization (80–95°C), high-pressure processing (HPP) and retorting (121°C) influence the properties of casing structures are only partially known. As low pasteurization of casings is a standard procedure and well implemented by most users, newer high-barrier multilayer casings are emerging on the market to fulfil two additional requirements: mechanical stress resistance and low transmission rates. As high thermal processing (90–121°C) and HPP are gaining in importance, the casing industry is offering alternative structures (mainly seven layers) with even higher barrier structures in order to withstand the temperatures and stress imposed on the casing material. Analogous literature in packaging is showing changes in permeability of similar structures (nylon and ethylene vinyl alcohol [EVAL]), mainly a decrease in WVTR, especially during retort processing (Halim *et al.*, 2009). As these results are based on non-oriented materials, and knowing that casings are typically biaxially oriented, it can be assumed that the suggested changes in film crystallinity will be somewhat different in casings.

Technical data sheets pertaining to casings obtained from manufacturers for applications in highly abusive situations should be double-checked under practical conditions after thermal or pressure processing. Retorted casings experience considerable thermal stress and possibly greater changes in crystallinity; consequently, their functional shelf lives should be verified over 5–12 months at

Table 14.4 Simplified classification of polyamide and multilayer casings according to their permeability/barrier amplitude and relationship to shelf life for refrigerated cooked emulsion-type sausages

	Layers	OTR	WVTR	Shelf life (months)
Monolayer casings	1	10–20	20–30	0.5–1.5
Low barrier casings	3	10–20	10–15	1.5–2.5
Medium barrier casings	5	8–10	5–10	3–5
High barrier casings	5 or 7	2–5	1.8–3.5	5–9

ambient temperature – this is a prerequisite for such applications. Furthermore, the influence and efficiency of counter-pressure retorts on such materials during processing are important considerations to be taken on board as these may slightly or dramatically alter the barrier properties of multilayer casings. For example, material expansion during thermal processing will increase, thereby causing additional stress to be applied to the oriented material. Casing expansion during thermal processing is an important quality parameter determining deformation and, therefore, the final product shape, stability of the clip closure, print distortion, permeability and final handling from retort cages. Consequently, monitoring in order to gain a comprehensive understanding of the full impact of these parameters on the final product is critical when developing highly shelf-stable products and should be a routine procedure.

Understanding volumetric changes during the filling-clipping process, thermal treatment, showering, cooling and storage of sausages in synthetic polymer multilayer casings is of critical importance in order that the correct type of casing is selected based on its suitability for a particular application (Savic and Savic, 2002). The sausage diameter (calibre) changes of an emulsion-type pasteurized sausage at different temperature levels (72°C, 82°C and 92°C) during all of the previously highlighted processing steps are shown in Fig. 14.1.

In meat-processing factories, higher pasteurization temperatures (80–95°C) are frequently used, but, consequently, it is often forgotten that volumetric changes are extreme under these processing conditions and result in a number of negative outcomes which are often not taken into account – for example, higher aroma loss, further sausage shape deformation, clip closure instability due to high internal pressures, etc. If an overly extendable casing layer structure is used for higher pasteurization, the casing wall thickness may decrease by as much as 10% during the

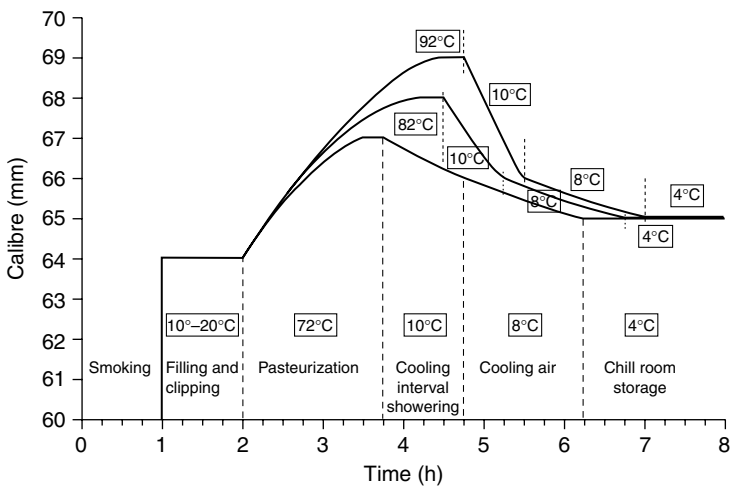

Fig. 14.1 Calibre changes of an emulsion-type pasteurized sausages at different temperature levels (72°C, 82°C and 92°C).

cooking phase and, subsequently, the final product diameter (calibre) may increase up to 5%. Therefore, permeability values have to be checked during the practical conditions of manufacture, or preferably stronger casings, offering slightly less convenience during clipping, may have to be used. The mechanical values supplied by most casing manufacturers in the form of technical specification sheets refer to values obtained at room temperature conditions; consequently, thermal mechanical resistance must be ascertained through testing governed at specific factory conditions. The best practical control of mechanical properties is through assessment of sausage diameter (calibre) chart fluctuation (Fig. 14.1). In counter-pressure retorts, the sausage diameter increase can be controlled over computer-monitored regulation of pressure, so that even at sterilization temperature (e.g., 121°C), a deformation equivalent using a low pasteurization temperature can be achieved. Uncontrolled sausage diameter growth is not only an undesirable factor for optimal casing functionality, but it also decreases the high-pressure gelation of the sausage meat batter. Elevation of internal sausage pressure would produce an added degree of structural stabilization and enhance desirable textural properties. Therefore, pasteurized (low, medium or high) emulsion-type sausages (Fig. 14.2) are produced through a combination of heat and pressure, which induces gel formation, the gels being formed differing significantly in their final structures and sensorial properties.

Understanding why the multilayer structure exists in terms of polymers used to create these casing structures and the gauges (thicknesses values) associated with each layer is extremely important in order to predict how these materials will technically behave for important packaging properties such as gas permeability (P) and water vapour transmission rate (TR). Once known, such properties can be predicted using different mathematical equations (Cooksey *et al.*, 1999). When using such equations, mainly conceived for calculating laminated

Fig. 14.2 Small calibre pasteurized emulsion-type sausages in high barrier casings with longer shelf life. Reproduced with permission from Podanfol.

and coextruded film performance, one must take into account that multilayer casings are coextruded and completely biaxially oriented, leading to lower TRs than those encountered for non-oriented materials (Cooksey, 2004). For a polyolefin, such as polypropylene, a 45% reduction in oxygen transmission rate is achieved using a 300% orientation.

When calculating P or TR for practical purposes, some basic interrelationships in polymer properties must be considered carefully. The primary factors which significantly alter all P values are the presence of water and the constant temperature change during cooking and cooling (Hernandez, 1994). Water molecules are present in high abundance on the inside and outside of casing structures (usually polyamide (commonly called nylon) on the inside and outer of casing surfaces) due to the fact that high relative humidity plays an integral part of all processing procedures in food factories. Consequently, the nylon layers are in direct contact with water (outside from water and vapour from cookers, and inside from the foodstuff), thereby resulting in relatively high permeability values which further increase above 20°C (Fig. 14.3).

In the same way, storage of ready-to-eat products contained in high-barrier casings is highly dependant on the relative humidity surrounding the product. At 4°C, the typical storage temperature for products like sausages, the oxygen transmission rate (OTR) does not vary considerably at different humidity levels. However, when sterilized (retorted) sausages in casings are stored at room temperature, different humidity levels can play an important role in the oxidation of certain food components.

Another advance in multilayer barrier structure is the selective or almost engineered adhesion properties of the inner casing layer. The ability to modify the inner layer, while keeping the overall casing structure intact, has enabled the

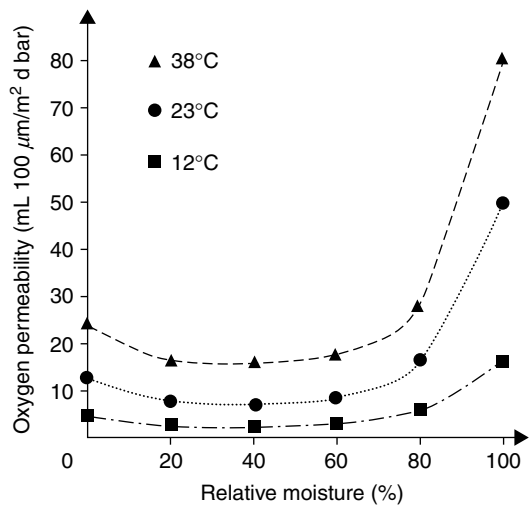

Fig. 14.3 Changes in oxygen permeability with increasing relative humidity at three different temperatures of a 50 μm casing wall. Reproduced with permission from BASF.

development of multilayer casings with selective adhesion properties for a wide range of sausage products formulated from a diverse scope of recipes, varying from protein-rich versions to highly extended versions with elevated water content (Savic, 2006). The availability of such 'tailor-made' casings is important for highly industrialized meat-processing and packing plants consisting of automated equipment, where even small incompatibilities in adhesion can cause problems on automatic peeling equipment, thereby leading to unwanted 'give-away' products, which will eventually present themselves on the slicing lines, resulting in considerable economic losses. This feature becomes even more pronounced for products such as cooked cured meats, like that of industrially manufactured hams. Therefore, combining a knowledge pertaining to the polymers used in casings with the technologies used for ham manufacture is necessary in order to find an adapted approach which provides an optimal production solution, but which needs to be operated on a factory-by-factory basis (Savic, 2010).

Smoke and water vapour permeable thermoplastic casings

The synthetic polymer casings developed in the early 1970s were monolayer polyamide casings with moderate barrier properties and PVDC casings with high-barrier properties. Only in the 1980s did multilayer shrinkable barrier casings possessing a high degree of performance appear on the market; yet these went on to capture the global meat-processing market a decade later. With this kind of acceptance of synthetic polymer casings, the first attempts to produce industrially usable semi-permeable to permeable polyamide casings took place in the 1990s. At the turn of the century, a number of developments emerged which resulted in a multitude of products coming into the marketplace, all possessing various degrees of permeability using novel types of nylon-based casings. Attempts were made to modify these new polyamide casings so as to approach permeability values similar to those for natural, collagen and fibrous casings. Currently, no one commercially available modified polyamide casing is available on the market which has achieved these properties. However, it is important to note that a new casing range, possessing permeability values which lie between those of artificial permeable casings (collagen and fibrous) and high- to low-barrier nylon casings have been created.

This new group of casings is called smoke permeable thermoplastic casings (technically described as modified polyamide casings); they possess higher permeabilities to water vapour, gases and smoke components. Alteration of permeability is achieved by blending and incorporating relatively high concentrations of chemical modifiers into the polymers, such as organic or mineral fibres, organic or inorganic fillers, various chemical compounds such as glycols, vinyl pyrrolidone, vinyl alcohol, etc. All altered blends of polyamide or co-polyamide with the aforementioned modifiers are mainly patented, and most of the known producers of casings possess one solution or another in order to deliver permeability values that are required by their customers for the production of various meat products. When developing these casings, manufacturers initially focused on smoke permeability, but, gradually, attention to water vapour and general

gas permeability issues followed, all of which resulted in the development of a diverse and large group of family casings (Table 14.5). The emergence of this casing group, defined by possessing unique permeability characteristics and not being commercially available previously, has brought about new adaptations in meat-processing procedures. In countries possessing highly traditional meat products, these adjustments were made only to match or mimic these meat products – in other words, only slight changes in meat-processing procedures were made to produce an almost identical product. In other countries, larger modifications were made, such as in the remodelling of processing procedures and reformulation of recipes in order to deliver new meat products to the market with previously unsighted properties.

Main types of smoke and water vapour permeable casings

One of the first smokeable nylon casings which appeared on the market in the 1980s was a polyamide structure which had undergone little or no modification. It was observed that the permeability of polyamide to smoke increased proportionally to increasing humidity. This observation led to the use of casings to deliver a modified smoking approach and showed the potential that modified casings presented over conventional types of casings. The casing used was a biaxially oriented monolayer and smoking was carried out in the presence of water or steam at temperatures between 60°C and 85°C (Erk *et al*., 1984).

This invention specifically relates to a smoke permeable, biaxially oriented casing comprising one polyamide polymer and at least one water-soluble synthetic polymer (preferably a polyvinyl alcohol) and possessing a water vapour permeability of between 40 and 200 g/m^2 /day. The polymer mixture could also contain additives which would be added to influence casing appearance, haptic characteristics, humidity storage capacity and peeling behaviour (Delius *et al*., 2004).

Table 14.5 Comparative transmission rates of common and novel types of casings

	Thickness (μm)	WVTR	0TR	Phenol VTR
Natural casings	600–1000	3000	450	200–400
Cellulose fibrous casings	80	1500	50	100–150
Cellulose casings	80	1000	90	100–160
Collagen casings	130	800	100	100
Collagen casings	80	1200	180	140
Collagen casings	60	1400	200	160
Collagen casings - small	35–40	2000–3000	400–500	300–400
Modified nylon casing (1)	35–40	200–500	20–200	90–160
Modified nylon casing (2)	35–40	100–250	25–80	n.a.
Modified nylon casing (3)	35–40	40–50	20–40	n.a.
Nylon casing (UPA-PA66)	35–45	12	10–20	n.a.
Nylon casing (OPA-PA6)	35–45	9	10–20	n.a.
Multilayer casing	35–45	2-–6	2-15	n.a.

Notes: WVTR (water vapor transmission rate): g/m^2 24 h; OTR (oxygen transmission rate): cm^3 /m^2 atm 24 h; Phenol VTR (phenol vapor transmission rate): g/m^2 24 h.

A related structure to the above invention described a monolayer or multilayered sausage casing made from a thermoplastic mixture which was composed of polyamide and at least one or more synthetic water-soluble polymer, including at least one organic and/or inorganic filler and was either non-oriented (non-stretched), monoaxially or biaxially oriented, as is the norm for most of the casings dealt with in this chapter. Water vapour permeability, mainly in the range of 40–200 g/m^2 /day (85% R.H. and 23°C), was described in this invention and was achieved using a nylon mixture with organic fillers comprising polysaccharide and/or organic fillers of quartz powder, titanium dioxide, calcium carbonate, talcum, mica or another form of aluminosilicate and other mineral fibres or glass microspheres in a biaxially oriented state. However, when such a casing is presented in either a non-oriented or monoaxially oriented state, its permeability remains higher – that is, in the range of 125–1100 g/m^2/day. Due to its mixed modified structure (some of the components have coarse particle size), the surface of the casing has a natural appearance (roughness and glossiness values measured are similar to natural materials such as fibrous cellulose or collagen casings). The casing described was specifically designed for the production of dried and/or smoked sausages (Stalberg *et al*., 2007).

Another innovative structure is a single-layer or multilayered smoke permeable casing (tubular form) comprised of at least one mixed layer of polyamide and natural fibres. On addition of appropriate additives, casing thicknesses lie between 5 and 200 μm and the water vapour permeability for such a casing is at least 25 cm^3 /m^2 /day/bar (85% R.H. and 23°C) for non-oriented, monoaxially or biaxially oriented casing states. A novel three-layered structure comprised of differentiated blend ratios for inner, middle and outer layers using polyamide with cellulose fibres and appropriate additives also exists. This multilayered structure allows for greater flexibility when adjusting parameters such as adhesion, surface appearance, etc. (Többen and Henze-Wethkamp, 2007). This casing form has a water vapour permeability of 25–32 g/m^2 /day (at 23°C, R.H. 85%) and an oxygen permeability of 27–44 mL/m^2 /day (at 23°C, R.H. 75%). The smoke permeability is not technically described for this casing format, but sensory evaluation has shown acceptable levels of smoke penetration onto the surface of the product, thereby providing a satisfactory smoke flavour.

A highly permeable single-layer casing (tubular film), described in this next invention is based on a polyamide/polyvinylpyrrolidone blend as per Mori and Arai (2007). A more complex and advanced blend is produced using a base matrix of polyamide and 4–5 wt % of hydrophilic compounds (such as polyvinylpyrrolidone, polyvinyl alcohol, polyalkylene glycol, polyvinyl alcohol ethers, polyvinyl ethers and cellulose ethers); this non-polyamide fraction is highly dispersed and forms a hydrophilic compound in a disperse phase with a linear particle size consisting of 0.1–3.0 μm in a direction perpendicular to the plane of the tubular film, and is either a polymer compound (mentioned above as non-polyamide fraction) or a low-molecular substance like salt. The casing arising from this ingredient blend showed water vapour permeability rates of about 450–515 g/m^2 /day (at 30°C, R.H. 65%) and permeability in respect to phenol (a smoke component) of

about 110 to about 160 g/m^2 /day (at 85°C) (Borodaev *et al*., 2004). Surprisingly, even though the water vapour permeability of collagen casings is much lower, the phenol permeability of this novel polyamide casing was almost equivalent to the collagen casing.

Another innovative casing is a permeable thermoplastic polymer casing possessing a moisture-vapour rate of more than 150 g/m^2 /day (ASTM E96), typically, and more preferably between 500 and 2000 g/m^2 /day, comprised of polyamide and polyether. This casing can be oriented or non-oriented and it can be composed of one, two or more layers of a single polymer or a combination of the aforementioned ones. The casing is permeable to smoke, CO_2, O_2 and other gases and suited to the production of a variety of sausages, especially dry sausages (Johansson, 2000). As this casing shows many similarities to the previously used collagen or fibrous casings, some comparative values will be described herewith. The sausages which can be produced with this casing are smokeable and the final product has a taste, odour and colour similar to products manufactured in the previously described conventional casings. The concentration of certain major smoke components, like, guaiacol, m-cresol and p-cresol are found at the same levels in sausages manufactured using these casings compared to sausages produced in collagen casings. Moreover, the end product (dried sausage) showed greater shelf life stability and required no repacking. The final point in weight loss from fibrous casings was achieved somewhat earlier than in novel nylon casings (Fig. 14.4) using identical conditions. An alternative drying procedure for plastic casings was suggested, as was described in the later invention by Wilfer (2010). He pointed out that a lower relative humidity could be used within the first few days of ripening without the danger of forming a dry rim around the dried sausage (Fig. 14.5). This invention also showed that hardness values for the dry sausage produced in this novel casing was comparable to that produced using conventional casings; the smokeability and smokiness being surprisingly comparable to that achieved using conventional casings. In this instance, *smokeability*

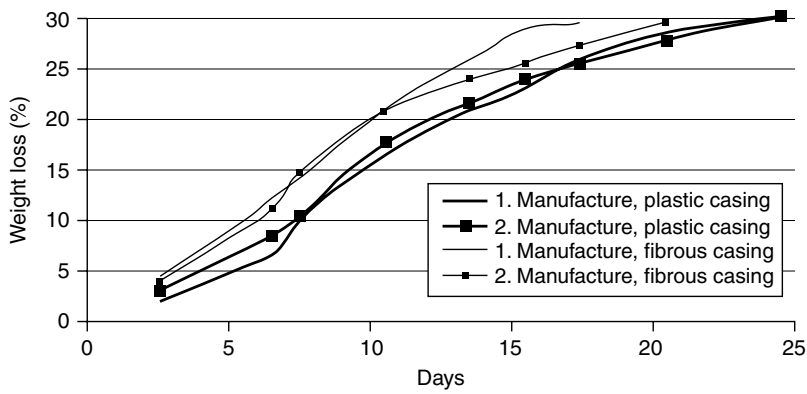

Fig. 14.4 Weight loss curve in novel modified polyamide casing (plastic casing) compared to fibrous casing (Johansson, 2000).

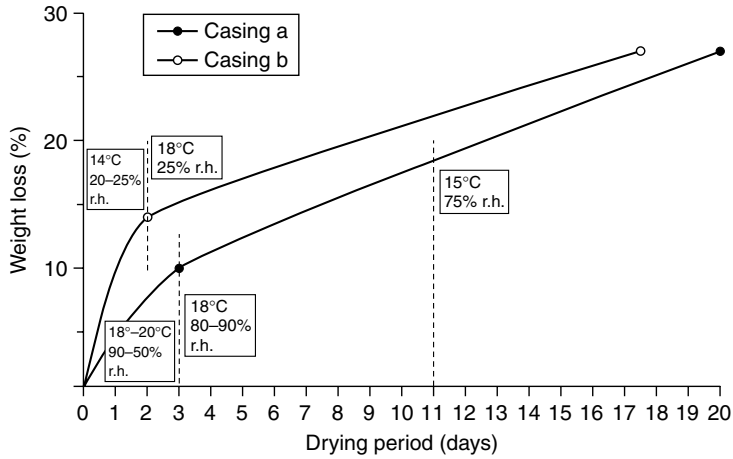

Fig. 14.5 Weight loss comparison for the production of a raw sausage in a fibrous cellulose casing (a) and in semi-permeable highly modified nylon casing (b) with a shorter ripening time (adapted from Wilfer, 2009).

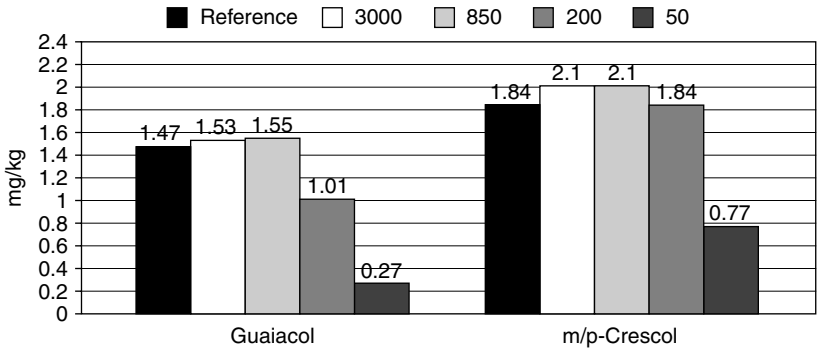

Fig. 14.6 Guiacol and m/p cresol contents in dry sausage (Johansson, 2000). Reference: sausage prepared in collagen casing; 3000, 850, 200 and 50 indicate different WVTR (g/m^2/24 h at 23°C, R.H. 50%) of novel casings.

refers to the permeability of the casing to smoke and the term *smokiness* refers to smoky taste, aroma and colour of the final, peeled product. The method of evaluating smokiness was determined through sensory evaluation. Chemical analysis of some smoke components (guaiacol and cresol contents) correlated favourably with sensory assessment. The smokiness (as determined through sensory evaluation) of the dry sausage was shown to be dependent on the vapour permeability of the novel nylon casing used under particular conditions (Fig. 14.6).

The next invention of highly modified permeable nylon casings describes a smokeable, biaxially stretched, heat-shrinkable film comprising of at least one polyvinyl alcohol resin, at least one polyamide resin and the optional use of an antiblocking agent. This innovative film was produced using a specific type of

polyvinyl alcohol with which the tubular film (casing) showed a substantial rise in water vapour and smoke permeability (McGarel, 2005).

Another somewhat different combination of polymers is described in a new invention, whereby the casing consists of at least one polyamide with polyvinyl alcohol (PVAL) and polyether block amide (PEBAX). This casing has a water vapour permeability that is at least 1000 $g/m^2/day$, yet its oxygen permeability, at a film thickness of 30 μm, is less than 2.1 $cm^3/m^2/day$ (at 23°C, R.H. 85%). This invention reveals neither meat technological characteristics nor any descriptive steps on how the casing could be used, nor does it mention results achieved for permeabilities, shrink values, final dried sausage characteristics, etc. (Schiffmann, 2007).

Application of smoke and water vapour permeable casings in production of meat products

Meat products which can be produced with semi-permeable nylon casings are diverse around the world and primarily include products previously manufactured in collagen, cellulose and cellulose fibrous casings. These novel semi-permeable nylon casings are roughened to achieve a matt appearance, thereby resembling cellulose fibrous or collagen surfaces, ultimately presenting a more natural look.

Raw sausages (air-dried in Mediterranean countries and smoke-air-dried in central and southern Europe) are traditionally produced in natural casings and industrially in collagen, cellulose and cellulose fibrous casings. At the time of writing, in 2012, these novel smoke permeable nylon casings are not suited to fully match the properties of collagen and/or fibrous cellulose casings for the production of classical dried salami-type products, but are close to meeting the criteria required for their uptake for raw sausage products requiring short ripening times (5–25 days, depending on sausage diameter).

The first examples of raw sausages which have already switched from cellulose casings to semi-permeable highly modified nylon casings are spreadable products, like German-types of Braunschweiger Mettwurst and fine and coarse Teewurst. These spreadable raw sausages were traditionally produced in cellulose casings, where fat migration through the casing from the sausage during storage was common. By switching to the novel nylon casings, this phenomenon was eliminated and, consequently, the surface hygiene of the product improved significantly, thereby allowing for easier product manipulation, improving appearance of the secondary package (flow pack), provision of greater convenience through better product grip and easier storage in the refrigerator after initial use. Many available novel nylon semi-permeable casings fulfil the criteria required for the production of spreadable raw sausage products, possessing water permeability values of 50–200 $g/m^2/day$, oxygen transmission rate of 30–200 $cm^3/m^2/day$ and a smoke permeability to allow for the creation of the typical regional tastes associated with the product (phenol vapour transmission rate 50–160 $g/m^2/day$).

The permeability values associated with these new casings combined with different mechanical, physical and chemical properties meant that new meat technological procedures were necessary to achieve the same end product, but often this resulted in unsighted advantages. In the case of a dry sausage ripened for 21 days of

diameter 63 mm, the drying procedure (which requires a final weight loss of 27%) employed for a porous nylon casing is totally different from that used for the dry sausage product packed in conventional fibrous cellulose casings. The difference in drying procedure lies in very low initial relative humidity of 20–25%, which can be used with the innovative nylon casing but is not possible when using fibrous cellulose casings (usual initial relative humidity with fibrous casings is 90–95%) because of the risk of forming a dry rim (firm border around the sausage with discolouration). In this particular case, the desired weight loss can be reached within 17.7 days using the porous novel nylon casing (water vapour permeability preferably between 70 and 300 g/m^2/day) compared to 20 days when using the conventional fibrous cellulose casing (water vapour permeability 1000–1500 g/m^2/day). This accelerated ripening of the raw sausage can be achieved without the addition of glucono-delta-lactone or other acidulants, usually added to accelerate the fermentation of dry sausages (Wilfer, 2010).

In conclusion, the application of this polyamide casing reduced ripening time by almost 12%; this finding in itself is extremely interesting. Therefore, more research and development is required in order to test new casing types in order to determine their true commercial potential.

14.3.2 Additive release transfer (ART) casings

Casings with inner impregnation have been in common use since the 1980s, when cellulose and cellulose fibrous casing manufacturers developed technologies to overcome conventional smoking processes by replacing them with casings which had been previously impregnated with colours, smoke or aromas which would then be transferred from the casing to the meat product during the cooking process. For instance, fibrous-reinforced regenerated cellulose casings impregnated with known acidic liquid smoke preparations transfer colour during cooking to the meat mass and in no way cause deterioration of the casing during subsequent product storage. In the early days of development, casings were impregnated thoroughly on both the exterior and interior sides of the casing (Goldberg, 1982).

While functioning to transfer ingredients to the product mass, these casings were, at the same time, permeable, allowing cooking losses to occur during production. The next step in their development was to develop casings with the same impregnation features but offering an additional barrier property. Today, such casings are of combined origin – that is, possessing an outer layer made of a thermoplastic material (barrier layer) and an inner absorptive layer (fibrous, textile or similar highly absorptive materials). As the combined casings are relatively cost intensive to produce, new developments with purely thermoplastic multilayer casings are emerging to fulfil the function of additive absorption and retention at the time of their preparation; at the time of their usage, these thermoplastic multilayer casings also function to release or transfer additives from the casing to the surface of the heat- and pressure-induced gel in the newly formed sausage (or formed cured meat). In the commercial literature *additive release transfer (ART) casings* are also called *functional casings* (Table 14.6).

Table 14.6 Types of additive release transfer (ART) casings

		Since (year)	Caliber availability (mm)	Outer layer	Inner layer	Transfer additive
A	Permeable casings – non-barrier					
	Fibrous cellulose casings	1980s	45–165	1-layer	1-layer	Smoke
	Cellulose casings	1980s		1-layer	1-layer	Smoke
	Collagen casings	1980s		1-layer	1-layer	Smoke
	Textile casings	1990s		1-layer	1-layer	Smoke
B	Combined casings – two-phase casings – barrier					
	Fibrous-plastic casings	1990s	65–145	Thermoplastic	Fibrous	
	Paper-plastic casings	2000s	60–280	Thermoplastic	Paper	
	Textile-plastic casings	2000	40–197	Thermoplastic	Cotton fiber	Color, smoke, aroma
	Double casings(2 separate casings)	2008	70–153	Thermoplastic	Fibrous	Color, smoke
C	Thermoplastic casings – multilayer casings					
	Inner layer porous		40–200	Polyamide	Special blends	Color, smoke, aroma
	Inner layer treated		40–200	Polyamide	Polyolefine	Color, smoke, aroma

Cellulose casings

Small-calibre cellulose casings impregnated with liquid smoke (smoke colouring and flavouring constituents derived from natural wood and including acids and neutralized acids, phenols and carbonyls) were one of the first casings used for the production of frankfurter-type meat products to impart food-grade colour to the surface of ready-to-eat frankfurters (Chiu, 1981).

Commercially produced, non-fibrous, small-diameter casings of regenerated cellulose made from viscose are the typical industrial casings used for the mass production of frankfurters. In order to reduce costs in the sausage manufacturing plant, a novel impregnated cellulose casing was invented. In this development, cellulose casings are sprayed on the internal surface with an innovative liquid smoke composition during the shirring process itself. This liquid smoke, containing low to moderate amounts of tar, is less expensive than currently available tar-depleted liquid smokes and is used in combination with at least one anionic surfactant; optionally, a wax component is applied to the inner surface of the cellulose casing, which is then shirred and maintains a uniform application of the liquid smoke composition on the inner surface of the small-calibre casings. This innovative cellulose casing is used to manufacture smoky-flavoured and -coloured foodstuffs (mainly frankfurters) without the requirement to use large volumes of liquid smoke to drench the encased product, thereby reducing cost. While small-diameter cellulose casings used for frankfurter production have been used to demonstrate the use of functional casings, other types of casings are included in the invention description, such as collagen casings and casings manufactured from plastics or nylons, including multilayer casings wherein the outer layer (non-food contacting surface) is a nylon or plastic, with the interior surface being cellulosic, or where the inner layer is made of a plastic or nylon capable of retaining the liquid smoke coating, while the outer layer is cellulosic. The inner nylon or plastic layer in a multilayer structure, or if used as a monolayer casing, will be of a nylon or plastic form that is porous and capable of retaining the liquid smoke (DuCharme and Merritt, 2004).

Cellulose fibrous-reinforced casings

Cellulosic fibrous casing composites were the materials of choice for any kind of impregnation application with additives which were used to enhance sausage or ham flavour, colour or odour. This flexible, non-woven composite possesses unique characteristics of high strength, low elongation, semi-permeability to gases and liquids and heat stability (93°C). The basic construction of fibrous composite casings can be modified in several ways and it is, therefore, no surprise that this was the material of choice for smoke impregnations for more than three decades (Nicholson, 1991). The natural functional behaviour of these casings influenced the further development of many industrialized sausage types worldwide after the 1950s. Dry sausage production relies on many advantages that fibrous casings have to offer, including the modification of its moisture permeability depending on whether the fibrous material is dry or wet; a wet fibrous casing has a moisture-vapour transmission rate of 12.700 g/m^2 /day/atm (at 38°C) and a dry one of only 2.300 g/m^2 /day/atm (ASTM E9666).

Fibrous-reinforced regenerated cellulose casings, capable of transferring a smoke colour to a meat product, were already developed in the 1980s. The fibrous casing was impregnated with a neutralized natural smoke colourant formed by neutralizing an aqueous natural liquid smoke colouring solution consisting essentially of a water-soluble alkaline neutralizing agent that was selected from the group consisting of propylene glycol, glycerine and mixtures thereof (Goldberg, 1982).

Collagen casings

Collagen casings containing smoke components which are encapsulated and then released during curing or cooking are the subject of this invention. The collagen matrix is extruded into a tubular film (collagen casing) with dispersed smoked component selected from the group consisting of a smoke colourant, a smoke flavourant or a mixture thereof. The encapsulating material is a lipid (fatty acid ester) and the smoke flavouring agent is liquid smoke (Kenneth, 1997). This lipid 'shell' melts during thermal processing (77°C) and the smoky odour is released to the frankfurter-type sausage. Active components selected from the group of smoke colourants, smoke flavourants or a mixture thereof, release the smoke component during curing or cooling of the wrapped food product and it is this encapsulation that is the core feature of this invention.

The fact that mechanical characteristics of coloured edible collagen casings may considerably alter after addition of food-grade colours to the collagen mass (Vinokic *et al*., 2006) suggests that usage of encapsulating agents and smoke flavours may also considerably influence the mechanical properties of such modified edible collagen casings for frankfurter production.

Textile casings

The most recent and relevant invention pertaining to textile casings is a tubular food casing that comprises textile material and is capable of absorbing, storing and transferring dyes, aromatic substances and/or flavourings to meat products stuffed within the casing. The textile material comprising the casing consists of natural fibres, such as cotton, linen, silk and wool, modified fibres, such as viscose staple fibres, or synthetic fibres, such as polyester fibres or polyamide fibres (or mixtures thereof). The dye, aromatic substance and/or flavouring is combined with a binder, or a binder mixture, which can be a food-derived protein, such as albumin, casein, zein, wheat protein, soybean protein, pea protein, a polysaccharide, or a derivate thereof, such as a cellulose ether and/or cellulose ester, alginic acid and/or alginate, chitosan, pectin, carrageenan or starch (or starch derivatives). The described functional casing possesses a water vapour transmission rate which is greater than 150 g/m^2 /24/atm and is therefore suitable for usage in the production of raw sausages (dry sausages).

When the casing described above is treated with an outer coating consisting of acrylates, methacrylates, vinyl acetates and/or PVDC, it becomes suitable for the production of cooked sausages, without allowing significant weight or cook loss to occur during thermal processing (Auf der Heide *et al*., 2008).

Combined casings (two-phase casing)

As permeable (non-barrier) casings allow water loss during thermal treatment, thereby allowing a certain degree of drying to occur and a decrease in water activity, innovative casing manufacturers designed a two-ply casing consisting of a classical absorbing inner layer (inner food contact layer of the casing) coated or surrounded by an outside layer (or casing) consisting of an impermeable outer thermoplastic polymer, thereby inhibiting any considerable cooking or storage loss. The main material combinations used in this format were: fibrous-plastic, textile-plastic and a double-casing consisting of two separate casings being shirred into one strand so that they could be used in one cycle.

Inner fibrous or fibrous/cotton and outer plastic structure

A popular and effective combination of materials used for casing manufacture is that comprising the use of inner fibrous or fibrous/cotton combined with the use of a plastic-based outer structure which provides impermeability to water vapour and/or gas. The inner layer, as highlighted, is comprised of individual fibres of a woven or knitted fabric, preferably a non-woven fabric impregnated with colourings and/or flavourings and consisting of cotton fibres, cellulose fibres, particularly regenerated cellulose fibres, viscose fibres or mixtures thereof. The outer plastic layer film has either an accentuated shrinkage between 70°C and 90°C or is made of non-shrinkable material for special applications such as the manufacture of D-shape hams. This outer plastic layer is made of polyethylene and polyamide, which is extruded on its inner face and in a wet state (PE/PA/PE structure). The advantage of this structure is the high absorptive capacity of the inner layer and the high-barrier properties of the outer layer. Ingredient impregnation of the inner layer can consist of spice mixtures, smoke flavours, caramel and a variety of colours and colour patterns which are employed to create a more natural appearance (Schäfer and Nohmi, 2001).

Another inventive solution in fibrous barrier structures is that provided by a three-ply casing which is comprised of a cellulosic first layer regenerated from a mixture of viscose and smoke components, a second adhesive layer and a third layer comprised of essentially any natural or synthetic film-forming material such as, nylon, polyethylene, polyvinylidene chloride, regenerated cellulose, collagen or a metallocene resin (Fig. 14.7). The internal cellulosic surface containing smoke components are integrally blended. Such laminated films (after seaming from flat to tubular states to become a functional casing) are not restricted to three-layer manufacture, but may, in fact, consist of any number of film layers that are self-binding or are bound together using intermediate adhesive layers (Appleby, 2001).

Double-casing (two separate casings on one shirred stick) with a transferable inner casing

This casing system is composed of two separate casings, one being an outer barrier casing (thermoplastic casing – either monolayer or multilayer) and an inner casing which carries a transferable colourant, aromatic and/or flavour substances. The specification for the outer casing is that it possesses low permeability to water

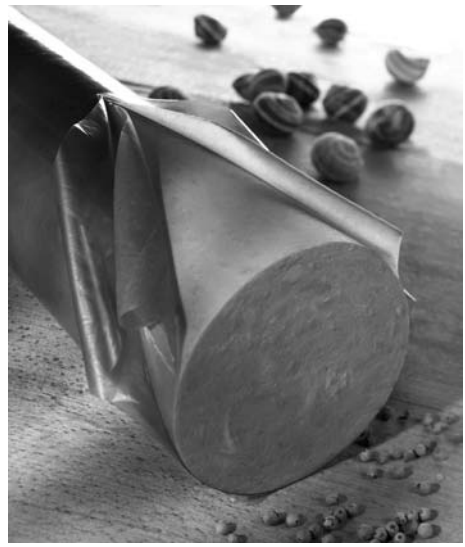

Fig. 14.7 A smoke release transfer fibrous-plastic casing (fibrous barrier smoke) after peeling. Reproduced with permission from ViskoTeepak.

vapour, oxygen and volatiles, while the inner casing is produced from regenerated cellulose, a mixture of thermoplastic starch and/or its derivative, polyurethane, paper textile fabric or non-woven fabric. In this example, the inner layer again may carry the colourant, aromatic and/or flavour substances such as a spice, spice mixture, a spice extract, liquid smoke, dry smoke, a natural or synthetic aroma and/or flavour enhancer. The binder used to hold these substances together would be a polysaccharide, a modified starch, dextran, pullulan, tragacanth gum, xanthan gum, gum Arabic, alginate methyl cellulose, among others. The two separate casings are merely held together at their respective starting point in the shirred tubular food casing by means of a plastic or metal clip. Such casings are used for cooked cured meats and large-diameter emulsion-type cooked sausages (König *et al*., 2009).

Synthetic multilayer polymer casings with a transferable inner layer

Synthetic multilayer polymer casings have been widely used, and globally distributed, since the 1990s and have proven to be popular with many small to middle-sized processed-meat manufacturers. However, the capacity for such polymers to function as release or transfer casings has not been as well established as it has for ART casings and, since 2000, it has only demonstrated limited success in this respect. However, indications are that the future may hold some promise and two possible approaches used for incorporating additives onto the inner surface of such films are as follows:

- Spreading the additive into, or onto, a flat film which consequently has to be dried. Thereafter, this ‘fixed’ and ‘dried’ film is converted from a flat state to a tubular form by means of seaming.

- Applying the additive into, or onto, a tubular film inner surface directly over a bubble principle (over rollers) or over a spraying system during shirring.

Multilayer casings used for absorption and retention of additives, such as flavourants, including liquid smoke, colourants and combination of those are modified in such a manner that the inner layer becomes receptive for applied liquids. The modification of this inner layer can take place in three different ways and are described in detail below.

1. A porous inner layer of different modified polymers (mostly patented) which are capable of absorbing liquids, carrying flavours, colourants and other functional additives.

Such a casing consists of a multilayer barrier structure with an inner layer having a three-dimensional polymer network, which possesses a high porosity of spaces connected to one another. This altered network is formed with the aid of porosity modifiers; such a modified inner layer has a liquid absorption capacity based on the overall composite of the film in the range up to 40% by weight. The porosity modifier used in this casing may be derived from numerous sources – soybean, peanut, corn oils, glycerols, sorbitols, glycols, mineral oils – while the inorganic filler may be silicon dioxide, talc (Mg_2SiO_4), aluminium oxide, among others. The food additive which is bound and distributed throughout the porous inner layer of the casing is a liquid smoke, containing 2–25% (by weight) of a sorbitan monolaurate, sorbitan monopalmitate, sorbitan monostearate mixture which is then released to the food during the cooking process (Henze-Wettkamp *et al*., 2009). The functional casing inner layer, possessing sufficient porosity and absorptivity, constitutes a layer which is 10–30 μm thick, preferably 15–20 μm. If the inner layer is too thin it exhibits little effect, whereas optimum layer thicknesses allows for efficient flavour transfer.

Another multilayer structure (biaxially oriented shrinkable casing) also possesses a liquid-absorbent inner layer and an outer impermeable barrier layer. The liquid absorptive layer has a high moisture-vapour transmission rate which is treated with liquid smoke and comprises a block copolyether ester polymer (Lee *et al*., 2009, 2010). The additives are colourants, flavourants and combinations of two or more thereof. The colourants consist of anthocyanin, annatto, betaine, caramel, paprika, turmeric, chlorella, cochineal, artificial colourant and combinations of these. The flavour additive may consist of baked flavours, barbecued, broiled, grilled, fried and similar flavours or, again, combinations of these. Furthermore, the additive is comprised of a liquid smoke of known origin.

2. An inner layer coextruded with starch or treated with protein coatings which allows for absorption, retention and later transfer of liquid additives onto the filled food mass (mainly meat emulsion, cooked cured meats and other foods).

A seamless, tubular food casing produced by co-extrusion (multilayer structure) which is comprised of a mixture of a thermoplastic starch and/or a thermoplastic starch derivative and at least one further polymer (homopolymer or copolymer) containing hydroxycarboxylic acid units, a polyester urethane, a polyether urethane, a polyester ether urethane or a polyalkylene carbonate as its inner layer, all further layers being used as barrier layers. Internally, the casing carries at least one transferable colouring, aromatic and/or flavouring substance. Such a casing is used for cooked emulsion-type sausages (larger diameter), cooked ham, cured goods and even processed cheese (König *et al*., 2007).

Another approach to producing the inner casing layer which will still allow additives to be coated and embedded into this layer in a stable and uniform manner is through the use of coatings, as pre-treatment binders, and then by applying the additive itself. So the process would consist of coating the casing with a binder, applying an additive onto it, drying and fixing it for a suitable storage period. For this purpose, a single-layer or multilayer casing (thermoplastic polymer) is coated with a binder or carrier. Such a casing is dried and in the second stage it is treated with at least one additive; both the carrier and the additive are transferable to the food filling, wherein the additive consists of fine to coarse foods or mixtures having a mean particle size of at least 60 μm and an additional binder layer covering and fixing the additive (additional fixing and final production step). The binder or carrier itself can be a wax, fat, oil or other hydrophobic or water-insoluble substance which softens or becomes liquid under the action of heat up to about 90°C and thus brings about a transfer of the additive, preferably tallow, drying oil, fatty acid ester, fatty alcohol or a mixture thereof. The additives are defined to be fine-grain to coarse-grain or piece-form, particulate aromas and/or flavourings, such as spices, herbs, vegetables, mushrooms, fruits, cereals, nuts and/or cheese. These additives can also be applied to a layer composed of a protein which then becomes coagulated by the action of heat. This food casing is used for cooked meat products, but owing to the vast choice of additives available, especially those possessing relatively large particle sizes, this casing is well suited for production of dry sausages (raw sausages) where the additive and its binder are transferred below room temperature (ripening temperatures between 12°C and 18°C) (König *et al*., 2009).

3. A physically treated inner layer, using either corona discharge, plasma or flame treatment, UV irradiation or other treatments to change the surface tension of the film and activating (polarizing) the inner surface, thus enabling surface retention of liquid additives.

This consists of a multilayer structure (barrier casing) with an innermost layer comprised of a polyamide and a cross-linked polyvinylpyrrolidone, thereby providing the casing on its inner surface with a porous layer structure capable of drawing away a smoke-curing liquid which will later go on to be transferred to the meat product during cooking. Moreover, this inner layer can be corona discharged in order to increase surface tension, thereby allowing for even better distribution

of the smoke-curing liquid and the avoidance of irregular spot development which occurs via factors such as shirring, manipulation and storage. The multilayer structure possesses high strength and low permeability to vapour and/or oxygen, thus addressing the issue of product storage stability (Mori and Arai, 2008).

Another multilayer structure exists whereby the liquid has been applied to the surface of a nylon film. However, prior to its application, the film surface is activated, such that the film surface has a dyne level of at least about 50 dynes. The amount of liquid capable of being absorbed by the nylon film is higher after activation. Film surface activation can be achieved using plasma treatment, flame treatment, corona discharge, UV irradiation, electron beam or gamma irradiation. The additive chosen for inclusion can consist of a colouring agent, a flavouring agent or both. Such approaches can be used to attach Maillard reagents and even antimicrobial agents (Samuels, 2004).

14.4 Effective selection and use of sausage casings for optimum product quality: possible meat product defects due to incorrect selection of casing types

The following sections look at ways of maximizing the benefits of using various types of casing in differing circumstances.

14.4.1 Storage and shelf-life stability of casings

With the appearance of ready-to-use (RTU) casings and functional (mainly ART) casings onto the market, it is critical that due diligence is applied with respect to their holding and storage, which is more critical than those required for the traditional dry and stable forms. RTU casings, whether cellulose fibrous, collagen or plastic, are pre-moistened in order to allow for immediate usage on filling and clipping equipment. Although coated with antimicrobial agents, monitoring of temperature during casing storage has become a precondition. As ART casings contain food ingredients, storage conditions must be adapted to the type of additive used within the casing in order to guarantee optimum functionality.

14.4.2 Preparation prior to casing use

Even though convenience plays a most important role in industrial operations, all processing aspects pertaining to casings must be carefully considered and the best solution chosen for that particular application. In general, hard casings which possess outstanding mechanical characteristics are resistant to high temperatures, induce high-pressure during thermal processing and allow production of cylindrical calibre-stable products; conversely, these same casings must be soaked for a longer period of time – that is, their water absorption is crucial in order to achieve recommended stuffing calibre (RSC). Softer and more elastic casings can be brought to

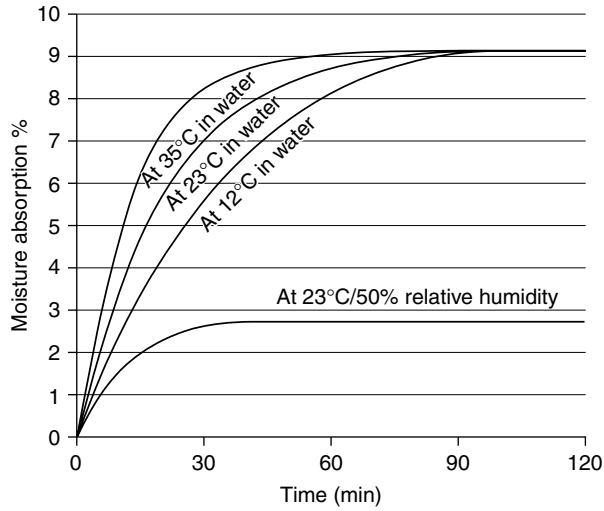

Fig. 14.8 Water and humidity absorption of polyamide-based casings (non-shirred form) in function of time (min) and at three different temperature levels.

the same RSC using a shorter casing soaking time; however, this causes the calibre of these casings to increase during higher thermal treatment (eventually high pasteurization) and is a critical processing factor which needs to be considered. Figure 14.8 provides an overview of how water temperature influences the soaking time of a multilayer nylon-based casing; even though these data refer to flat casing in water, it is generally understood that shirred casings, due to their compression, would need longer periods of time to reach a higher level of polyamide saturation (with most polyamides, the highest moisture absorption is 9%).

Frequently, basic knowledge of polyamide absorption properties is lacking in food-processing plants, even when ready-to-use (RTU or pre-moistened) casings are delivered; when pre-moistened on the casing manufacturers site these may dry out when not properly stored or improperly handled. One must constantly bear in mind that most polyamide-based multilayer casings have at least one outer nylon layer which is highly hygroscopic and needs to absorb moisture before it can perform mechanically to its highest potential. Some polyamides used in the outer layer are softer and more elastic so that they can be used without prior soaking, but usually at relatively low expansion ratios (the difference between the initial casing diameter and the final filling diameter is in this case low and the internal pressure of the meat mass is lower).

14.4.3 Ease of casing use on clipping equipment

Determining the correct clip size and clip grade is mostly a question of synchronizing clipping equipment and casing manufacture. Mechanically stronger casings which enhance better meat batter gelation due to high-pressure filling/clipping are

usually less convenient to clip; however, if such casings are soaked correctly and thoroughly, most of the modern processing equipment will cope with such casings. Improvements in clipping equipment design and operation allow for easier monitoring and registration of closure pressure, clip designation, cycle speed and full adjustment to achieve a highly reproducible clipped sausage or meat product.

14.4.4 Thermal treatment, smoking, cooling (showering) and drying

The correct choice of thermal treatment and subsequent cooling (initially with interval showering followed by air cooling) of synthetic polymer casings is of the utmost importance in order to attain optimal results. The cooking diameter (Fig. 14.1) is usually 3–10% higher than the recommended for filling or stuffing calibre. This expected increase is normal and influenced by the following factors:

- expandability of the food mass (batter) at higher temperatures
- distributed air content in the food mass (mainly dependent on comminution method and physical and chemical characteristics of the meat batter)
- pasteurization temperature and temperature curve used
- filling/clipping of the casing with or without prior soaking
- thickness and mechanical properties of the film/casing type
- hanging or horizontal position of the logs during the cooking phase.

Some products will expand at higher ratios during cooking, while others expand at much lower ratios, and, as a consequence, greater product casing wrinkling problems will be encountered with products which have expanded to a greater extent. Overall, cooling either in connection with showering, interval showering or using a combination of showering and air cooling is important for final casing shrinkage, thereby giving a tight surface appearance to the sausage. If wrinkling problems are encountered, the cooling rate should be slowed so as to allow the casing to follow the food mass contraction and, additionally, sufficient moisture should be available to activate the shrinkage of the outer nylon casing layer.

14.4.5 Peeling of the casings

Retail sausages

End consumer peeling characteristics and associated criteria are concentrated on easy-to-use features, like cutting behaviour, peeling direction (preferably spiral), optimized tear resistance and medium adhesion to withstand slow manual casing removal.

Industrial use (peeling and slicing operations)

As the use of highly sophisticated automatic peeling machines become a reality, peeling behaviour becomes critical on such lines. Optimal adhesion, longitudinal

peeling and high tear resistance become a combined bundle of characteristics required to achieve maximum results while minimizing wastage. Peeling direction is crucial for many applications where adhesion of the meat mass to the casing is highly dependent on muscle protein activation; if meat adhesion is excessive when peeling in one direction, it may be correct when peeling in the opposite direction due to the flow profile (longitudinal orientation) of meat batter during stuffing (Savic and Savic, 2002).

14.5 Meat industry requirements for new casing types

While advances in the development in edible collagen casings have improved products dramatically in recent decades, they still lag behind natural casings, particularly in relation to sensorial characteristics associated with natural casings. It is also obvious that synthetic polymer-based casings will draw near the properties of cellulose and cellulose fibrous casings. Currently, the meat industry needs a real cost-effective alternative for the manufacture of small-calibre frankfurters with the following features: high-speed linking, smoke permeability at elevated temperatures and relatively low weight loss during short-term production storage, impeccable peeling behaviour on automatic peeling equipment and eventual smoke impregnation on inner casing surfaces to shorten or eliminate traditional or liquid smoking processes. Initial results in highly modified semi-permeable nylon casings give hope that these demanding criteria can be met in the years to come.

Additive release transfer casings need further cost-effective solutions in synthetic polymer execution in order to provide colour, smoke and flavour to meat products peeled at the manufacturing site for high-speed slicing and packing lines. Present solutions with two-phase casings (fibrous and plastic) are cost intensive, so further research is required to be carried out in this area.

14.6 Future trends

The scientific advances in polymer science are bringing about ever growing and significant developments in the creation and application of new materials; a promising future is envisaged. Further developments in the manufacture of synthetic polymer casings are anticipated, along with the development of application technologies. As the cost of direct production and environmental control investments necessary to maintain manufacture of cellulose fibrous and collagen casings is high, the allure of finding new solutions via novel polymers, and mixtures thereof, to fulfil current meat technology criteria is significant. In the next decade, novel casings with multiple functions will emerge as a result of already initiated processes of adapting meat products to novel casing types.

As soon as any convenient and cost-effective new and functional casing is developed, the meat industry will be quick to move on its implementation as a means of alleviating the pressure of high-cost manufacture, which is currently

being demanded by the retail chain buyers. Casings will be adapted even more to industrial applications in a future manufacturing environment which utilizes highly automated equipment in order to reduce labour costs and the need for highly trained personnel. Therefore, convenience in use and high reproductive values in production will be issues which predominate in the foreseeable future. Limits between films used to produce tubular casings on line (TSA – transfer sealing automats) in meat factories and casings will slowly merge into one vast area encompassing flat (seaming films to become casings within the meat factory) and tubular films (synthetic polymer casings). Both areas (flat and tubular form films) will demand large-scale development in the future.

14.7 Sources of further information and advice

LANG and EFFENBERGER (2006) review, in the third German edition of their book, all types of sausages casings with technical background in manufacturing, usage and available commercial types, with a buyer's guide.

SAVIC and SAVIC (2002) present a book which attempts to collect old and new knowledge on main sausage casing types and principles of their production and use. The book presents the classical and more recent research data, bringing together all the relevant current knowledge and theories on the structure and function of most sausage casing types.

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15

Packaging of ready-to-serve and retail-ready meat, poultry and seafood products

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Abstract: The development of food product ranges in the areas of convenience-style, ready-to-eat, ready-to-heat-and-serve and so on over the past 20 years has been significant. As product ranges have grown, each offering ever more convenience to the consumer, the demand for new processing and packaging technologies has had to keep pace, with some interesting developments having emerged. This chapter attempts to address these issues.

Key words: ready-meals, convenience-style food products, processing, packaging, microwaving, heating, smart packaging.

15.1 Introduction

Ready-to-serve meals, also referred to as ready meals, microwave meals, television dinners or convenience meals, are generally accepted to be pre-packaged frozen or chilled complete meals that require little preparation apart from reheating prior to serving. These meals can include meat, poultry, seafood, pasta, rice and vegetable dishes or a combination thereof and can be classified as traditional, continental, ethnic, vegetarian or low-calorie/healthy option dishes (Henchion, 2000). While the frozen ready-to-serve meal once dominated the convenience market in Ireland, a recent study by Mitchell *et al*. (2011) reported that 75% of consumers would now choose a chilled ready meal over its frozen counterpart. The busy 'grab and go' consumer who may not even have time to purchase a ready-to-serve meal can avail of an impressive display of prepared, cooked meat, poultry and seafood products universally available at hot food counters.

Food product development and preservation techniques have come a long way since 1810 when the Frenchman Nicolas Appert first developed a process for preserving foodstuffs in sealed jars and crockery. The American engineer Percy

Spencer, who patented the microwave oven after an experiment with a magnetron melted a chocolate bar in his pocket in 1945, would probably not have believed that his invention would become an integral part of almost every household in 2011. In 1953, C. A. Swanson & Sons, inspired by airline meals, produced the first successful ready-to-serve meal in the United States, which consisted of a Thanksgiving meal of turkey, cornbread dressing, frozen peas and sweet potatoes served in an aluminium tray. The choice of cook-chill ready-to-serve muscle-based products available on the supermarket shelves has increased dramatically since chicken Kiev and cordon bleu were launched by Marks & Spencer in 1979.

The retail chilled ready-to-serve meal market in Ireland was worth €38.72 million in the 12-month period between March 2009 and 2010. While this was a 10.2% fall in value from the previous 12 months, the volume of product sold rose by 4.4% during this time. Italian cuisine accounted for almost 44% of the total chilled meals sold; Indian-style, ready-to-serve meal ranges also registered growth while the sale of traditional and Chinese meals declined between 2009 and 2010. Private label or own-brand meals contributed 72% of total sales of chilled ready-to-serve products (Hussey, 2010).

Clever marketing strategies, competitive pricing and special 'meal deals' have ensured that the ready-to-serve muscle-based food product sector is not left behind in these unstable economic times. Most regular television viewers will be familiar with the mouth-watering Marks & Spencer's advertisements cleverly aired around celebratory occasions (Valentine's Day, Mother's Day, Christmas and Easter) offering ready-to-serve three-course seasonal meal solutions at highly attractive prices. Entertaining at home has never been easier with the introduction of premium ready-to-serve product lines – for example, Tesco Finest and Marks & Spencer Dine-In range – which are marketed as suitable for serving to guests at dinner parties.

The aim of this chapter is to summarize the packaging techniques and materials currently used in the development of ready-to-serve and retail-ready meat, poultry and seafood products. The microwave heating process and associated problems will be addressed. Finally, some packaging technologies will be evaluated for their potential use in muscle-based heat-and-serve products.

15.2 Key drivers

The convenience ready-to-serve and retail-ready muscle-based food sector has been driven by a number of factors including social and demographic shifts, changes to the family unit and increased income levels. The increasing number of women leaving the home and entering the workforce and therefore experiencing increased time pressures at evening meal times has led to a greater demand for ready-to-serve muscle-based products. The main meal of the day now takes an average of 30 minutes to prepare compared to the two and half hours in the 1930s. There has also been a loss in cooking skills which in turn has created a greater reliance on ready-to-serve meal solutions.

There has been a steady decline in the average number of people per household, with recent statistics estimating that there are now only 2.4 persons in the European homestead. While young adults are choosing to set up home on their own, they

are delaying settling down, marrying and starting families. A rise in the number of divorces and single-parent households has also resulted in smaller family units. The modern family unit has changed over the years too, with family members leading more fragmented and individual lives. This has led to a decline in formal eating and the workplace has become an increasingly popular eating location. The marked growth in the number of people over the age of 65 is set to continue. Older consumers who may be living alone are more likely to buy convenient ready-to-serve and retail muscle-based meals. The elderly population also benefit from smaller portion sizes and added packaging features designed for easy opening and easy handling. The multicultural society of today, which has enticed consumers to explore new food experiences, is reflected in the diverse range of ready-to-serve and retail-ready products now available. Consumers now have access to authentic Caribbean-, Moroccan- and Jamaican-style, ready-to-serve meal dishes on their weekly shopping trip.

Due to space restrictions and high labour costs it is now common practice for large food service operations to supply food retailers, hospital, school, university, factory and defence-force canteens and meals on wheels organizations with prepared or part-prepared meals on a daily basis. Such organizations who are responsible for catering for the young, sick and elderly rely on high-quality food products and innovative packaging solutions and techniques to ensure safe, nutritional, organoleptically acceptable meal experiences. The future of ready-to-serve and retail-ready muscle-based products may remain bright if manufacturers take note of the fact that the most significant change occurring in the food and drinks market is the convergence of three main trends – health, premium or indulgence and convenience (freshly prepared and 'on the go') – with the emergence of ethical as a fourth key trend. Industry experts foresee the most important trends for future new product development within the health trend to be wellness and weight management, along with natural or organic product demands (Meziane, 2007).

15.3 Packaging requirements

The goal of food packaging is to contain food in a cost-effective manner that satisfies industry requirements and consumer desires, maintains food safety and minimizes environmental impact. The complex requirements of society today have put even greater demands on the packaging industry. Consumer demand for minimally processed foods with fewer additives and preservatives, heightened food safety concerns, market globalization, increased regulatory enforcements and new distribution trends are major driving forces for food packaging innovations (Sonneveld, 2000; Suppakul *et al*., 2003).

Traditionally, the basic functions of food packaging have been divided into four categories (Robertson, 1993):

- protection – to protect the food product against the deteriorative effects of the external environment

- communication – to communicate as a marketing tool, as well as provide storage conditions and cooking instructions
- convenience – to assist the consumer with greater ease of use and time-saving convenience
- containment – to contain products of various shapes and sizes.

Containment, protection and preservation, which are considered the principal technical packaging functions, are achieved by retarding deterioration, extending shelf life and maintaining the quality and safety of packaged food. Shelf life has been defined as the period of time during which the food product will remain safe, be certain to retain desired sensory, chemical, physical and microbiological characteristics and comply with any label declaration of nutritional data (Brown and Williams, 2003). The shelf life of a food product is controlled by three factors (Robertson, 2009):

- product characteristics, including the physical, chemical, biochemical and microbiological nature of the product, as well as formulation and processing parameters, also known as intrinsic factors
- environment in which the product is exposed during distribution and storage, also known as extrinsic factors and includes relative humidity, light, temperature and consumer handling
- properties of the package.

The first step in the development of a food packaging system that will minimize undesirable changes in quality and maximize the development and maintenance of desirable properties is a knowledge and understanding of the deteriorative reactions that influence ready-to-serve and retail-ready meat, poultry and seafood products. Ready-to-serve and retail-ready muscle-based products are susceptible to several problems during storage, reheating and warm-holding, such as warmed-over flavour, colour and texture changes, food/package interactions and moisture changes.

15.3.1 Lipid oxidation and warmed-over flavour

Lipid oxidation is the process by which molecular oxygen reacts with unsaturated lipids to form lipid peroxides. The process involves the formation of lipid radicals from unsaturated lipids, the uptake of oxygen, rearrangement of double bonds and the eventual breakdown of lipid peroxides to produce a variety of products including alcohols, aldehydes and ketones (Gardner, 1975). Cooked meat, poultry and seafood products are more susceptible to lipid oxidation due to the disruption of cell membranes initiated by the cooking process. The resultant breakup of cell compartments permits the interaction of pro-oxidants with unsaturated fatty acids and oxygen, the generation of free radicals and propagation of the oxidative reaction (Asghar *et al*., 1988). Tims and Watts (1958) used the term ‘warmed-over flavour’ to describe the rancid or stale off-flavour that develops as a result of lipid oxidation in cooked meat in refrigerated storage. Susceptibility to oxidation increases with increasing unsaturation and, because membranal phospholipids are

highly unsaturated, it is generally believed that lipid oxidation in muscle foods is initiated in this fraction (Gray and Pearson, 1987). Fish and seafood products which contain high levels of polyunsaturated fatty acids (PUFA) are most susceptible to lipid oxidation, followed by poultry, pork, beef and lamb (Cross *et al*., 1987). The process of warm-holding, where hot foods are stored above 65°C, can lead to the development of warmed-over flavour as well as physical deterioration of retail-ready muscle-based products (Creed, 2010).

15.3.2 Colour changes

Appearance, especially colour, is one of the most important quality attributes influencing a consumer's decision to purchase. Percentage residual headspace, oxygen transmission rate of packaging film, product to headspace volume ratio, light intensity and product composition are critical factors affecting colour stability of ready-to-serve and retail-ready cured-meat products (Møller *et al*., 2003). Nitrosylmyoglobin, formed from a reaction between nitrite and myoglobin, is denatured to nitrosylmyochrome after cooking and is responsible for the characteristic pink colour of cooked cured-meat products (Juncher *et al*., 2003). Exposure of nitrosylmyoglobin and nitrosylmyochrome to light even at very low oxygen levels promotes oxidation to metmyoglobin, which imposes a dull greyness to the neat product (Møller *et al*., 2000). The appearance of sauces used to complement cooked meat-based ready-to-serve and retail-ready products can also be influenced by cooking and pasteurization techniques. *Sous vide* processing lightened (increased the Hunter L/b ratios) the colour of tomato and basil, hollandaise, béarnaise and mushroom sauces, but the redness (Hunter a\b ratios) remained unchanged (Fagan and Gormley, 2005).

15.3.3 Moisture loss

Protection or prevention of moisture loss in ready-to-serve and retail-ready muscle-based products is best achieved by moisture-resistant packaging materials and strict temperature and humidity control during storage. In chilled or frozen food products, water loss in the form of desiccation, dehydration or evaporation can result in quality loss. Dehydration in frozen meat products leads to freezer burn. Freezer burn causes the exposed lean meat surface to become rancid, discoloured and physically damaged (Brown and Williams, 2003). Inevitable moisture loss which occurs during reheating of ready-to-serve muscle-based meals can be reduced by the addition of a gravy or sauce.

15.3.4 Food/package interactions

The most common food/package interactions are the migration of low molecular weight substances such as stabilizers, plasticizers, antioxidants, monomers and oligomers from plastic packaging materials into food. Some migrants can affect the organoleptic quality of the food product as well as being hazardous to human health. Reheating, especially higher temperature conventional oven

heating, can greatly accelerate the migration of volatile and non-volatile components from the polymer-based ovenable tray or lidding material (Creed, 2010). High-fat ready-to-serve muscle-based food products in intimate contact with the packaging material are most susceptible to migration compounds during extended storage. An investigative study concluded that migration of food contact materials from a wide range of plastics used in food packaging rarely exceeded acceptable levels (Czerniawski and Pogorzelska, 1998). The migration of antimony from PET oven-proof trays to pasta, vegetable, potato, meat poultry and fish-based ready-to-serve meals was found to exceed limits determined by the European Commission in 50% of the products tested at 180°C, but was well below accepted tolerable daily intake (Haldimann *et al*., 2007). Furthermore, low molecular weight compounds (volatile and non-volatile) may migrate from food into packaging materials through the sorption mechanism, changing the barrier characteristics of the packaging material. Volatile substances such as flavour and aroma directly affect food quality while non-volatile compounds such as fat and pigments affect the package. Complete polymerization is required during the manufacture of plastic packaging materials for the food industry.

In the European Union (EU), legislation on food packaging is set out in Regulation (EC) No. 1935/2004 of the European Parliament and of the Council on materials and articles intended to come into contact with food. Commission Directive 2002/72/EC relating to plastic materials and articles intended to come into contact with foodstuffs, lays down limits with respect to the concentration of certain substances in packaging or of migrants in foodstuffs or corresponding food stimulants. These regulations are based on the toxicological data of substances. EU Directive 2002/72/EC stipulates that a maximum of 10 mg/dm^2 or 60 mg/kg of physiologically non-hazardous substances may transfer from the packaging to the foodstuff (global migration) (Galić *et al*., 2011).

15.4 Microwave reheating

While it is widely accepted that the microwave oven has greatly contributed to the daily lives of modern society, the physical phenomena involved in the heating method are complicated and pose significant challenges for those responsible for the development of microwaveable packaging materials (Tang and Resurreccion, 2009). Most of the ready-to-serve or retail-ready muscle-based products available on the supermarket shelves are suitable for both conventional and microwave oven cooking or reheating. Conventional ovens work by heating a chamber which holds the food product. The food product, which equilibrates to the temperature of the oven through conduction, is heated from the outside and the centre of the product is the last part to heat up (Gallo, 2009). Microwave oven heat uses the dielectric properties of the food to reheat or cook the food. When the food is placed in the chamber it is exposed to microwave energy which excites the polarized molecules of the food. The polarized molecules attempt to align themselves with the electric field in which they are placed. The molecules are unable to keep pace with the

moving electric field, which alternates many millions of times per second, and the microwave energy generated is converted into heat (Coles, 1993). A number of factors can influence temperature distribution in microwaved foods. These include:

- The shape, thickness and density of the food: overheating can occur with irregularly shaped foods. Rounded, regular shapes will tend to heat more evenly than those with sharp corners or dishes of varying thickness. Denser foods will take longer to heat than those with a more open, porous composition.
- The thermal properties (specific heat capacity and thermal conductivity, penetration depth) of the food: specific heat is a measure of the ability of a material to hold heat compared to water, which has a specific heat capacity of 1.0. It is defined as 'the quantity of heat required to raise the temperature of a unit mass of substance by 1°C'. Specific heat varies with temperature, especially at the freezing point of foods. The thermal conductivity of each component of a food product must be known to avoid overheating of some areas while other parts of the dish remain cool. There is a marked discrepancy in microwave penetration depth of ice and water which causes large variations in temperature within a frozen, microwaved product.
- The dielectric properties of the food which govern how well a material absorbs microwaves vary according to the composition (carbohydrate, fat, protein, salt and water content) of foods. There are three relevant dielectric properties:

  1. Dielectric constant, ε', which is a measure of the ability of a material to store microwave energy.
  2. Dielectric loss factor, ε'', shows the ability of the material to dissipate this energy as heat.
  3. Dielectric loss tangent, tan δ, is defined as the ratio of dielectric loss factor to dielectric constant or the ability of a material to be penetrated by microwave energy and to dissipate this energy in the form of heat.

- The starting temperature of the food (refrigerated, room temperature or frozen). The higher the starting temperature, the faster the food will cook. Freezing has a major effect on the way materials heat because of the different dielectric properties of ice and water. Ice is highly transparent to microwaves and heats poorly while water is highly absorptive and heats easily.
- Nature of the food product (single or multi-component). Physically dividing the containers for multi-component ready-to-serve meals facilitates microwave reheating as the microwave recognizes each compartment as a single tray. This achieves more uniform heating since the shape, mass, specific heat capacity and dielectric loss of each meal component may be individually controlled. Foil shielding and product placement in the container may also ensure more uniform heating.
- The composition, shape and size of the food container. The net weight of most microwaveable food products is 250–400 g and the size of most containers is approximately 15 cm × 20 cm, with depths of 2.5–3.0 cm. The shape of the container should be oval or round and kept as regular as possible.

The temperature profiles of rectangular trays show that the corners are the hottest, the centre is the coolest and the side walls are slightly cooler than the corners. If corners are unavoidable they should be rounded as much as possible and side walls should have generous draft angles. The bottom of the container should be of generous proportions and the bottom centre should be slightly bowed or raised to minimize the volume above it as it is the slowest area to heat. (Coles, 1993; Schiffmann and Schiffmann, 2005)

15.5 Packaging materials

Temperature processing requires complete harmony between the food product and the packaging material. The key to successful food packaging is to select the package material and design that best satisfy competing needs with regard to product characteristics, marketing considerations (including distribution and consumer needs), environmental issues and cost (Marsh and Bugusu, 2007). Dual-ovenable (conventional and microwave) product/package systems need to be more rigorous to perform in extreme temperatures, thus reducing material selection options for the packaging technologist. Microwave packaging systems suitable for ready-to-serve meat, poultry and seafood products can be divided into two broad categories: active or passive. Passive microwave packaging containers serve to hold the food and allow microwaves to pass through the material without contributing any heat to the product. Most of the conventional packaging materials, which include paper, glass and plastics, are microwavable. Active packaging materials interact with the microwaves to provide a source of heat inside the pack (Schiffmann and Schiffmann, 2005). The most common active packaging materials are susceptors which transfer heat to the surface of the food or foil shields which prevent the microwaves from reaching all or part of the food. The requirements of passive microwaveable packaging materials must:

- allow rapid microwave heating
- provide a barrier to oxygen, moisture and microorganisms
- be compatible with the product
- withstand processing and storage conditions
- not impart any taint during processing or cooking
- retain solvent and odour in the packaging material during storage and cooking
- provide physical strength
- be cost-effective
- not ignite or smoke during normal use
- not affect the colour of the food
- not allow the migration of any materials into the food during processing, cooking or reheating.

15.5.1 Passive packaging materials

Paper and paperboard

Paper features heavily in the packaging of ready-to-serve muscle-based products in the form of informative cardboard sleeves surrounding the container and lidding material which must be removed before temperature processing. A large number of paperboard containers, in the form of trays, plates and cartons are also available for microwave cooking. Generally, the paperboard used is solid bleached sulphate (SBS), but solid unbleached sulphate (SUS), also known as natural or kraft paperboard, can be used (Huss, 1998). The paperboard is coated with various polymers which provide chemical resistance and sealability. The thin layer of high-temperature plastic provides a relatively inexpensive method to produce containers that capitalize on the structural strength of the paperboard while adding the barrier required to prevent moisture and fat in the food from entering the paperboard. The choice of coating depends on the maximum temperature the container is likely to be exposed to. Paperboard is coated by either extrusion coating of a molten polymer resin, adhesive or extrusion lamination of a previously fabricated polymer film or roll coating of a polymer solution (Schiffmann and Schiffmann, 2005). Low-density polyethylene (LDPE)-coated paperboard has a low melting point and is only considered suitable for microwaving frozen vegetables and frozen desserts. High-density polyethylene (HDPE)- and polypropylene (PP)-coated paperboard have better temperature and grease resistance than LDPE, but are still not rigid enough for conventional oven heating. Extrusion-coated polyester, which is the most common dual-ovenable paperboard material, is grease resistant, has a maximum temperature use of 205°C and modest gas barrier characteristics.

Glass

The use of glass as a packaging material for muscle-based, ready-to-serve and retail-ready foods which require reheating is limited. While soda lime glass is not suitable for microwave use, borosilicate glass containers are microwave transparent but should be used sparingly. The cylindrical shape of most glass containers or jars causes focused interior heating. The food content of large jars tends to become intensely hot at the top surface and where it is in contact with the glass, while the centre remains significantly cooler during the heating process. Microwave heating of smaller jars causes hidden hot spots in the centre of the container, while the surface and outer area of the food product remain relatively cool. Large temperature differences between the product and glass can lead to thermal expansion differences causing the glass to break or crack. Conservative heating instructions and stirring is advised for all food heated in glass containers. The restriction at the neck and the cylindrical shape of most glass food containers can cause bumping and eruption of the contents especially if there are large food particles present (Schiffmann and Schiffmann, 2005).

Plastics

The two major categories of plastics are thermosets and thermoplastics. Thermosets are polymers that solidify irreversibly when heated and cannot be remoulded; they are

employed mainly in automobile and construction applications. Thermoplastics are polymers which soften upon exposure to heat and return to their original condition at room temperature. Thermoplastics are ideal for food packaging since they can be moulded and shaped into various products (bottles, trays) and plastic films and they are recyclable. Many types of plastics are used for packaging food products including polyolefins, polyesters, polyvinyl chloride, polyvinylidene chloride, polystyrene, polyamide and ethylene vinyl alcohol. Polyolefins and polyesters are the most commonly used plastics in the food industry (Marsh and Bugusu, 2007):

- **Polyolefins** – Collective term for polyethylene (PE) and polypropylene (PP) and other less popular olefin polymers. Polyethylene, a simple inexpensive plastic, is manufactured by addition polymerization of ethylene. There are two categories of polyethylene: high-density and low-density. Low-density polyethylene (LDPE) is flexible, strong, tough, easy to seal and moisture-resistant. It has a low melting point with a maximum temperature resistance of 101°C, making it only suitable for microwaving frozen vegetables and frozen desserts. LDPE is not suitable for conventional oven use. High-density polyethylene (HDPE) has better temperature and grease resistance than LDPE, but is not widely used for microwave packaging due to the high cost of production and poor sealability. Polypropylene is harder, denser and more transparent than polyethylene. While the maximum temperature resistance of polypropylene (PP) is 160°C, making it suitable for hot-filling, boil-in-bag applications and microwave heating, it is still not rigid enough for conventional oven heating.
- **Polyesters** – Polyethylene terephthalate (PET), polycarbonate and polyethylene naphthalate (PEN) are condensation polymers formed from a reaction between carboxylic acid and alcohol which produces ester monomers. PET, which is recyclable, is the most commonly used polyester in food packaging. PET, formed when terephthalic acid reacts with ethylene glycol, provides a good barrier to gas and moisture, and resistance to heat (205°C), mineral oils, solvents and acids but not bases. PET exists as both an amorphous (transparent) (APET) and a semicrystalline or crystalline (opaque white) (CPET) thermoplastic material. Amorphous PET is more ductile and softer than crystalline PET (Marsh and Bugusu, 2007). Crystallized polyester (CPET) trays are manufactured by thermoforming an extruded PET sheet containing an added nucleating agent. CPET forms a high-gloss, rigid tray with a temperature resistance of 230°C. Since the trays are not optically clear after the crystallization process, they are usually pigmented in black, white and ivory (Huss, 1998). Polycarbonate, formed by polymerization of a sodium salt of bisphenol acid with carbonyl dichloride (phosgene), is clear, heat-resistant and durable. Polyethylene naphthalate (PEN), a condensation polymer of dimethyl naphthalene dicarboxylate and ethylene glycol, has greater gas and water-vapour barrier properties and performs better at higher temperatures than PET. Polycarbonate and PEN are mainly used as a gas barrier replacement in bottle manufacturing.
- **Ethylene vinyl alcohol** – Ethylene vinyl alcohol (EVOH) is a copolymer of ethylene and vinyl alcohol. EVOH has excellent barrier properties to oil, fat

and oxygen. However, EVOH is moisture sensitive and is mostly sandwiched within multilayered co-extruded films.

- **Polystyrene** – Polystyrene (PS) is an addition polymer of styrene, is clear, hard and brittle with a relatively low melting point. It can be mono-extruded, co-extruded with other plastics, injection moulded, or foamed to produce a range of products. Foaming produces an opaque, rigid, lightweight material with impact protection and thermal insulation properties creating a popular take-out packaging material for the food service area.
- **Polyvinyl chloride** – Polyvinyl chloride (PVC), an addition polymer of vinyl chloride is a heavy, stiff, ductile, medium strength, amorphous and transparent material. PVC has excellent resistance to chemicals (acids and bases), grease, oil, good flow characteristics and stable electrical properties. Uses in food packaging include bottles and packaging film manufacture. The thermoforming properties of PVC render it useful for blister pack manufacture such as those used for meat products. PVC flexibility can be modified with the use of plasticizers – for example, adipates.
- **Polyvinylidene chloride** – Polyvinylidene chloride (PVdC) is an addition polymer of vinylidene chloride. PVdC is heat sealable and an excellent barrier to water vapour, gas, fatty and oily products. It is used in flexible packaging as a monolayer film, a coating, or part of a co-extruded product. PVdC also has applications in hot-filling, retorting, low-temperature storage and modified atmosphere packaging (MAP).
- **Polyamide** – Commonly known as nylon, polyamide is formed by a condensation reaction between diamine and diacid and repeating units of the polymer are held together by amide links. Nylon 6, with six carbons, is a typical packaging material. Nylon has good chemical resistance, toughness and low gas permeability properties. Mechanical and thermal properties are similar to PET and uses include boil-in-the-bag packaging.
- **Silicon oxide coatings** – A mixture of silicon oxides (SiOx) are applied to the surfaces of plastic films by vacuum deposition or electric beam evaporation. The resulting film is transparent, retortable and recyclable with excellent barrier properties (Kirwan and Strawbridge, 2003).

Most of the plastic packaging materials used for heat-and-serve and retail-ready muscle-based food product containers or trays are multilayered plastic structures (Table 15.1). Plastics may be combined by lamination or co-extrusion. Lamination involves bonding two or more plastics together or bonding plastic to a material such as paper or aluminium. Bonding is achieved using adhesives. Lamination enables reverse printing where printing is buried between layers and therefore not subject to abrasion. Lamination can also add or enhance heat sealability. In co-extrusion, two or more layers of molten plastics are combined during film manufacture. Co-extrusion is more rapid than lamination; however, materials used must possess thermal characteristics which allow co-extrusion. Recycling of laminated and co-extruded plastic materials is complex due to the combinations of materials used in such technologies (Marsh and Bugusu, 2007). The use of multilayered

Table 15.1 Semi-rigid packaging materials for in-package microwave processing

Material	Food type/Application
PP/EVOH/PP	Sauces, ready meals and pasta
PP/PVdC/PP	Sauces, ready meals and pasta
CPET/APET	Sauces, ready meals and pasta
(HT)PS[a] /EVOH/filled PP/PP	Sauces, dips
PS/PVdC/PS	Ready meals developed for use on thermoform/ fill/seal lines
PS-PPO[b] /EVOH/PP	Sauces, ready meals and pasta

Source: Adapted from Coles (1993).
[a] Heat-resistant polystyrene.
[b] Ultra-high heat-resistant structure.

materials employs the benefits of several different polymers which can be varied to meet specific needs. They usually consist of three or more layers – for example, an oxygen barrier polymer, a structural or 'carrier' polymer and a tie layer that bonds the barrier layer to the structural layer. Typical membrane lidding materials suitable for ready-to-serve muscle-based products are thin multilayered high-barrier structures – for example, PET/PP/EVOH/PP, PET/PVdC/PE, PET/SiOx/PE, PET/Al foil/PP, PET/SiOx-PET/CPP, amorphous PA/PA/PP and PET/PA-EVOH-PA co-extrusion/PP (Coles, 1993).

Metal

Aluminium has excellent physical protection and barrier properties, formability and decorative potential, recyclability and consumer acceptance, making it one of the most common metal-based packaging materials in the ready-to-serve and food service industry. Aluminium is a lightweight, silvery white metal derived from bauxite ore, where it is present in combination with oxygen as alumina. Magnesium and manganese are often added to improve strength. Aluminium foil is manufactured by rolling aluminium metal into very thin sheets followed by annealing to achieve dead-folding properties. Aluminium foil is available in a range of thicknesses where thinner foils are used to wrap food and thicker foils are used in tray manufacture. Foil provides an excellent barrier to moisture, air odour, temperature, light and microorganisms. Foil is inert to acidic foods and does not require lacquer or other protection (Marsh and Bugusu, 2007).

While aluminium foil plates, trays and containers are widely used for conventional oven cooking and reheating, the material has always been controversial for microwave oven use (Schiffmann and Schiffmann, 2005). The two major concerns are potential for damage to the magnetron and arcing. A study conducted by the Fraunhofer Institute for Process Engineering and Packaging IVV in Freising, Germany, on behalf of the European Aluminium Foil Association (EAFA), with support of the US Aluminium Foil Containers Manufacturers Association (AFCMA), concluded that microwave heating of food packaged in aluminium foil trays or in plastic containers with aluminium foil or aluminium laminated lids is perfectly viable. The study did not reveal any hazardous results from any

of over 200 food portions heated in aluminium packs. While in some cases the uniformity of heating was better in aluminium containers than in packages constructed from other materials, the heating process took up to three times as long, making it unlikely that aluminium will become the microwave heating container of choice in the future (Annette, 2008).

15.5.2 Active packaging materials

Active containers or packages contain elements that interact with the microwave field to create conditions other than simply holding the food products during microwave processing. The three basic classes of active container are shields, susceptors and field modifiers and patterned susceptors (Schiffmann and Schiffmann, 2005).

Microwave susceptors, shields and field modifiers

A susceptor is defined as a material which converts microwave energy into thermal energy to promote preferential heating of food through contact (Perry and Lentz, 2009). They are mainly used to provide localized effects such as crisping and browning. Field modification containers redirect the energy of the microwave field in a predictable manner to provide different heating rates to singular ingredients of multi-component meals and to overcome the problems of overdone edges and underdone centres in food products.

Browning is a surface colour change, usually associated with bread and pastry, brought about by the Maillard reaction. The Maillard reaction requires an amino acid (glycine), a reducing sugar (fructose), a surface temperature of 100°C, a water activity of 0.8 and a neutral or slightly alkaline pH to proceed. Temperatures in excess of 190°C are required to dehydrate the surface of the food product to achieve a water activity value of 0.1 in order for crisping to occur. During microwave heating, the internal vapour pressure of the food product is raised and a phenomenon known as water pumping occurs. Water pumping is a process whereby water is forced to the surface where it condenses, saturates the food product and prevents crisping from taking place (Schiffmann and Schiffmann, 2005). The cool temperature, high humidity and water-pumping action associated with microwave processing, which are not conducive to Maillard browning or crisping, has led to the introduction of susceptors in pastry-based heat-and-serve and retail-ready meat, poultry and seafood products (salmon en croute, steak and kidney, chicken and mushroom pies). Susceptors are also employed in the packaging industry to alleviate the problem of uneven heat distribution associated with microwave heating, especially in multi-component meals. Metalized conductive coating susceptors are used extensively for microwave food packaging applications. Vacuum-deposited aluminium is the most common metalized susceptor. Susceptors comprise four basic layers (Schiffmann and Schiffmann, 2005):

1. A polymer film heating surface (often 0.012 mm of heat-set, biaxially orientated PET, which will be in direct contact with the food) onto which is deposited

2. a thin metal layer (usually vacuum-deposited aluminium).
3. an adhesive to bond the film to
4. a substrate (usually paper or paperboard) to provide structural stability.

Field modifiers are used in microwave shielding, field distribution and field intensification. Microwave shielding involves the incorporation of aluminium foil as part of the microwave container to protect areas which may be susceptible to overheating. Single compartments of a multi-component ready-to-serve meal can be protected by shielding during microwave heating. When aluminium is properly designed in strips and geometric patterns, it can act as a field intensifier where it locally intensifies microwave energy. This is particularly useful for uniform heating of larger frozen food products. It can also act as a field distributor by transferring the microwave energy to areas in the food that may otherwise be deficient – for example, the underside centre of a pizza.

It is anticipated that developments in susceptor technology will feature heavily in future packaging innovations for the microwave food market (Bohrer, 2010). Micro-Grill™ is Inline Packaging's latest innovation in microwave susceptor. The Micro-Grill™ turns an ordinary microwave into a convenient and quick panini sandwich grill. It incorporates performance-enhancing features, which include focused energy pattern for enhanced grill lines, increased susceptor heat output to ensure superior crisping and vertical and horizontal moisture vent channels.

Heinz, in association with Graphic Packaging International (GPI), has developed the microwaveable susceptor bowl which can 'take a pie crust from frozen to flaky in just five minutes'. The microwaveable bowl is made with GPI's MicroRite™ Quik-Crisp® material, which consists of susceptor-coated polyester laminated on a paperboard base. The pie crust is crisped with a MicroFlex® Q patch placed on the inside of the outer carton, which crisps the top of the pie while leaving the filling moist and tender. GPI has also developed parallel technology in the form of QuiltWave™ to enhance the browning and crisping of microwave food products, through the use of laminated quilts or pockets. The laminated quilts expand when exposed to microwave energy, providing close contact with the food product to produce the crisping effect.

15.6 Packaging techniques

The development of packaging systems for the ready-to-serve and retail-ready meat, poultry and seafood sector can be particularly challenging. The muscle-based prepared meals are usually multi-component products with different physical and thermophysical properties. While each component will react differently to chilled and frozen storage, as well as various reheating processes, the entire meal must reach a consistently high quality before appearing on the plate of the consumer. The reheated ready-to-serve meal must also be microbiologically safe, be appetizing in terms of its visual and sensory qualities and contribute to the recommended levels and balance of nutrients in the consumer's diet (Creed, 2010).

A number of packaging techniques have been developed over the years in an attempt to achieve safe, nutritious and organoleptically acceptable ready-to-serve or retail-ready meat, poultry and seafood products.

15.6.1 Modified atmosphere packaging (MAP)

Modified atmosphere packaging (MAP) has been defined as the packaging of a perishable product in an atmosphere which has been modified so that its composition is other than that of air (79% nitrogen, 20.9% oxygen and 0.03% carbon dioxide) (Hintlian and Hotchkiss, 1986). The established modified gaseous environment gradually changes in the pack as a result of interactions between the product and packaging environment, usually instigated by microbial and oxidative reactions. Like most foods, ready-to-serve and retail-ready muscle-based products spoil rapidly in air due to moisture loss, reaction with oxygen and microbial contamination. Applying MAP to ready-to-serve food products may present considerable practical difficulties (Spencer, 2005). Partitioned trays designed to keep meal components separate in a complex dish prevent the flow of gas across and through the product, making the application of MAP inefficient or even impractical on high-speed, high-throughput packaging machines. Another problem unique to ready-to-serve meals is the three-dimensional textural components of some ingredients, such as pasta shapes or a bed of rice. These meal components retain large amounts of air in their interior spaces, causing a problem that can be compounded by enveloping the trapped air with overlaid sauces (Spencer, 2005). Extension of product shelf life in MAP depends on a number of factors including knowledge of the food product, initial microbial load of the product, good manufacturing hygiene, storage temperature, gas composition and gas permeability of the packaging material.

The choice of gas used for MAP is dependant upon the food product being packaged. The three main commercially used gases in modified atmosphere packaging are oxygen (O_2), carbon dioxide (CO_2) and nitrogen (N_2). The effectiveness of carbon monoxide (CO) and Argon (Ar) has also been investigated for use in modified atmosphere packaging (Spencer, 2005). Oxygen is a colourless, odourless gas which promotes several types of deteriorative reactions in foods including lipid oxidation and pigment oxidation. Most common spoilage bacteria and fungi require oxygen for growth. Therefore, to increase product shelf life, pack atmospheres should contain a low concentration of residual oxygen. In some foods, however, a low concentration of oxygen can result in quality and safety problems such as pigment (myoglobin) oxidation in meats resulting in metmyoglobin formation (brown colour), senescence in fruits and vegetables and growth of food poisoning bacteria – for example, *Listeria monocytogenes* and *Escherichia coli*.

Carbon dioxide is a colourless gas with a slightly pungent odour at very high concentrations. Carbon dioxide dissolves readily in water to form carbonic acid. It is also soluble in food lipids and other organic compounds. The solubility of carbon dioxide increases with decreasing temperatures (Mullan and McDowell, 2003). The high solubility of carbon dioxide can lead to 'pack collapse' in foods containing

high amounts of water and fat, such as beef, fish and poultry, due to the reduction of headspace volume (Parry, 1993). The decline in the pH of the food product, as a result of the formation of carbonic acid inhibits the growth of most microorganisms. Carbon dioxide is also thought to alter membrane function and inhibit or decrease enzymatic reactions of bacterial cells (Daniels *et al*., 1985; Sivertsvik *et al*., 2002).

Nitrogen is an inexpensive, readily available, relatively inert gas with no odour, taste or colour. Nitrogen does not support the growth of aerobic bacteria but does not inhibit the growth of anaerobic bacteria. Nitrogen is used MAP as a filler gas either to reduce the proportions of the other gases or to prevent pack collapse, thus maintaining acceptable pack shape. MAP using CO_2/N_2 gas mixes at compositions of 25–50% CO_2 and 50–75% N_2 along with a gas to product of 2:1 is widely used to maximize the shelf life and inhibit the development of oxidative off-flavours in ready-to-serve and retail-ready meat, poultry and seafood products (Mullan and McDowell, 2003). MAP resulted in an extension of shelf life of refrigerated precooked chicken (Chouliara *et al*., 2006), precooked shelled red claw fish tails stored at refrigerated temperatures (Chen and Xiong, 2008) and ready-to-eat sushi and cold noodle products stored at ambient temperatures (Chen *et al*., 2003).

Important considerations when selecting plastic packaging materials for MAP applications include food contact approval, gas and vapour barrier properties, optical properties, antifogging properties, mechanical and heat-sealing properties. MAP lidding films may require piercing before product reheating.

15.6.2 Aseptic packaging

Aseptic packaging of foods can be defined as a process where a pre-sterilized food product is filled and hermetically sealed in sterile packaging materials under an aseptic environment without reheating for sterilization. Aseptic processing requires (Buchner, 1993):

- sterilization of the products before filling
- sterilization of the packaging materials or containers and closures before filling
- sterilization of aseptic installations before operation – UHT unit, lines for products, sterile air and gases, filler and relevant machine zones
- maintaining sterility in the total system during operation – sterilization of all media entering the system, air, gases, sterile water
- production of hermetic packages.

Aseptic processing and packaging systems are separate but integrated operations where the packaging step relies on the processor to provide a quality sterile product. Presterilization of food products consists of heating the food to the desired UHT temperature, maintaining this temperature for a predetermined time period to achieve sterility. The food is then cooled to ambient temperature or an elevated temperature to the desired viscosity for filling. Indirect heating methods for liquids with particulates include tube-type heat exchangers, scrape-type heat exchangers,

rotaholders, ultra-high-pressure sterilization and microwave sterilization (Buchner, 1993). Various methods for the sterilization of packaging materials are currently used in aseptic packaging systems. These include dry heat, saturated steam, superheated steam, UV light and ozone, hydrogen peroxide, pulsed light and ethylene. The effects of a number of processing techniques on packaging materials have been reviewed by Ozen and Floros (2001). An aseptic packaging technique employing the form-fill-seal system relies on the temperatures reached by thermoplastic resin during the co-extrusion process used to produce the multilayer packaging material to produce a sterile product contact surface (Han and Scanlon, 2005).

While the main form of aseptic packaging is the carton and is typically composed of paper (70%), polyethylene (LDPE) (24%) and aluminium (6%) with a tight polyethylene inside layer, pouches, cups, trays and plastic cans also be aseptically packaged. The paper component of the package provides stiffness, strength and the 'brick'-like shape of the package. Polyethylene is used on both the outer surface (printing surface) and the innermost layer of the package, forming a tight seal. An ultra-thin layer of aluminium foil provides a barrier against light and oxygen eliminates the need for refrigeration and prevents spoilage without the use of preservatives (Annette, 2008). The Tetra Wedge Aseptic (TWA) microwaveable 200 S pack launched by Tetra Pak in 2005 was the world's first microwaveable aseptic packaging. The innovative package, which uses polyethylene terephthalic silicon oxide (PET SiOx) as an oxygen barrier, is designed to ensure product safety and integrity as well as original flavour, colour and texture for 6 months without the need for refrigeration or preservatives. The distinctive new shape of the TWA microwaveable 200 S offers the benefit of even heating, easy handling and accurate pouring over conventional stand-up plastic pouches (AP-Food technology.com, 2005). Aseptically packaged, ready-to-serve meals are one of the newest aseptic technologies to appear on the market. Vetetee Rice (Japan) launched its Dine-In range of cooked rice in shelf-stable microwaveable plastic trays, sealed with a clear plastic lid (Annette, 2008).

15.6.3 Retort packaging

The term 'retort pouch' is used to describe a flexible or semi-rigid package made from heat-resistant laminated plastic, into which food products are placed, sealed and sterilized at temperatures up to 121°C. The resulting product is then sterile and shelf-stable. The retort pouch has been developed as an alternative to metal and glass for packaging of processed food products. Retort pouches are used in several countries for a wide range of processed ready-to-serve shelf-stable muscle-based products, such as solid meat packs, beef curry, beef stew, meatballs in tomato sauce, sauces and soups. Laminate films used for retort pouch manufacture vary depending upon their specific application. The materials must be inert, heat sealable, dimensionally stable and heat resistant (usually 115°C–125°C). They should have low oxygen and water-vapour permeability and be physically strong to resist penetration by the foodstuff contained and abuse during handling and storage (Kirwan and Strawbridge, 2003). There are two main types

of pouches: gussetted and pillow packs. Gussetted pouches have an insert in the bottom allowing them to stand in an upright position. They are considered weaker than pillow-style pouches due to heat sealing through four layers at the corner seal. However, gusseted pouches take up less space on the supermarket shelf and can advertise the product clearly in the upright position (Potter, 2008).

Pouches are made from either aluminium foil-based plastic laminates or foil-free plastic laminate films, which are suitable for microwave heating. Most pouches are constructed from either three- or four-ply (layer) laminates with an inner food contact heat-seal layer (normally polypropylene), barrier layer (aluminium, ethylene vinyl alcohol (EVOH), silicon oxide or aluminium oxide), an optional nylon layer and an outer polyester layer. Adhesives with a polyurethane-base between the layers of film hold the laminates together. Polypropylene, on the inner layer of the pouch, acts as a sealing layer and can provide strength and flexibility to the pouch. Aluminium provides a complete barrier to oxygen, light, moisture and aroma. The optional nylon layer can increase the strength of the pouch due to its puncture and abrasion resistance. The outer polyester layer is heat resistant and provides an area for printing, often reverse printing. In aluminium foil-free pouches the product is visible and microwaveable. The aluminium layer is replaced by silicon oxide and aluminium oxide coatings. The silicate layer is a fine layer of flexible glass which exhibits gas and moisture barrier properties equivalent to aluminium foil.

During retort processing, all components including inks and adhesives must be able to withstand high temperatures, be suitable for water immersion, steam processing, air processing, or a combination of each. Products may be hot (> 63°C) or cold filled into the pouch. The level of gas in the headspace of the pack can be reduced by steam flushing prior to sealing. In oxygen-sensitive foods, pouches may be back flushed with nitrogen. Headspace can also be reduced by physically flattening the pouch without moving the product into the seal area causing contamination. Thermal processing will preserve the pack contents by destroying spoilage microorganisms and inactivating enzymes capable of activity in the pack during subsequent storage. Development of an appropriate safe retorting process is dependant on product type and characteristics. Chilled foods with a greater than 10-day shelf life where psychrotrophic *Clostridium botulinum* is of concern, the Advisory Committee on the Microbiological Safety of Food recommend a heat process of 90°C for 10 min or equivalent. Alternatively, one, or a combination of the following controls may be imposed (Potter, 2008):

- a pH of 5.0 (or less) throughout the food and throughout all components of complex foods
- a minimum salt level of 3.5% in the aqueous phase throughout the food and throughout all components of complex foods
- an a_w of 0.97 or less throughout the food and throughout all components of complex foods
- a combination of heat and preservative factors, which can be shown consistently to prevent growth and toxin production by psychrotrophic *Clostridium botulinum*.

15.6.4 Vacuum packaging and *sous vide* processing

Vacuum packaging is a packaging technique where 97–99.7% of the atmospheric gases are removed from the package. The packaging material used for vacuum packaging must possess high gas and moisture barrier properties and must be capable of heat sealing perfectly to deliver adequate containment (Robertson, 2006). Polyester/PVdC/LDPE and PA/PVdC/LDPE are suitable for long-term storage of vacuum-packed cooked muscle-based products. The oxygen permeability of the vacuum packaging film for cooked and processed meat products should be below 15 cm^3 /m^2 /24 h atm (Perdue, 1998). *Sous vide* processing, which originated in France in the 1970s, is the application of a pasteurization thermal process to food products in a hermetically sealed vacuum pouch or tray (Nyati, 2000). After heating, the product is cooled to 4°C within two to three hours of pasteurization, stored, distributed and retailed under chill conditions and reheated before consumption (Peck and Stringer, 2005). The shelf life of *sous vide*-processed products is variable and ranges from 1 week to 3 months, depending on the food being processed, the process adopted and regulations governing the product manufacture (Carlin, 2000). The shelf life of a *sous vide*-processed salad with meat in mayonnaise ready-to-serve product was extended to 52 days when stored at 4°C compared to non-processed control samples which showed signs of spoilage after only 10 days under the same storage conditions (Levkane *et al*., 2010). *Sous vide* processing of foods seals in the volatile flavour compounds within the package, resulting in superior flavour profiles. There is increased tenderness and moistness, improved colour retention and reduced nutritional loss as nutrients are not leached out in cooking waters. While vacuum packaging inhibits the growth of aerobic spoilage microorganisms in minimally processed *sous vide* muscle-based products, the generally preservative-free environment is ideal for the growth of some psychrotrophic and spore-forming organisms such as *Clostridium botulinum* and *Bacillus cereus*, which may have survived the heating process (Hyytia-Trees *et al*., 2000). A survey of 2168 *sous vide*-processed, commercially available meals representing 24 different meat, fish or vegetable ready-to-serve products concluded that the health risks associated with these products is minimal as long as low temperatures are maintained during storage. The presence of psychrotrophic, toxin-producing strains of *Bacillus* or *Clostridium* was rare or non-existent (Nissen *et al*., 2002).

15.6.5 Pasteurization treatments

A number of post-processing in-package strategies have been proposed to reduce the risk of *Listeria monocytogenous* and other harmful *Listeria* strains, *Clostridium botulinum* and *Bacillus cereus* in ready-to-serve and retail-ready meat and poultry products. Of the total product recalls, 57% of those due to the presence of *Listeria monocytogenous* in the United States in 2000 were associated with ready-to-serve meat products (Dawson, 2008). *Listeria monocytogenous* can grow at refrigeration temperatures, low pH environments, high salt

concentrations and low water activities. Two variants of the disease are recognized today (Borch and Arinder, 2002). The invasive form mainly affects the susceptible immuno-compromised population groups such as the elderly, organ transplant patients, unborn babies and cancer patients. The mortality rate for infected persons is 20–25% and can result in septicaemia, meningitis and still births. Non-invasive listeriosis causes febrile gastroenteritis. It has been documented that *Listeria monocytogenous* may become established in the processing environment, surviving cleaning and disinfection routines (Suihko *et al*., 2002). Therefore, post-heat treatment contamination is a major concern during peeling, slicing or cold filling of ready-to-serve meal components. The recent consumer and marketing demands for home-style production of ready-to-serve meals have led to less severe processing techniques and less use of preservatives, thereby increasing the risk of *Clostridium botulinum*. The heat-resistant psychrotrophic strains of *Bacillus cereus* which have been identified in many foods, including cooked meat products (Konuma *et al*., 1988), are of concern for prepared ready-to-serve foods stored at refrigerated temperatures.

Thermal pasteurization

Thermal pasteurization is the application of an in-package surface heat treatment in the form of dry heat, water, steam or infra-red sources on ready-to-serve and retail-ready meat and poultry products to reduce the risk of pathogens and increase shelf life. The rate of surface heating can be affected by the meat surface roughness, product composition, packaging film and product size. Temperature ranges for most post packaging pasteurization units range from 70°C to 96°C and dwell time can range from 30 sec to 10 min, although longer times have been reported. A study by Murphy and Berrang (2002) found that pasteurization of cooked, vacuum-packed chicken breast strips in steam and hot water at 88°C for 25 and 35 min lowered *Listeria innocua* populations by 2 and 7 log cfu/g, respectively.

High-pressure processing

Another in-package technology used to kill harmful bacteria with little or no change in meat quality is high-pressure processing. High-pressure processing damages the microbial cell membrane which leads to the collapse of intracellular gas vacuoles, cell elongation and cessation of microorganism movement (Cheftel, 1995; Hoover *et al*., 1989). High pressure also causes deprotonation of charged groups and disruption of salt bridges and hydrophobic bonds, resulting in conformational changes and denaturation of proteins. Enzymes are inactivated as a result of the conformational changes at enzyme active sites. During the high-pressure process, the packaged food products are placed in a high-pressure vessel, after which the vessel is sealed and filled with water. The pressure is then raised to a set point, usually between 300 and 700 MPa for meat products, by pumping water into the sealed vessel, and held constant for an allocated time. High-pressure processing is suitable for high-value meat products or where thermal treatments are

not an option. Other functions of high-pressure processing are the prevention of spore germination and meat marination.

Flexible and semi-rigid packaging materials are best suited to high-pressure processing to prevent package deformation. Water vapour and oxygen permeabilities of several laminated plastic films (PP/EVOH/PP, OPP/PVOH/PE, KOP/CPP, PET/Al/CPP) were not affected by high pressures between 400 and 600 MPa (Masuda *et al*., 1992). A cast-co-extruded 100 μm PA/glue/free radical linear PE (40 μm/20 μm/40 μm) was found to be incompatible with high-pressure treatments due to delamination (Lambert *et al*., 2000). Food packages with headspace and some easy-open films are not suitable for high-pressure processing (Belcher, 2006).

Irradiation

Food irradiation involves exposing pre-packaged products to gamma, X-ray or electron beam irradiation (Belcher, 2006). In the United States, the Joint FAO/IAEA/WHO Expert Committee approved the use of radiation treatment of foods up to 10 kGy dose in 1980 (Ozen and Floros, 2001). Radiation inactivates microorganisms by damaging their genetic material. A photon of energy or an electron beam randomly strikes the genetic material of the cell, forms a lesion in the DNA and breaks the DNA strand. Large numbers of single-strand lesions may result in the death of the microbial cell (Dickson, 2001). While it is an effective method in reducing microbial populations, inhibiting sprouting and controlling insect infestation, commercial applications have been limited due to consumer concern about the safety of radiated food products.

All packaging materials permitted for use for irradiated foods in the United States are regulated by the FDA under 21 CFR 179.45 (Belcher, 2006). The packaging materials should not transmit toxic substances and undesirable odours or flavours to foods. Irradiation of packaging materials may generate gases, such as hydrogen, and produce low molecular weight hydrocarbons and halogenated polymers. Radiolysis products formed upon irradiation of a polymer or adjuvant could migrate into food and affect odour, taste and safety of the food product (Galić *et al*., 2011). Two EU Directives (1999/2/EC and 1999/3/EC) relating to irradiated food were implemented in Ireland in September 2000 by S.I. No. 297 of 2000 (Food Safety Authority of Ireland, 2006). Some of the chemical and physical properties of polymer materials may be altered after exposure to irradiation. Irradiation can lead to chain scission and/or cross-linking of polymers. The main reaction during irradiation in most plastics, such as PE, PP and polystyrene (PS) is generally cross-linking. Cross-linking can decrease elongation, crystallinity and solubility as well as increase the mechanical strength of polymers. Chain scission decreases the length of polymers. Irradiation of cellulose, the main component of paper, results in a loss of mechanical properties (Ozen and Floros, 2001). Another concern related to radiation exposure of plastic films is the formation of free radicals. It is thought that these free radicals become trapped in crystalline regions of the polymer and change the mechanical properties of the plastic materials (Buchalla *et al*., 1993).

15.7 Active packaging applications

Smart packaging is a broad term encompassing a range of new packaging concepts, most of which can be placed in one of two principle categories: active packaging and intelligent packaging (O'Grady and Kerry, 2008). A number of active and intelligent packaging technologies for meat-based products have been extensively reviewed (Hogan and Kerry, 2008; Kerry *et al*., 2006; O'Grady and Kerry, 2008). Active packaging can be defined as a mode of packaging in which the package, the product and the environment interact to prolong shelf life or enhance safety or sensory properties while maintaining the quality of the product (Suppakul *et al*., 2003). Self-heating and self-venting systems used in the ready-to-serve muscle-based sector are examples of active packaging technologies. Active packaging also involves the incorporation of certain additives, either loose within the pack, attached to the inside of the packaging material or incorporated into the packaging material with the aim of maintaining or extending product quality and shelf life (Kerry *et al*., 2006). Oxygen absorption (Cryovac® OS scavenging film, Cryovac Sealed Air Corporation; ZERO$_2$®, CSIRO, Division of Food Science Australia in collaboration with VisyPak Food Packaging, Visy Industries, Australia) and antimicrobial films may have potential for use in ready-to-serve and retail-ready muscle-based products.

Intelligent packaging systems are designed to monitor specific attributes of packaged foods, or environment within the pack, and provide information about the quality or safety of the product during transport and storage (O'Grady and Kerry, 2008). Comprehensive reviews of intelligent packaging technologies for meat-based products have been presented (Hogan and Kerry, 2008; Kerry *et al*., 2006; O'Grady and Kerry, 2008). Time-temperature indicators (OnVu ICE labels, BASF Future Business GmbH; Fresh-Check®, TEMPTIME Corporation, NJ, USA) and sensor technology (Toxin Guard™ , Toxin Alert, Ontario, Canada) may have potential applications in intelligent packaging of ready-to-serve muscle-based products.

15.7.1 Active packaging systems

Self-heating packaging systems

Even though self-heating of ready-to-serve meat, poultry and seafood products are still in relative infancy and remain niche, the basic self-heating technology has been in operation since the First World War. The most common and safest self-heating technology is based on the exothermic reaction between calcium oxide (quicklime) and water. Calcium oxide is inexpensive, readily available and the by-products of the reaction are environmentally acceptable. The can or meal container is divided into three compartments or chambers. The food product is surrounded by an outer chamber containing the calcium oxide which in turn is separated from a third water-filled chamber by a thin breakable membrane. When the membrane is pierced the water and calcium oxide are mixed and the resulting reaction releases heat which heats the surrounding food product. This technology

is targeted at the consumer who likes the outdoor life, camping excursions, fishing trips and the emergency services.

Hot Pack™ self-heating meals contain a 300 g portion of a ready-to-eat meal in a pouch with a dish, knife, fork, salt, pepper, serviette and a 45 mL sachet of water to activate the heater. The heater is a Non-Magnesium Flameless Ration Heater® and is exclusively available from Canland UK Ltd. The ration heater is composed of finely powdered iron and magnesium metals and table salt. A small amount of water is added to the ration heater to activate the heating reaction. Eight recipes of Hot Pack™ meal are available, which include Lancashire hot pot, chicken casserole, sausage and beans, meatballs and pasta in tomato sauce and chicken dopiaza curry.

HOTBOX self-heating meals offer the convenience of just turning the box over and pressing a button to release the water into the calcium oxide compartment to activate the heating mechanism (Fig. 15.1). The HOTBOX is then returned to its original position and allowed to stand for 12–15 min, after which time it is ready-to-eat. Heating will continue for 15–20 min to ensure that the meal remains hot and therefore perfectly suitable for outdoor consumption. Eating utensils are included and the packaging is biodegradable. The range of HOTBOX products includes self-heating drinks (hot chocolate, cappuccino and lemon tea) and soups (mushroom, tomato and five vegetables) as well as beef stew, chicken tikka masala, chilli con carne and ravioli with tomato sauce.

Hotcan® self-heating meals are heated by piercing the top of the can to a maximum depth to release water into a calcium oxide compartment. Hotcan® meals are available in sausage and beans, chilli con carne, chicken curry and spaghetti Napoli varieties. La Bruite shelf-stable/self-heating meal lines and EverSafe complete meal kits have been recently introduced on the US market (Annette, 2008). Thermotic Developments are designing a new heat transfer process, direct steam heating, by ensuring that excess present water is present with the calcium oxide/water reaction. This system transfers heat to the product by injecting steam directly into and through the food (Butler, 2008).

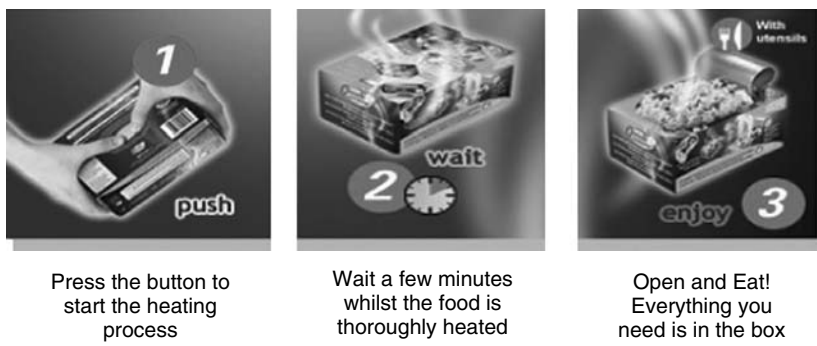

Fig. 15.1 Heating instructions for HOTBOX revolutionary self-heating meals. Reproduced with permission from HOTBOX (www.hotbox.co.uk).

Self-venting packaging systems

The introduction of various self-venting technologies has helped to improve the uniformity of heating, shorten the heating time and maintain the moisture level during microwave cooking of the heat-and-serve muscle-based products. The self-venting system enables food products to be cooked, distributed, displayed, reheated and served in the same package. The technology also allows products to be microwave cooked from their raw state. Three main technologies are used for self-venting packages (Schiffmann and Schiffmann, 2005):

- one-way valve self-venting system – vents the steam through a valve moulded into the packaging film;
- non-valve hole self-venting systems – holes, micropores or larger slits, usually covered with a barrier film, which can be exposed by the removal of adhesive film tape or labels by the consumer, by the internal steam pressure or by the shrinkage of the film lid allowing the steam to vent;
- non-valve cohesive-seal or seal break self-venting systems – have a weak point at a tray or lid seal or use a device that is microwave interactive to melt a hole in the material allowing steam release.

Cryovac Sealed Air and Multivac developed Cryovac® Simple Steps™ vacuum-skin packaging for microwave applications. The Kepak Group (Ireland) in conjunction with the Food Packaging Group at University College Cork recently developed the extensive 'Global Cuisine' convenience range of precooked meat joints and ready-to-serve, muscle-based meals using CRYOVAC® Simple Steps™ vacuum-skin packaging technology. This innovative packaging system enables products to be cooked, shipped, stored, displayed, sold, reheated and served in the same package. The outer sleeve is simply removed and the product is reheated in the microwave for 3–7 min (depending on the product), without any film peeling or puncturing requirements. During the heating process the vacuum-skin film expands to form a bubble, trapping moisture and flavour, which subsequently self-vents and relaxes over the food product (Fig. 15.2). The 'stay cool' tray handles and easy-open features reduce the risk of burns when serving.

The M Vent Pouch has been developed by Borogue using Steripeel™ technology in conjunction with TPN FlexPaks advanced sealing technology, which enables the unique self-venting performance of the pouch during microwave cooking (Anonymous, 2010). Birds Eye has recently launched a 'Simply Bake to Perfection' frozen-to-oven range of products. The 'Bake to Perfection' packaging is made from a strip metalized PET/PET laminate that allows for oven cooking from frozen. The fish cooks in the pouch and vents steam during the cooking process while the laminate features a heat-seal coating on the inner surface for quick sealing (Partos, 2010).

Smart ovens

Smart cooking is a new cooking innovation combining the cooking capabilities of a convection oven with microwave and grill cooking. The smart cooking process

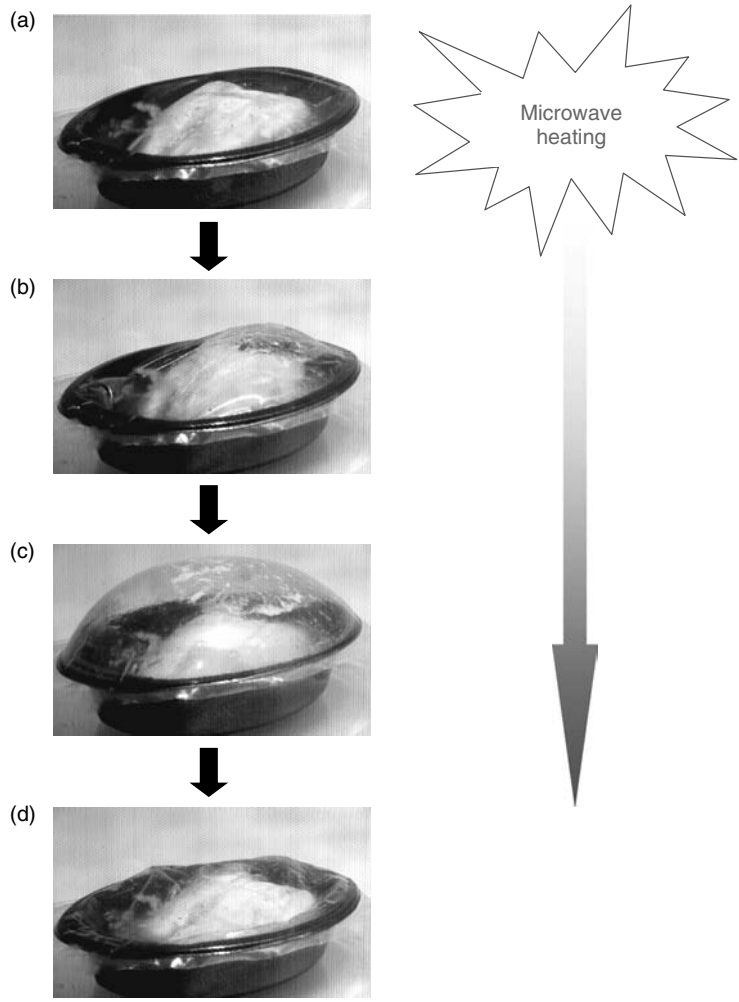

Fig. 15.2 Schematic representation (a–d) of the bubble formed by the self-venting film during microwave reheating of a Global Cuisine ready-to-serve meat-based product. © Food Packaging Group, UCC.

is made possible by the development of more sophisticated barcode symbologies and the Smart oven – for example, the Samsung BCE 1197 oven or the Breville BOV 800XL oven. A portable data file (pdf) 417 is a two-dimensional barcode (smart code) which can carry up to 1.1 kilobytes of data in the space of a universal product code (UPC) barcode (Fig. 15.3). It allows the encoding of additional information not possible with linear barcodes, such as nutritional information, addresses of food manufacturers, cooking instructions and even graphics (Yam *et al*., 2005). The pdf 417 located on the ready-to-serve product is scanned by the built-in smart code oven scanner which converts the code into a cooking

Fig. 15.3 Ready-meal packaging (Marks & Spencer) containing a SmartCode for use with a Smart Oven.

instruction. Every smart code contains a unique set of instructions which provide the Smart oven with the correct temperature, microwave power and cooking time for each individual food product (O'Grady and Kerry, 2008).

15.8 Future trends

In recent years most research and development has been motivated by the need to address three major trends in the food packaging industry, namely, the health trend, the green movement and the food safety trend. This is set to continue in the ready-to-serve and retail-ready food sector. The three main trends must also incorporate new and improved levels of convenience to alleviate the pressures of increasingly hectic lifestyles and to fit with the needs of an ageing global population. Advancements in greener packaging will become a baseline requirement for packaging manufacturers after Marks & Spencer's commitment to reduce packaging by 25% by 2012. Further applications of aseptic packaging techniques may negate the need for additives and preservatives in ready-to-serve muscle-based products and lead to considerable reductions in packaging waste. Continued research into edible films and their application as edible coatings is expected. The addition of edible coatings to fried food products can reduce the absorption of fat by up to 40% as well as providing a vehicle to carry extra nutrients to the food, resulting in healthier products. The health benefit of edible films should be further exploited and employed in the retail-ready food sector where fried foods are regular menu features.

Further developments in wireless technology in the ready-to-serve packaging sector are expected after Fulton Innovation recently developed a soup container that can reheat its contents without any external heat source. The container features charging technology printed onto the packaging which allows the consumer to heat the soup by placing the pack on a wireless charged work surface and choosing low, medium or high temperature (Anonymous, 2011). While a vast number of active and intelligent packaging technologies are commercially available, few have, to date, been adopted by the ready-to-serve sector. Oxygen scavenging and antimicrobial films suitable for conventional and microwave heating in conjunction with innovative packaging technologies may have the potential to extend the retail shelf life of many muscle-based, ready-to-serve products.

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16

In-package pasteurization of ready-to-eat meat and poultry products

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Abstract: In-package thermal pasteurization is a widely used processing method to eliminate foodborne pathogens in packaged ready-to-eat foods. The chapter first gives an overview of the microbial concerns that are associated with ready-to-eat foods. It then describes factors such as product configuration in package and processing time–temperature combinations that affect the effectiveness of in-package pasteurization. The chapter concludes with a review of published studies to further the technology of in-package pasteurization.

Key words: ready-to-eat, in-package, pasteurization, pathogens, food safety.

16.1 Introduction

Ready-to-eat (RTE) foods are a group of food products that are pre-cleaned, pre-cooked, mostly packaged and ready for consumption without prior preparation or cooking. According to the 2009 US Food code (FDA, 2009), RTE foods should be in an edible form without an additional preparation step to achieve food safety. Foods in this category usually contain raw materials of animal origin, such as eggs, fish, meat, poultry and ratites, and must be cooked to allow the lowest internal temperature to reach a minimum temperature, for a minimum holding time, during manufacturing to destroy microorganisms of public health concern. In an industrial setting, the cooking step is achieved by thermal processing using steam,

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hot water, microwave, or infrared. The thermal process should be designed by a thermal process authority and should ensure a minimum lethality (F_0) for the target microorganism (usually a foodborne pathogen). A properly processed and packaged RTE food should be free of the target foodborne pathogen and ready for consumption.

Among common foodborne pathogens, such as *Listeria monocytogenes*, pathogenic *Escherichia coli* and *Salmonella* spp., *L. monocytogenes* is the major concern for refrigerated RTE foods. This microorganism is a rod-shaped, non-spore-forming, Gram-positive bacterium widely distributed in the environment (Ryser and Marth, 1991). It can grow at temperatures between 1°C and 45°C, but optimally between 30°C and 37°C. *L. monocytogenes* has been isolated from a wide range of food products including pates, milk, soft cheeses, ice cream, coleslaw, RTE meat and poultry products, and smoked and lightly processed seafood products. As this microorganism is capable of growing at refrigerated temperatures, it can grow to a dangerously high level without causing significant changes to the quality indicators of RTE foods under normal refrigerated conditions. Temperature abuse, such as in a malfunctioning refrigerator, would allow *L. monocytogenes* to grow at accelerated rates that lead to unexpectedly high levels of *L. monocytogenes* in refrigerated RTE foods.

L. monocytogenes is a life-threatening pathogenic microorganism that causes listeriosis. People with weak, suppressed or compromised immune systems are most susceptible to the infection of *L. monocytogenes*. It is generally recognized that the mortality rate of listeriosis can be as high as 20% for pregnant women, children, elderly and immuno-compromised patients. *L. monocytogenes* also causes premature delivery, miscarriage, or stillbirth in pregnant women. The ability of *L. monocytogenes* to grow at refrigerated temperatures makes the presence of *L. monocytogenes* in refrigerated RTE foods a significant health hazard to consumers susceptible to *L. monocytogenes* infection. In recent years, several outbreaks of foodborne listeriosis that linked to the consumption of RTE meats have occurred in the United States, resulting large number of cases of illnesses and even deaths and the recalls of large quantities of meat and poultry products (CDC, 1998, 2000, 2002). The serious health risk implicated by *L. monocytogenes* in RTE foods led both the US Food and Drug Administration (FDA) and the Food Safety and Inspection Services of the US Department of Agriculture (FSIS-USDA) to establish a 'zero-tolerance' policy for *L. monocytogenes* in RTE foods since 1989. This policy stipulates that the detection of any *L. monocytogenes* in either of two 25 g samples of a RTE foods renders the food adulterated.

L. monocytogenes is killed by thermal processing commonly used for RTE food manufacturing. However, *L. monocytogenes* from the environment may contaminate the products after the thermal processing. In a study by Lawrence and Gilmour (1994) examining the incidence of *L. monocytogenes* in the environment of a cooked poultry plant, 15% of the environmental samples collected over a 6-month period contained *L. monocytogenes*. Therefore, any post-processing processes, such as peeling, slicing and packaging of RTE foods, may cause cross-

contamination of *L. monocytogenes* between the product and the environmental surfaces such as equipment, utensils and personnel. It is well recognized that post-thermal processing exposure of RTE products to food contact surfaces in the production lines is a common cause for recontamination of *L. monocytogenes* in what otherwise would be *Listeria*-free products. In a survey conducted by the National Food Processors Association examining the prevalence of *L. monocytogenes* in eight categories of RTE foods from retail markets in the states of Maryland and California in a period of 14–23 months, the prevalence rates of *L. monocytogenes* in RTE foods ranged from 0.17% to 4.7%, and the level of *L. monocytogenes* in positive samples ranged from < 0.3 MPN/g to 5.2 log cfu/g (Gombas *et al*., 2003). In 2006, FSIS-USDA, based on a comprehensive *L. monocytogenes* risk assessment, issued new compliance guidelines to meat processors in an attempt to reduce the incidences of foodborne listeriosis caused by *L. monocytogenes*-contaminated RTE meats (FSIS-USDA, 2006). The new guidelines require meat manufacturers to adopt one of the 'Three Alternatives' for post-lethality exposed RTE meats to control *L. monocytogenes* and ensure the safety of RTE meats. To meet the requirement of Alternative 1, the manufacturers need to use a post-lethality treatment (which may be an antimicrobial agent or process) to reduce or eliminate *L. monocytogenes* and an antimicrobial agent or process to suppress or limit the growth of the pathogen. To meet the requirement of Alternative 2, the manufacturers may choose to apply either a post-lethality treatment or an antimicrobial agent or process to control the growth of *L. monocytogenes* in RTE meats. To meet the requirement of Alternative 3, the manufacturers may choose not to apply a post-lethality treatment nor an antimicrobial agent or process to control the growth of *L. monocytogenes* in the post-lethality exposed product, but rely solely on sanitation measures to control *L. monocytogenes* in the post-lethality environment. If a manufacturer adopts Alternative 3, its products will be subjected to the most frequent FSIS-USDA verification testing among the three alternatives. Due to these stringent measures for controlling *L. monocytogenes*, a steady declining trend in the incidence of foodborne listeriosis has been observed in the United States since 1996 (Fig. 16.1; CDC, 2010).

In the compliance guidelines published by FSIS-USDA (2006), several physical treatments to eliminate *L. monocytogenes* in RTE foods were recommended for post-lethality, post-packaging products. These treatments include thermal pasteurization, such as hot water or steam pasteurization, and non-thermal treatments, such as ultra-high-pressure process or ultraviolet light (UV) irradiation. The UV treatment can be used as either a post-lethality treatment or antimicrobial agent or process depending on whether it eliminates, reduces or suppresses growth of *L. monocytogenes* (FSIS-USDA, 2006). Due to the limitation of equipment, product and production efficiency, the use of high-pressure process or UV treatments for post-lethality-exposed RTE foods has not been widely adopted by the food industry. Thermal treatment remains the most used post-lethality treatment for RTE foods. One way to ensure that a product is not exposed to potential contamination sources after the initial lethality treatment is to ensure that the post-lethality heat treatment is applied to the product after it has already been packaged. This

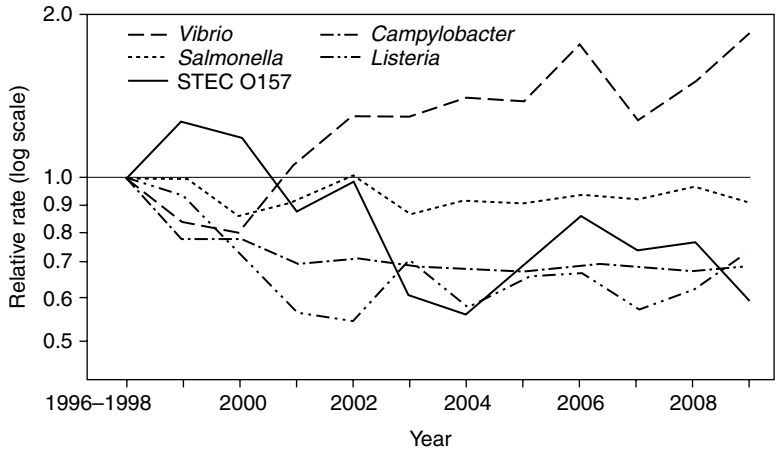

Fig. 16.1 Relative rates of laboratory-confirmed infections with *Campylobacter,* shigatoxin producing *E. coli* O157, *Listeria, Salmonella* and *Vibrio* compared with 1996–1998 rates, by year – Foodborne Diseases Active Surveillance Network (FoodNet), United States, 1996–2009 (CDC, 2010).

chapter summarizes the application of in-package thermal pasteurization for use in decontaminating RTE meat and poultry products that are susceptible to microbial recontamination during post-thermal processing processes.

16.2 In-package pasteurization

In-package pasteurization serves as the final kill step in product processing to eliminate vegetative pathogens such as *L. monocytogenes*, pathogenic *E. coli* and *Salmonella* spp. in RTE foods. The temperature-time conditions needed to achieve the desired efficacy of an in-package thermal process is determined by the food types, dimension and contact surfaces. For most RTE foods, the dimension and contact surfaces are the most important factors that determine the temperature and time of heating (Fig. 16.2). For single-piece products (Fig. 16.2a), the contamination of pathogens primarily occurs on the food surfaces, including cracks and crevices beneath the surface. The goal of in-package pasteurization for this type of product is to inactivate the pathogens on the product surface. Since the primary heating target is the surface, a short heating time and/or low heating temperature may be sufficient to kill pathogens located on the surfaces. For products with multiple contact surfaces in a package (Fig. 16.2b), such as hot dogs, diced meat cubes and sliced hams, the objective of in-package pasteurization would be to eliminate the pathogens in a location of the product package that is the 'coldest' spot, which usually is at the contact surfaces among food pieces. Therefore, higher heating temperatures and/or longer heating time may be needed to eliminate the bacteria located at the contact surfaces.

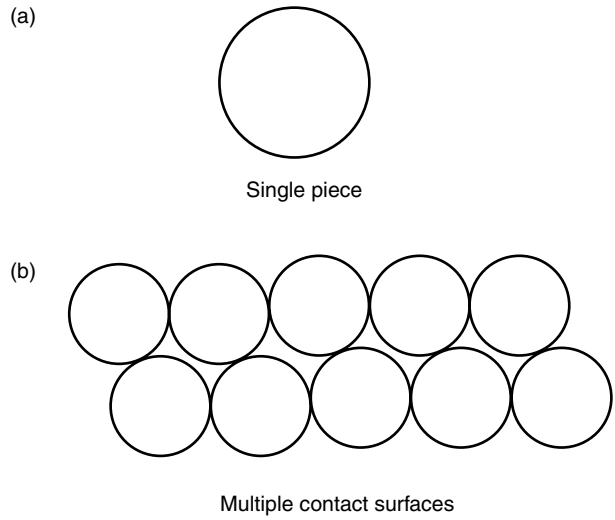

Fig. 16.2 Single piece (a) or multiple contact surfaces (b).

16.3 Time–temperature for in-package pasteurization

The efficacy of in-package pasteurization in inactivating vegetative pathogens largely depends on the pasteurization temperature and time applied to the food product. Product variety, size and arrangement in packaging and heating medium are some of the factors that need to be considered for selecting the appropriate heating temperature and time. Many studies have been conducted to determine the temperature and time needed for in-package pasteurization to achieve desired reduction of foodborne pathogens of concern in various meat and poultry products. Muriana *et al*. (2002) examined hot-water immersion in pasteurizing deli-style meats (turkey, ham and roast beef). The meats were inoculated with a four-strain mixture of *L. monocytogenes* (10^7 cfu/mL) and vacuum-sealed in shrink-wrap plastic packaging bags. The packages were submerged in a circulating water bath maintained at 90.6°C, 93.3°C or 96.1°C and heated for 2–10 min. A reduction of 2–4 log *L. monocytogenes* in the deli meats was observed. Apparently, the > 90°C heating for 2–10 min for in-package pasteurization of deli meats by hot-water immersion was effective in eliminating *L. monocytogenes* from the deli meats, considering that the level of *L. monocytogenes* in product, if presented, after packaging is < 1 cfu/g. However, a significant population of bacteria survived the heating in the experiment. This pointed to a significant aspect of heat transfer during hot-water immersion heating. For solid foods, heat is transferred by conduction, which is a relatively slow process. Although the surface temperature of the deli meats may have reached a level that is capable of inactivating the bacteria on the surfaces, the temperature under the surfaces may not be high enough to inactivate the bacteria located in the cracks and crevices.

This may be a reason why some bacteria survived the hot-water immersion process. When designing an in-package pasteurization process, it is necessary to identify the coldest spots in RTE foods and select a heating temperature and duration for these spots that completely inactivates the target bacteria. For individually packaged single-piece products, it is necessary to increase the heating temperature at sub-surface locations to a point that is lethal to the bacteria without recooking the products. As a reference, the cold spot is normally located about 2–3 mm below the surface, and the temperature at the spot should be allowed to reach 65°C (149°F) or above (Fig. 16.3). Murphy *et al*. (2005) demonstrated that a complete inactivation of *L. monocytogenes* could be achieved by atmospheric steam heating (Fig. 16.4). These researchers also examined the thermal resistance of the *Listeria* cocktail in selected deli meats and reported that the D-values for a *Listeria* cocktail at 160°F (or 71.1°C) were 4, 5.5 and 9.3 sec in roast beef, smoked ham and smoked turkey, respectively, with z-values ranging from 5.1°C to 7.9°C. The D and z-values of *L. monocytogenes* in these RTE meats can be used to determine the temperature and time of in-package pasteurization that are needed to achieve the desired reduction of *L. monocytogenes* in roast beef and smoked ham and turkey.

For whole package pasteurization, Murphy *et al*. (2003b) conducted a study to determine the lethality of *L. monocytogenes* in cooked chicken breast fillets and strips during in-package pasteurization by steam under atmospheric conditions. Fully cooked chicken breast fillets (120 g) and strips (454 or 127 g) inoculated with *L. monocytogenes* (10^{7-8} cfu/g) were vacuum packaged and then subjected to steam heating at 90°C. These packages contained multiple contact surfaces and, therefore, a thorough cooking process was needed to inactivate the inoculated *L. monocytogenes.* Figure 16.5 illustrates the temperature history during

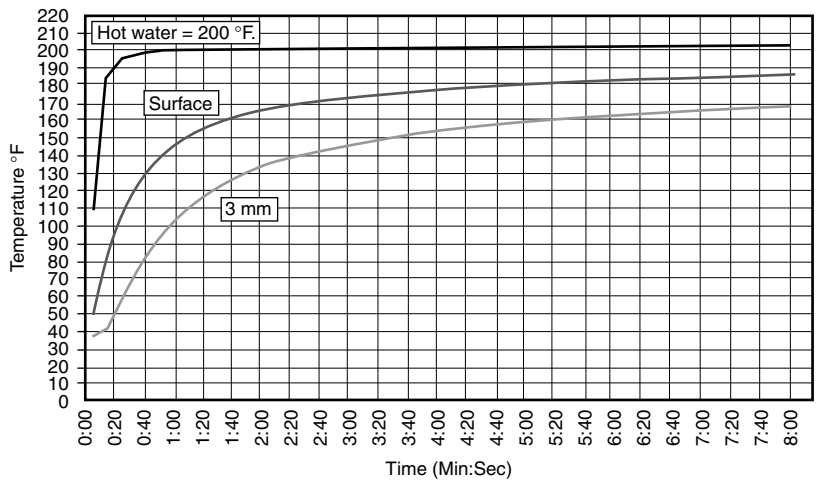

Fig. 16.3 Temperature histories in the heating chamber, on the product surface, and at 3 mm below the surface of deli meats (Alkar-RapidPak, Inc., 2010).

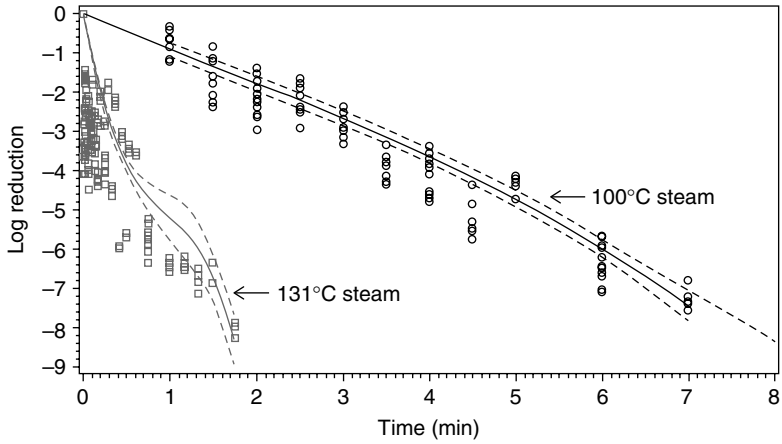

Fig. 16.4 Thermal inactivation of *L. monocytogenes* during post-lethalty steam-heating for surface pasteurization of individually packaged bologna (14 cm in diameter and 1.5 cm in thickness) (adapted from Murphy *et al*., 2005).

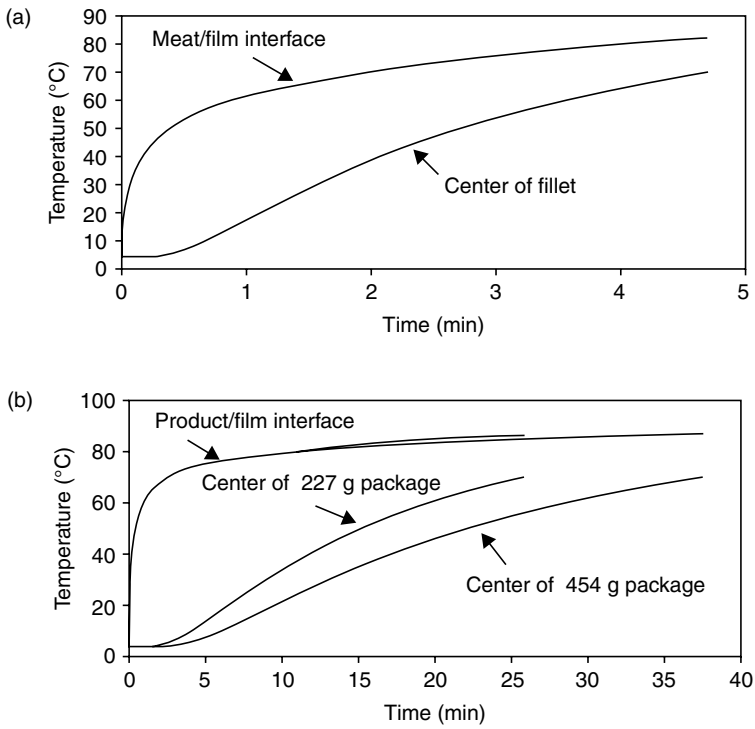

Fig. 16.5 Temperature histories during steam in-package pasteurization of fully cooked and vacuum-packaged chicken breast fillets (a) and strips (b) (adapted from Murphy *et al*., 2003b).

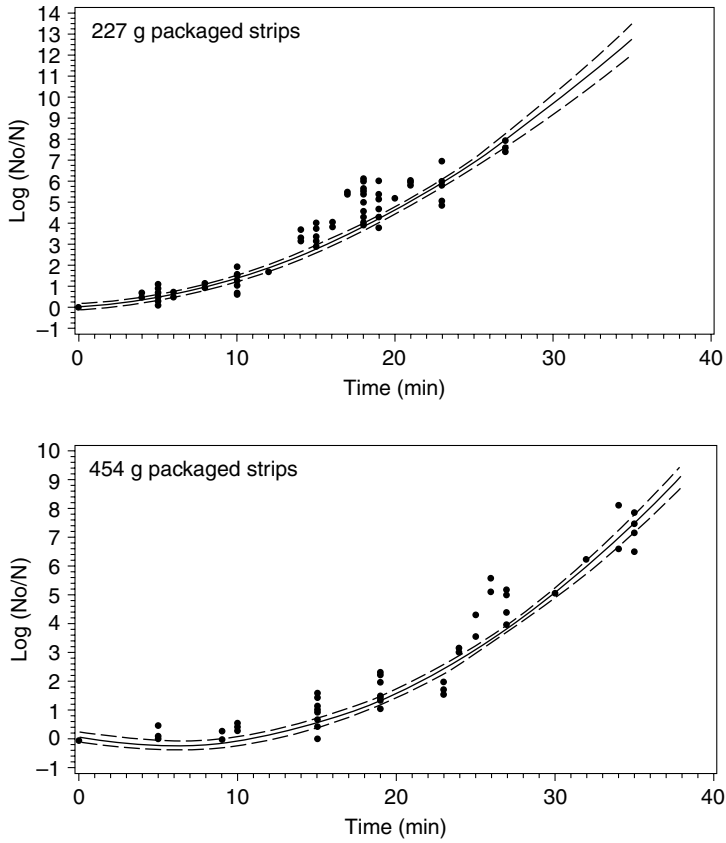

Fig. 16.6 Log reductions of *L. monocytogenes* in fully cooked and vacuum-packaged chicken breast strips during steam in-package pasteurization (adapted from Murphy *et al*., 2003b).

steam in-package pasteurization for the chicken fillets and strips, and Fig. 16.6 depicts the results of achieved log-reductions of *L. monocytogenes* in chicken-strip packages. Apparently, for products with multiple contact surfaces, much longer heating times were needed to inactivate the bacteria. The inactivation of *L. monocytogenes* in fully cooked turkey breast meat and in RTE bologna (14 cm diameter × 1.5 cm thickness) during in-package pasteurization were examined by Murphy *et al*. (2003a, 2005). They reported that a 50 min heating time was needed to achieve 7 $\log_{10}$ cfu/cm^2 inactivation of *L. monocytogenes* on the surface of turkey breast meat in hot water maintained at 96°C, and *L. monocytogenes* cells were reduced by 2 log in bologna after treated for 2.5 min in ambient steam at 100°C. McCormick *et al*. (2003) determined the D-values of *L. monocytogenes* and *S.* Typhimurium in low-fat turkey bologna at various surface pasteurization temperatures. The inoculated products were aseptically packaged in

polyethylene pouches, vacuum-sealed and treated in a heated water bath maintained at various temperatures. The D-values for *L. monocytogenes* were 124 and 16.2 sec at 61°C and 65°C, respectively, the D-values for *S.* Typhimurium were 278 and 81 sec at 57°C and 60°C, respectively, and the z-values were 4.44°C and 5.56°C for *L. monocytogenes* and *S. Typhimurium*, respectively. Min *et al*. (2007) surface-inoculated fully cooked chicken breast strips with heat-resistant *L. innocua* to a level of approximately 10^7 cfu/g. The inoculated chicken breast strips were vacuum packaged and heated in a water bath at 60°C, 70°C, 80°C and 90°C for different durations. The authors reported a 7.0-log reduction of *L. innocua* in chicken breast products was achieved after 30, 10, 7 and 5 min heating in hot water at 60°C, 70°C, 80°C and 90°C, respectively.

Studies have also been conducted to examine the use of food additives in combination with in-package pasteurization. Mangalassary *et al*. (2007) evaluated the inactivation effect of nisin/lysozyme with in-package pasteurization on *L. monocytogenes* in ready-to-eat low-fat turkey bologna. Bologna samples were treated with nisin (31.25 AU/cm^2), lysozyme (0.5 AU/cm^2) and a mixture of nisin and lysozyme (31.75 AU/cm^2) before inoculation with *L. monocytogenes* to a final population of 0.5 log cfu/cm^2 on the product surface. Samples were vacuum packaged and subjected to 60°C, 62.5°C or 65°C heat treatment. The nisin-lysozyme and nisin treatments reduced the time required for 4-log-reductions at 62.5°C and 65°C. Nisin-lysozyme reduced the heating time by 23% and 31% as compared to control sample for achieving a 4-log reduction at 62.5°C and 65°C, respectively.

Although *L. monocytogenes,* pathogenic *E. coli O157:H7* and *Salmonella* spp. are pathogens of concern for RTE meat, the application of in-package pasteurization is not limited to inactivating these microorganisms. It also can be used to reduce the spoilage microflora on a product to extend product shelf life. Dykes *et al*. (1996) reported a study combining in-package thermal pasteurization and surface application of organic acids to extend the shelf life of vacuum-packaged Vienna sausages. In this study, Vienna sausages, surface-applied with a mixture of acetic acid (2%), ascorbic acid (0.1%), citric acid (0.25%) and lactic acid (0.8%) and also vacuum packaged (500 g/bag, 8 sausages/layer) were heated in a water bath maintained at 70°C (± 3°C) for 30 min to achieve a final center temperature of 65°C at the end of heating, and then stored at 8°C for up to 71 days. The microbiological shelf life, defined as the time needed to reach 5×10^6 cfu/g for lactic acid bacteria or total aerobic counts, was extended from 14 days for untreated samples to 56 days for heat-treated samples, representing a four-fold increase in the microbiological shelf life of the product. The application of organic acid mixture alone did not extend product shelf life to any meaningful degree.

Steam or hot-water immersion heating may affect the sensory attributes of RTE foods (Murphy and Berrang, 2002). Equally effective in inactivating bacteria, post-lethality in-package steam or hot-water immersion heating did not significantly affect water activity and shear force of chicken breast strips. However, increased water loss (or water purge) was observed in in-package heat-treated chicken breast strips after prolonged heating (35 min) at high temperature (88°C).

The process of in-package pasteurization can be evaluated by computer simulation. Huang (2007) developed a computer simulation method to simultaneously determine the coefficient of surface heat transfer and thermal diffusivity of beef frankfurters in single-layer packages during hot-water immersion heating. Figure 16.7 shows the accuracy of computer simulation results. In combination with the thermal inactivation kinetics and the General Method (GM), the simulated temperature histories were used to estimate the survival of *L. monocytogenes* in beef frankfurters during hot-water immersion treatment (Fig. 16.8). The calculated log-reductions of *L. monocytogenes* in packaged beef frankfurters by computer simulation were 1–2 logs higher than the

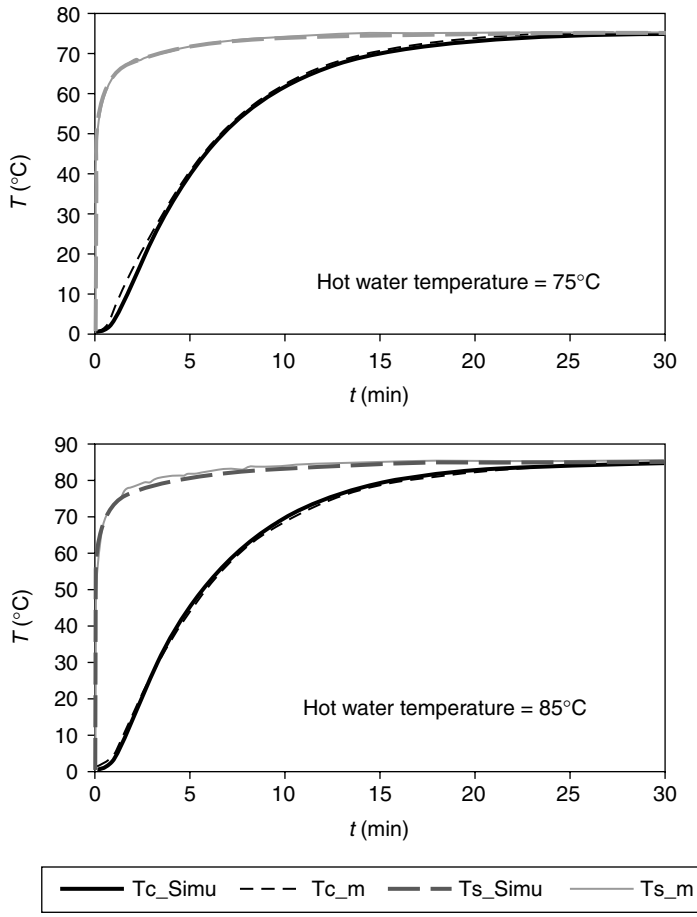

Fig. 16.7 Computer simulation of heat transfer during in-package pasteurization of beef frankfurters by hot water immersion heating (Tc_Simu and Ts_Simu: simulated temperature histories at the geometric center and on the surface of frankfurter packages; Tc_m and Ts_m: measured temperature histories at the geometric center and on the surface of frankfurter packages) (adapted from Huang, 2007).

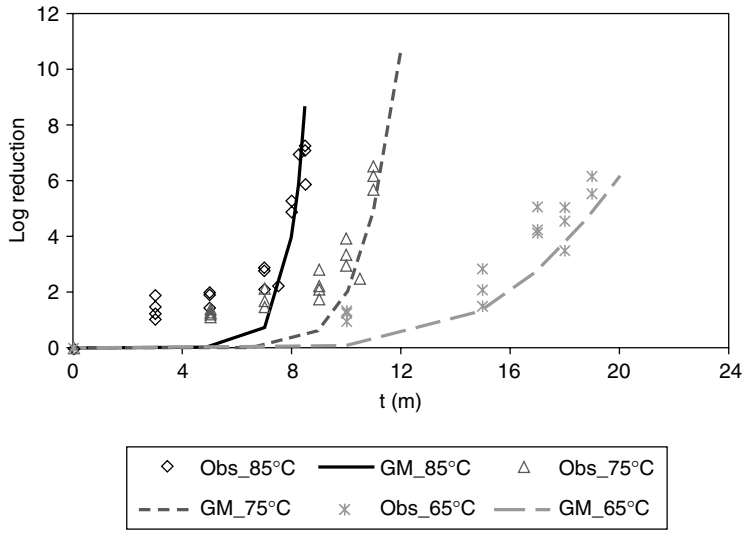

Fig. 16.8 Computer simulation of in-package thermal pasteurization of beef frankfurters to inactivate *L. monocytogenes* (Obs and GM: observed and calculated log reductions of *L. monocytogenes* under 65°C, 75°C and 85°C hot water bath; GM: General Method.) (Adapted from Huang, 2007.)

experimental observations during the initial stage of heating, but the simulation results were more accurate as heating progressed. In general, the computer simulation results for estimating the inactivation of *L. monocytogenes* in packaged beef frankfurters were slightly more conservative than the experimental observations, suggesting the computer simulation method can be used to design and evaluate the hot-water immersion or steam heating process for in-package pasteurization of RTE meats.

16.4 Equipment

The equipment that can be used in-package pasteurization of RTE foods is relatively simple in design and can be operated in continuous or batch operations. Basically, it consists of a water or steam tunnel through which packaged products are passed through and pasteurized. These systems are commercially available (Alkar, Lodi, WI, and Unitherm Food Systems, Inc., Bristow, OK). Figure 16.9 shows a schematic design of a continuous hot-water immersion system, and Fig. 16.10 illustrates the design of a hot-water spray or a steam injection system. In hot-water immersion and hot-water spray systems, hot water is used as the heating medium, while in steam injection systems saturated steam is injected through the nozzles. In all these systems, the temperature of the heating medium is generally below 100°C.

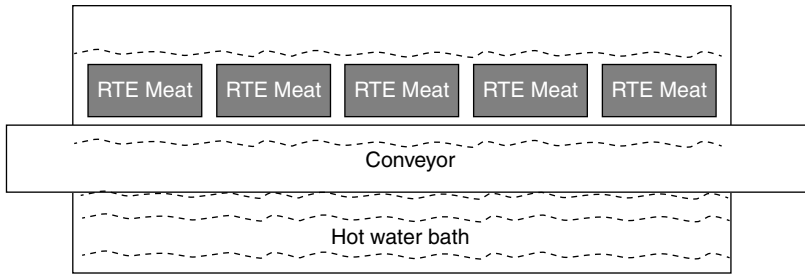

Fig. 16.9 Hot water immersion system for post-package pasteurization.

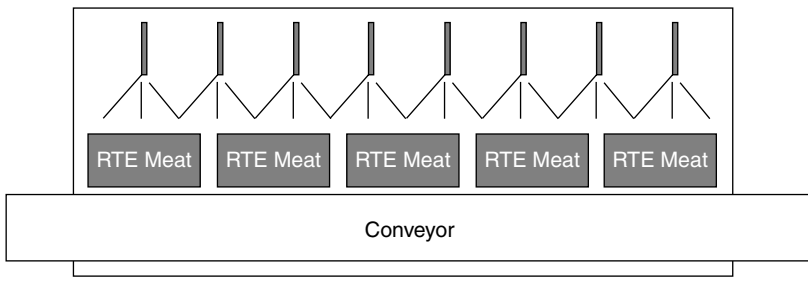

Fig. 16.10 Hot water spray or steam inject system for post-package pasteurization of RTE meats.

16.5 Practical considerations

As post-lethality-exposed products are fully cooked, subjecting the products to another cooking process can adversely affect the final quality. Unless the hygienic conditions in the post-lethality areas are poor and unsuitable for manufacturing RTE products, the bacterial load in the products prior to final packaging is usually low. Therefore, post-lethality thermal pasteurization is usually used in combination with antimicrobial agents (FSIS Alternative 2 intervention processes) to achieve maximum reduction of bacterial counts and retention of product quality. In all applications, it is recommended that the manufacturers should validate the process before commercial production.

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17

Environmentally compatible packaging of muscle foods

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Abstract: This chapter gives an overview of different types of meat packaging, approaches to source reduction and discusses specific research using bio-based packaging. Use of less packaging materials through thinner films and smaller packages without loss of strength or function are covered in some detail. Bio-based packaging materials can be extracted from biomass, synthesized from bio-derived materials and produced by micro-organisms including packaging materials cast and heat extruded. These bio-based materials include polysaccharide-, protein- and lipid-based compounds. Commercially available and research prototype packaging materials are also reviewed.

Key words: environmentally friendly packaging, bio-based, biodegradable, source reduction, edible.

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17.1 Introduction

Large amounts of packaging waste are discarded into municipal waste systems each year. Each of the 16 countries in the EU listed in Table 17.1 produces over 100 kg of packaging waste per person per year (http://www.ehsni.gov.uk/waste/regulation-and-legislation/regulations_packaging.htm) and over 29 million tons of plastic packaging waste were generated in the US in 2005 (EPA, 2007). Comstock *et al.* (2004) estimated that 70% of all packaging was devoted to food and that in the 1990s less than 10% of packaging material was recycled by consumers. In order to reduce the amount of synthetic polymer waste, a considerable amount of research has been devoted to source reduction, recyclable materials, and the production of biobased polymer films derived from natural sources.

Table 17.1 Packaging waste per person per year in Europe

Country	Packaging waste (kg per capita per year)
Ireland	>200
France	
Germany	176–200
Italy	
The Netherlands	
Luxembourg	
United Kingdom	150–176
Spain	
Denmark	
Sweden	100–150
Austria	
Portugal	
Belgium	
Finland	<100
Greece	

Source: European Environment Agency, 2005.

17.2 Types of meat packaging materials

The US Department of Agriculture-Food Safety and Inspection Service website (http://www.fsis.usda.gov/Fact_Sheets/Meat_Packaging_Materials/index.asp) has a description of the materials used for meat packaging. Part of this description is given below:

What are some materials used in meat packaging?

- *Plastic wraps and storage bags:* consumer plastic wraps and bags are made from three major categories of plastics: polyethylene (PE), polyvinylidene dichloride (PVDC), and polyvinyl chloride (PVC). The plastic resins are petroleum derivatives. Plasticizers, colorants, or anti-fog compounds may be added.
- *PE film:* the most-used transparent flexible packaging material; made from PE, a synthetic clear compound formed by polymerization of ethylene, a monomer from the petrochemical industry, susceptible to be polymerized under different conditions, i.e. at low, moderately high, and extremely high pressure. It is low cost, transparent, tough, heat-sealable, moisture-proof and resistant to low temperatures. Examples of consumer products made from PE include Gladwrap™ and Handiwrap™.
- *Polyvinylidene chloride:* a thermoplastic polymer which can withstand higher temperatures than PE; especially useful for covering utensils when microwaving foods; moisture-proof and transparent (example: Saran Wrap™).
- *PVC*: PVC replaced cellophane as the preferred meat wrapping used in supermarkets; a member of the vinyl family based on a monomer compound found

in petroleum. Low cost, protects against moisture loss, but has some oxygen permeability so it allows meat to 'bloom' (stay red and fresh looking).

- *Aluminum foil:* the foil is 98.5% aluminum with the balance coming primarily from iron and silicon to give strength and puncture resistance. The molten alloy is rolled thin and solidified between large, water-cooled chill rollers. During the final rolling, two layers of foil are passed through the mill at the same time. The side coming in contact with the polished steel rollers becomes shiny; the other side comes out dull. It does not make any difference which side of the foil contacts the food.
- *Freezer paper*: a white paper coated on one side with plastic to help keep air out of frozen foods, thus protecting against freezer burn and loss of moisture.
- *Parchment paper*: parchment is an odorless and tasteless paper made from cotton fiber and/or pure chemical wood pulps. It may be waxed or coated and is greaseproof or grease resistant. Parchment paper is primarily used in baking as a pan liner or to wrap foods for cooking.
- *Wax paper*: triple-waxed tissue made with a food-safe paraffin wax which is forced into the pores of the paper and spread over the outside as a coating.
- *Oven cooking bags*: both the bags and their closure ties are made from heat-resistant nylon. They can be used in a microwave oven or in a conventional oven set no higher than 400°F.
- *Bacon wrapper paper*: this paper is a glassine, greaseproof, or vegetable parchment paper, or a laminated product made from these papers and other materials and it is used for wrapping bacon.
- *Foam trays and other trays*: these are made from expanded polystyrene; formed when foaming agents are added to polystyrene and passed through a die.

One of the most popular forms of retail meat packaging is fitting a tight PVC stretch film over a polystyrene tray or a gas-flushed package; this is referred to as case-ready meat: it uses a barrier tray with a breathable lidding, all of which are made of a combination of plastic resins. Vacuum-packaged meats without trays are also used in many cases.

The meat packaging industry meets the needs of consumers, which are to receive products in their safest and most wholesome form possible, through the use of advanced technology in materials and packaging systems. The constraints of cost and efficiency are now compounded by the need for packaging to also be as environmentally friendly as possible. Meat packaging must maintain a high standard of product quality while minimizing the environmental impact of the packaging due to the highly perishable nature of meat. These challenges are being met through source reduction, use of recyclable materials, and the development of biobased packaging.

17.3 Source reduction

Source reduction is at the top of the priority hierarchy according to the Environmental Protection Agency (EPA) model for reduction of municipal solid

waste (see Fig. 17.1). This model was initiated when the Pollution Prevention Act of 1990 was enacted. According the *Consumer's Handbook for Reducing Solid Waste* (US EPA, 1992), source reduction can do more than prevent solid waste from entering the waste stream; it can also reduce energy usage for raw material production, conserve resources, and reduce waste handling costs and energy associated with handling the waste. The packaging industry has used this method to reduce material costs and lightweight materials to save on transportation costs for years. It makes economic sense as well as being better for the environment. According to garbologist, Dr Rathje, 'Source reduction is to garbage what preventative medicine is to health: a means of eliminating a problem before it can happen'. However, according to Lingle (1990), source reduction should be included in the package design only when it can be achieved without compromising transportability, product protection, package utility, consumer appeal, and product identification.

17.3.1 Examples of source reduction

Case-ready meats have been popular in the retail meat sector; however, the headspace necessary to maintain the modified atmosphere in the package has caused the package to be inefficient for shipping and material utilization. The Cryovac Division of the Sealed Air Corporation has recently developed and tested a tray with reduced headspace. The tray is more shallow than the typical case-ready tray and has a patented combination of a double lidding material. Prior to sealing, the double film is separated on the roll and allows the atmosphere to travel along the space between the two films. The upper film is a barrier film and the lower film is permeable so that if it touches the beef there is still opportunity for oxygen to reach the beef and maintain the red color of the meat. The reduced head-space allows

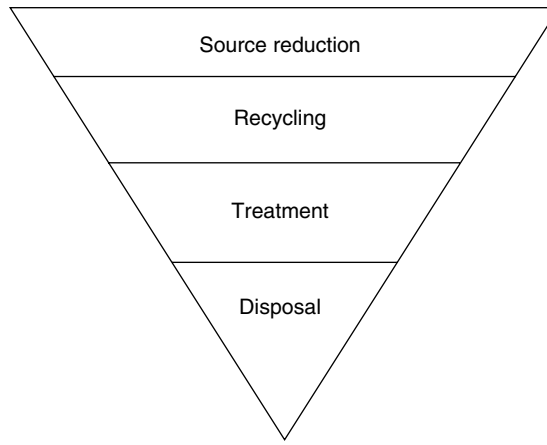

Fig. 17.1 EPA pollution prevention hierarchy (http://www.epa.gov/oppt/dfe/images/pwb-case1-images/hierlrge.gif).

more cube utilization in the shipping package configuration (palletization) and/or may also reduce the total size of the shipping case which reduces the amount of corrugated usage. Either way, less material is used, and greater efficiencies are achieved in shipping by reducing the headspace of the primary package.

Microwave products have traditionally been criticized as having excess packaging, much of which needs to be thrown away after cooking the meal. Smithfield's package containing a selection of different meat entrees has incorporated a cook-in package that allows one package to be used for processing, shipping, marketing, and consumer use. It replaces a package that used a tray with a boil-in-bag insert and also used thicker lidding material. This package won the 2003 Flexible Packaging Association Gold Award, in part due to source reduction (Hartman *et al.,* 2003).

Lightweighting materials has been a common method used for source reduction of packages such as metal cans, and glass and plastic bottles but is more difficult for materials such as flexible films which are already light. In fact, flexible packaging has been used to replace many of the heavier materials (cans, bottles) to take advantage of their light weight and the associated cost savings based on reduced shipping expenses and source reduction. However, one disadvantage faced by the change to flexible materials is that multilayer materials, which are perceived as environmentally unfriendly, are needed to obtain the equivalent strength and barrier properties of the previously used materials. One solution has been the use of metalized films: substrates such a polyester or polypropylene that have been coated with aluminum using vapor-deposition processes. They offer excellent barrier properties with significant source reduction advantages compared with aluminum foil laminates. Another method of lightweighting is referred to as down-gauging. Thinner films which can still provide the functions provided by thicker films are commonly used in the industry as a cost-saving measure (Forcinio, 1994). For example, the yield of a 32-gauge polyethylene terephthalate (PET) film was 50% more per pound of resin than its standard 48-gauge counterpart and if biaxially oriented, provided equivalent mechanical strength properties. This thinner material also produced less overall scrap and reduced waste by 250% (by volume) compared with a 1 mil (25.4 μm) thickness polypropylene (Rice, 2007).

Source reduction can also be achieved by replacement of a barrier layer with a thinner material or replacement with a multifunctional material. Meat packaging usually relies on coextruded, multilayer materials which are often five to eight layers. A new blending technology, referred to as 'smart blending', has been developed that can mechanically interlock materials thus eliminating the need for discrete barrier layers. This technology can be used to lightweight materials as well as down-gauge while still providing equivalent strength and barrier properties to those provided by multilayer coextruded materials. The films are produced via chaotic advection using a smart blender. For example, low-density polyethylene (LDPE) and ethylene vinyl alcohol (EVOH) have been blended to produce a film with mechanically interlocked platelet morphologies depending upon the degree of blending performed. The barrier properties of this film were similar to

three-layer EVOH control film and may be further improved with a newer generation smart blender (Kwon and Zumbrunnen, 2003).

The distribution package is often one of the best points to impact source reduction. The corrugated shipping container offers several options for reducing the total material usage. For example, a higher density linerboard may be used to replace a thicker linerboard yet provide the same strength properties. This type of board makes use of shorter recycled fibers but packs them closer together to produce a higher density liner material with strength equal to longer virgin fibers. Some manufacturers can also reduce the total fiber content in their box linerboard by using innovative converting methods. For example, one company has produced boxes with up to 20% less fiber than standard boxes but with equivalent strength using high compression linerboard (Zachary, 1991). Another idea may be to reduce flap dimensions or redesign the corrugated case for better cube utilization in the shipping environment. Stretch wrap may be down-gauged if a stronger material such as a PE copolymer is used or the wrap may be eliminated or replaced with an adhesive spray to stabilize the load (Lingle, 1990). Others have elected to eliminate the corrugated case entirely, opting to use slip sheets when possible. Whenever such changes are made it is important to test the newly designed distribution packaging to determine if more damage can occur to products, thus negating any benefits gained by source reduction.

17.4 Recyclable materials

Use of recyclable food packaging materials has received considerable attention in recent years because of the possibility of reducing waste and saving material resources. The US Food and Drug Administration (FDA) regulates the use of recycled materials for food packaging. The FDA has issued a favorable opinion on the suitability of a specific process for producing post-consumer recycled (PCR) plastic to be used in the manufacturing of food-contact articles to more than 100 submissions by various companies in recent years (CFSAN, 2006). The materials include polystyrene (PS), PE, polypropylene (PP), PET, polyethylene naphthalate, high-density polyethylene (HDPE), and acrylic polymers. The main concerns in the use of recycled materials are the chances of transfer of contaminants from the post-consumer materials to the food and use of materials not regulated for food-contact packaging including the adjuvants. The FDA has prepared a document entitled *Guidance for Industry - Use of Recycled Plastics in Food Packaging: Chemistry Considerations* to assist manufacturers of food packaging in evaluating processes for recycling plastic into food packaging (CFSAN, 2006). If a manufacturer wants to use recycled plastics for a food-contact application they must first submit critical information to the FDA, which includes providing a complete description of the recycling process, a description of the source of the recyclable plastic and description of any source controls in place intended to ensure that only plastic that initially complied with the applicable regulations is recycled (foodproductiondaily.com, n.d.). Use of functional barriers is an option to avoid direct

contact between recycled packaging material and food (Franz and Welle, 2003). A functional barrier is a layer of virgin polymer placed between the recycled material and the food so that it limits contamination from the recycled materials (Fig. 17.2). Huber and Franz (1997) studied the sensory qualities of the commonly recycled polymers and found that in most cases the properties were closer to those of the virgin polymers. Microbiological contamination from recycled plastics should not be of concern because processing of recycled plastics involves high temperatures and the use of sanitizers and cleaning agents which would eliminate any level of microorganisms in the material (Health Canada–Food & Nutrition, 2003).

Recyclable packaging materials that can be used in meat packaging applications include PE, PP, PS, and PVDC. A study conducted by the Cryovac Division of the Sealed Air Corporation, the National Cattlemen's Beef Association, and the National Pork Board found that case-ready products have grown to 60% of the meat retail market (Mize and Kelly, 2004). The most popular format of case-ready packaging consists of a clear or colored barrier-lined tray made of PS or PP and PE that is paired with a clear or printed barrier film (Belcher, 2006). Therefore using recycled polymers as mentioned above has a great potential in meat packaging applications. Table 17.2 shows examples of submissions approved by the FDA for the use of recycled materials in meat packaging applications.

Food-contact paper and board based on recycled fibers can also be used in some meat packaging applications. Recycled Kraft paper is used as a butcher wrap and other paper materials are sometimes used in meat packaging to absorb the grease

Fig. 17.2 A section through a multilayer package with recycled plastics as a non-food-contact layer (Crockett and Sumar, 1996).

Table 17.2 Dates of US FDA approvals for recycled materials in contact with meat

Date of approval	Company	Polymer	Use
19 November 1992	Lewis System	PE and PP	Containers for storing refrigerated poultry, red meat, and seafood
1 July 1993	Dolco Packaging Corporation	PS	Poultry trays
29 December 1998	Dolco Packaging Corporation	PS	Meat and poultry trays
1 August 2000	Polystyrene Recycling Company of America	PS	Manufacturing trays for holding poultry and meat

Source: CFSAN (2006).

(www.papernuts.com, n.d.). Recycled Kraft paper is produced from old corrugated cartons and paper processing waste which are fully recyclable. The corrugated industry has designed new, voluntary modular systems for case-ready meat (www.corrugated.org, n.d.). The corrugated modular systems make the shipping of all case-ready meat more efficient than the use of other containers. More than 75% of corrugated boxes are recycled, giving corrugated paper and board the highest recycling rate of any packaging materials used today (www. corrugated.org, n.d.).

There is an immediate need, globally, to minimize landfill and to reduce the negative environmental impact resulting from the disposal of plastics, including food packaging materials; the use of PCR plastics would help this cause to a great extent. Consumers are often encouraged to buy items in recyclable packages. Therefore use of recyclable materials in meat packaging applications is an integral part of making them more environmentally friendly.

17.5 Biobased materials

Several published sources (Petersen *et al,* 1999; Weber *et al.,* 2002; Com-stock *et al.,* 2004; Cutter, 2006) have divided biobased material films for packaging into three major categories.

1. Polymers extracted directly from biomass.
2. Polymers synthesized from bio-derived monomers.
3. Polymers produced by micro-organisms.

In most cases, materials under category 1 require some synthesis step to create a film, even if this step is as simple as casting. In fact, many of the given examples of materials from category 1 (i.e. soy protein, collagen, gelatin, zein, gluten) require chemical modification to form a film. Thus, most of the examples listed under category 1 could in fact be placed in category 2. Sources for biopolymers include:

- Polysaccharides
  - starches (corn, wheat, potato, pea, rice)
  - cellulose
  - gums (guar, locust bean, alginate, carrageenan, pectin)
  - chitin (chitosan).
- Proteins
  - animal (gelatin, collagen, whey, casein)
  - plant (wheat gluten, corn zein, soy protein).
- Lipids (waxes, triglycerides, phospholipids, glycerol)
  - lactic acid (polylactate, PLA)
  - hydroxyalkanes (polyhydroxyalkanoate, PHA)
  - bacterial polymers (celloluse, curlan, pullulan, silk-like fibers, PHA).

Most published research has focused on the use of plant material to form films and this remains an active research area (Jane and Wang, 1996). As new applications for

such materials emerge, characterization of renewable biopolymers is very important in order for these materials to be used for packaging applications. An obvious question about the use of biopolymers as packaging materials is why these materials should be used instead of petroleum-based materials that are highly functional and relatively inexpensive. The advantages of using biopolymers for food packaging include: reduction of dependence on petroleum-based packaging, use of a renewable agricultural resource that can be mass-produced, use of biopolymers as carriers to deliver shelf-life extenders such as antimicrobials or antioxidants, and biodegradability. Biopolymers can originate from a variety of sources including plant and animal material. Since the forming of a film requires cross-linking of molecular units to impart strength and flexibility, proteins and carbohydrates are often the best candidates for biopolymer films due to their chainlike molecular structure. Biobased food packaging (and biobased packaging in general) can be categorized as edible coatings, flexible films, and solid or semi-solid containers; edible coatings and flexible films will be discussed further in the sections below. Biobased film applications for meat include films functioning as a moisture barrier, oxygen barrier, texture modifier, breading adhesion aid, mold suppressor, integrity enhancer, bacterial inhibitor, physical protectant, oil barrier, antimicrobial carrier, and antioxidant carrier (Table 17.3).

17.5.1 Edible coatings

Edible coatings can physically protect meat or carry a quality-enhancing component (antimicrobial, antioxidant, flavorant, colorant). However, since the coating will either be consumed as a coating or become part of the meat product, it must be non-toxic and palatable from a flavor and texture standpoint. A variety of polysaccharide-based coatings have been tested for use on meat products including coatings made from starches, alginates, carrageenan, cellulose, pectin, agar, and chitosan. Carrageenan coatings have prolonged poultry meat quality (Pearce and Lavers, 1949; Meyer *et al.,* 1959) and the quality of fish (Stoloff *et al.,* 1948). Edible coatings for meat products have been tested since the 1940s and the use of biobased films to perform specific preservation functions has been evaluated for meat products. Pearce and Lavers (1949) reported using carrageenan to protect frozen poultry and Klose *et al.* (1952) incorporated an antioxidant into a gelatin coating to slow the development of oxidative rancidity in cut poultry meat prior to freezing. The major functions of edible coatings for meat products are reducing moisture loss, minimizing oxidation, reducing purge loss, and maintaining color quality (Gennadios *et al.,* 1997). Edible coatings are most often applied to meat as a solution and the coating is 'set' while on the surface of the product. Application can be by spraying, dipping, brushing, or some variation of these such as foaming. Commercial polysaccharide films have been used for quite some time in Japan (Labell, 1991). When used on meat products that are subsequently cooked the polysaccharide films dissolve, resulting in higher yields by reducing moisture loss while improving meat texture (Stollman *et al.,* 1994). High amylose starch films can be flexible, heat-sealable, oil resistant, and water soluble while they protect

Table 17.3 Biopolymer film and coatings applications tested for meat and poultry products

Material	Meat product	Application	Reference
Methyl cellulose	Pork and poultry	Breading adhesion	Bauer *et al,* 1969
Carboxy-methyl cellulose	Sausage	Mold suppression	Luck, 1968
Alginates	Beef, pork, chicken	Texture modifier, moisture barrier	Allen *et al.,* 1963
Alginates	Lamb carcass	Inhibit microbial growth	Lazarus *et al*.,1976
Alginates	Beef	Moisture/oxygen barrier	Williams *et al,* 1978
Carrageenan	Poultry	Moisture/oxygen barrier	Pearce and Lavers, 1949
Microbial pullulon	Diced meat	Oxygen barrier	Yuen, 1974
Chitosan	Chicken	Inhibit microbial growth	Acton *et al.,* 2000
Gelatin	Meats	Mold reduction, barrier	Kiel, 1961; Kiel *et al.,* 1960
Gelatin	Frozen chicken	Oxygen barrier, antioxidant carrier	Klose *et al.,* 1952
Gelatin	Smoked chicken	Moisture barrier	Moorjani *et al.,* 1978
Gelatin	Breaded meat	Oil barrier	Olson and Zoss, 1985
Gelatin	Meat cuts	Moisture/oxygen barrier	Whitman and Rosenthal, 1971
Corn zein	Cooked turkey	Oxygen barrier, antioxidant carrier	Wong *et al.,* 1992
Corn zein	Sausage	Moisture/oxygen barrier	Turbak, 1972
Wheat gluten	Sausage	Moisture/oxygen barrier	Turbak, 1972; Mullen, 1971
Wheat gluten	Turkey	Antimicrobial carrier	Schilling and Burchill, 1972
Wheat gluten	Bologna	Antimicrobial carrier	Dawson *et al.,* 2002
Whey protein	Frozen chicken	Physical protection	Alcantra *et al.,* 1997
Collagen	Beef steak	Moisture/oxygen barrier	Farouk *et al,* 1990
Collagen	Beef cubes	Moisture/oxygen barrier	Conca and Yang, 1993; Rice, 1994; Conca, 1995
Soy protein	Sausage	Moisture/oxygen barrier	Turbak, 1972
Soy protein	Chicken	Antimicrobial carrier	Dawson, 1998
Albumen/gelatin	Chicken parts	Breading adhesion	Suderman *et al.,* 1981
Albumen/soy/wheat protein	Meat parts	Batter/breading adhesion	Baker *et al.,* 1972

meat during storage and dissolve during thawing and cooking (Gennadios *et al.,* 1997). Wong *et al.* (1994) demonstrated that starch coatings inhibited moisture migration in food during storage and also found the starch coatings inhibited microbial growth by binding free water to lower the water activity of packaged meat. Starch-film moisture migration inhibition properties were verified by Cannarsi *et al.* (2005) who compared beef steaks packaged in either starch or PVC

films. Alginates are environmentally friendly coatings derived from seaweed. Gel formation can be initiated by the use of divalent cations such as calcium, manganese, and magnesium. They have been applied to meats to reduce moisture loss, improve color, slow oxidation, slow microbial growth, improve batter adhesion, carry protectants, and maintain texture. Cellulose casings are widely used in the manufacture of comminuted meat emulsions as a 'molding' container during cooking. These casing are normally peeled after cooking and degrade upon disposal via microbial degradation. Several researchers have also investigated cellulose coatings for meats to maintain quality during deep-fat frying (Meyers, 1990; Baker *et al,* 1994; Cutter and Sumner, 2002). Stubbs and Cornforth (1980) used calcium pectinate coatings on beef to reduce cooler shrinkage and bacterial growth during storage. Agar coatings have been used to carry antibiotics on the surfaces of poultry (Meyer *et al.,* 1959) and beef (Ayers, 1959) and to carry antimicrobials onto poultry meat surfaces (Natrajan and Sheldon, 2000a, 2000b). Another biopolymer under development for meat packaging/coating is chitosan, a carbohydrate derived from chitin found in the skeleton of shellfish. This is a waste product of commercial shell-fishing and can be processed to form a coating that has antifungal and antibacterial properties. Chitosan coatings were shown to reduce the total bacterial population on chicken drumsticks by 1 log (90%) compared with non-coated chicken drumsticks (Acton *et al.,* 2000). Kanatt *et al.,* (2004) added irradiated chitosan to minced lamb meat to reduce the lipid oxidation effects of irradiation on the lamb meat. Post-irradiation oxidation of irradiated lamb was 39% (leg meat) and 59% (rib meat) lower after 7 days of storage compared with non-treated meat.

Lipids have also been used to coat meat products primarily to inhibit moisture loss, reduce freezer burn, reduce off odors, and maintain color. Edible waxes have been used to reduce physical damage due to handling (McGrath, 1955) and to extend refrigerated shelf-life (Letney, 1958). Patents to protect frozen meats from freezer burn using lipid-based emulsions have been developed (Anderson 1960, 1961a, 1961b) and antioxidants added to lard and tallow coatings have also protected a variety of frozen meats from rapid oxidation, freezer burn, and moisture loss (Sleeth and Furgal, 1965). Waxes (carnauba, bees, and candelilla) have also been used to protect meat from frozen storage deterioration (Daniels, 1973; Cutter and Sumner, 2002). The freshness and organoleptic properties of beef, pork, and fish products have been preserved using fat coatings (Schneide, 1972), lipid-based films (Stemmler and Stemmler, 1976), and a mono-, di-, and triglyceride mixture (Heine *et al.,* 1979).

Protein edible coating materials include gelatin, collagen, casein, whey, soy, wheat, corn, and egg (Ben and Kurth, 1995). Edible casein coatings reduced moisture loss and delayed oxidation of frozen salmon (Khwaldia *et al.,* 2004). Villegas *et al.* (1999) maintained color stability and slowed oxidation of ham and bacon with gelatin dipping prior to freezing. Gennadios *et al.* (1997) enhanced the oxidative stabilizing effect of gelatin coatings on meats by adding antioxidants to the coatings. Gelatin coatings have been used to carry antioxidants/antimicrobials that slowed microbial growth and moisture loss, inhibited oxidation, and

stabilized meat during frying of poultry meat products (Klose *et al.,* 1952; Childs, 1957). Collagen coatings form an edible skin on meat products during heating that reduces moisture loss and absorbs smoke flavors during cooking (Cutter and Miller, 2004). Baker *et al.* (1994) reported that collagen coatings also reduce gas and moisture transfer by meats. Oxidation and off-flavor development were slowed with corn zein coating of cooked pork chops (Hargens-Madsen, 1995; Cutter and Sumner, 2002) or soy protein coating of cooked turkey (Herald *et al.,* 1996; Cutter and Sumner, 2002). Wheat gluten and soy protein coatings reduced cooked beef patty moisture loss during refrigerated storage (Wu *et al,* 2000).

Combining edible materials into one coating can increase the functionality of the coating through the combination of the component properties. For instance, the moisture barrier properties of lipids can be combined with the stability of starches or proteins. Ben and Kurth (1995) conducted several studies combining lipids with casein to protect meat surfaces from moisture loss and oxygen. These researchers also used protein gels to replace adsorbent pads and thus reduced packaging waste while maintaining product moisture. Wong *et al.* (1992) combined chitosan with lauric acid to create a moisture barrier film.

17.5.2 Flexible films

Nearly all commercial flexible films are produced by a heat-extrusion method, with the exceptions being meat casings produced from collagen and other soluble materials. Proteins investigated for biopolymer films include wheat gluten, corn zein, whey, pea protein, meat proteins, egg proteins, and soy. Starches studied include alginate, polysaccharides, cellulose, carrageenans, microbial polysaccharides, and chitosan. There has been widespread research activity on developing biobased packaging utilizing modified starch materials. PLA is produced from starch (primarily corn) fermentation followed by condensation of lactic acid resulting in a biopolymer. Commercial raw materials produced include polylactate produced by Cargill Dow (trade name NatureWorks PLA) and by Mitsui (trade name LACEA). Other starch-based raw materials for packaging include Nova-mont (Mater Bi), Biotec (Bioplast), and Earth (Earth Shell). These last materials require chemical modification of native starch materials and have been tested as molded containers (Salvage, 2000). Several researchers have evaluated the antimicrobial effects on raw beef of PLA alone (Chellappa, 1997) or PLA with nisin (Mustapha *et al,* 2002). Allanson (2000) inhibited *Escherichia coli* O157:H7 on ground beef and sausage during long-term refrigerated storage with PLA films at pH 3 and Krishnamurthy *et al.* (2004) reduced *E. coli* O157:H7 and *Salmonella typhimurium* on beef during storage after irradiation. Much of the research on biopolymer films has involved the production of films using the solvent casting method; in contrast thermal processing methods such as compression molding and extrusion have received limited attention. Jane and Wang (1996) and Huang *et al.* (1995) reported on an extrusion/molding technique, whereas Paetau *et al.* (1994), Jane *et al.* (1994), and Paulk *et al.* (1995) used compression molding to produce films from soy protein isolates. Other studies discuss the compression molding of starch

and corn zein films. Some of these films were reported to be rigid and brittle due to the absence of a plasticizer in the pre-processed mixture. The reducing agents in the Jane and Wang (1996) patent break the disulfide bonds in protein fractions to enable processing of the soy protein isolates. The disadvantage of the chemically modified protein is that the individual molecules are not structurally bonded, and water resistance decreases drastically. Cross-linking has been found to stabilize polymer chains and decrease vapor and gas permeability in protein-derived films (Kumins, 1965). Guilbert (1986) improved the barrier characteristics of films from various proteins (dried gelatin, casein, albumin, and ovalbumin) by the addition of organic acids. Various cross-linking agents and treatments that have been used in cast films include formaldehyde, glutaraldehyde, cysteine, transglutaminase, ultraviolet radiation, and glyoxal. Qualitative comparisons have been made between cast and heat-pressed protein films using scanning electron microscopy (Dawson, 1998). Casting films involves the evaporation of an organic solvent, ethanol in the case of corn zein, which results in a very porous material (Fig. 17.3a). The formation of heat-pressed films using corn zein or soy protein resulted in a homogenous structure with fewer voids seen at ×1000 magnification (Fig. 17.3b). Batch processing of films has shown that soy protein-glycerol, wheat-glycerol, and corn zein-glycerol mixtures can be thermally compacted. The press applied pressures of approximately 10 MPa for several minutes using a processing temperature of 150 °C for soy mixtures. To reduce water permeability, soy film has been laminated with the corn zein-glycerol mixtures at 125°C. Extruders were also used to mix the soy-glycerol heated (135°C) in a single-screw extruder set at 70 rpm. The addition of water during the compounding step was found to facilitate extrusion and tempering the extruded sheet on chilled rolls to cool the film rapidly resulting in higher quality films. Strantz and Zottola (1992) and Kim and Pometto (1994) tested the survival and growth of bacteria on meat packaged in PE with added corn starch due to concern over the corn starch supplying substrate for bacterial growth. These workers found that added starch, at levels of up to 28%, did not accelerate bacterial growth, impair color stability, or affect heat-sealing ability of the film. The mechanical properties of the PE film during refrigerated or frozen storage

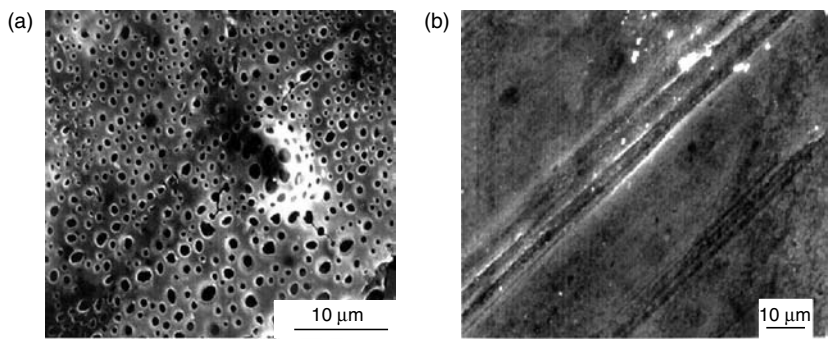

Fig. 17.3 (a) Cast corn zein film; (b) thermally compacted corn zein films.

were not affected by adding starch (Kim and Pometto, 1994). Strantz and Zottola (1992) also inoculated beef and bologna with three food pathogens (*S. typhimurium, Bacillus cereus, Staphylococcus aureus*) and reported that growth and survival were not affected by a 6% added-starch PE film. Both studies concluded that the addition of starch to PE film did not affect microbial growth or functionality of the film when used for meat packaging.

Proteins and polysaccharides generally are good oil, oxygen and aroma barriers but poor moisture barriers with a few exceptions. Waxes are good moisture barriers but tensile strength (TS) and flexibility are challenges for most biobased materials when compared with petroleum-based films. TS values for the soy films ranged from 0.8 to 5.0 MPa for soy protein-glycerol-water films depending upon the concentration of the components and the percentage elongation ranged from 6 to 123% (Cunningham *et al.,* 2000). These values for TS and percentage elongation with biobased films compare favorably made with other biopolymers but do not yet equal the physical properties of most PE films (Table 17.4). In general, protein films are good gas barriers at low humidity but permeability increases with increases in relative humidity (McHugh and Krochta, 1994). The properties of these films have been summarized in several references (Guilbert, 1986; Gennadios and Weller, 1990; McHugh and Krochta, 1994).

The use of biocides in combination with biobased packaging is an environmentally friendly approach to shelf-life extension for meat products which combines bio-friendly packaging with natural antimicrobials. The ability to deliver an antimicrobial substance directly to the surface of a food product has obvious benefits. Research has demonstrated that microbial stability of food can be improved by maintaining high concentrations of preservatives at the food surface (Torres *et al.,* 1985). Numerous studies have reported the addition of antimicrobials to biobased coatings and films. For example, Meyer *et al.* (1959) added antibiotics to carrageenan, reducing bacteria on poultry meat by 99%. Organic acids (Siragusa and Dickson, 1992, 1993) and nisin (Cutter and Siragusa, 1996, 1997) added to calcium alginate were more effective than direct addition of the antimicrobials for reducing several pathogens on beef tissue. Nisin-impregnated calcium alginate was also used to reduce pathogens on pork (Fang and Lin, 1994). Potassium sorbate and

Table 17.4 Comparison of the physical properties of thermally compacted, solvent cast, and synthetic commercial films

Film type	Thickness (mm)	TS (MPa)	Percentage elongation
Heat-set soy protein	0.38	5.0	123
Cast wheat gluten	0.101	2.6	276
Cast whey protein-glycerol	0.11	13.9	31
Cast soy protein isolate	NA	37	4.0
Cast corn zein	0.089	0.4	<1.0
Cellophane	0.36	114	20
HDPE	0.025	17.3–34.6	300
LDPE	0.025	8.6–17.3	500

lactic acid were incorporated into an edible cornstarch film to inhibit the growth of pathogens on poultry meat (Baron and Sumner, 1994). Films using soy and corn proteins have been formed by heat extrusion to carry antimicrobials within their structure (Padgett, 1998). Creating films from natural plant materials by the heat-extrusion method enables films to act as carriers of antimicrobials to the food product surface (Dawson, 1998). Temperature and film type can influence the rate of release of substances from the film. Redl *et al.* (1996), found that temperatures from 4°C to 20°C did not cause morphological changes in sorbic acid diffusivity in edible wheat gluten film. In addition, the rate of diffusion of nisin from cast wheat gluten films and heat-pressed wheat gluten films has not been shown to be significantly different (Teerakarn *et al.,* 2002). In addition, the initial nisin concentration may be reduced as a result of the temperature used for heat-pressed formed films, as nisin can be inactivated by prolonged exposure to high temperatures. Hoffman *et al.* (2001) verified this by showing that there is a loss of nisin activity at 149°C in non-aqueous systems. The effect of exposure temperature and nisin release was studied by Dawson *et al.* (2003), and it was revealed that as temperature increases so does rate of nisin release. This experimentation also demonstrated that more nisin migrated from heat-pressed wheat-gluten film than from corn zein film (Dawson *et al.,* 2003). This phenomenon is possibly explained by electron microscopy work that revealed that heat-pressed films have a more polymerized and compressed surface as opposed to the porous surface of cast films.

Nisin and lysozyme, in combination with ethylene diamine tetraacetic acid (EDTA), when incorporated into the structure of soy and corn protein films inhibit the growth of selected strains of Gram-positive and Gram-negative bacteria (Padgett *et al.,* 1995). Nisin has also been incorporated into protein films and PE films and found to retain its antimicrobial activity (Hoffman *et al.,* 1997). Three to 4 log reductions in *Listeria monocytogenes* (Dawson, 1998) and 2-3 log reductions in *E. coli* (Padgett, 1998) were found when the bacteria were exposed directly to the film. The addition of combinations of antimicrobial compounds to packaging films was shown to provide inhibition against *Listeria* and *E. coli* species (Figs. 17.4a and 17.4b) (Hoffman *et al.,* 1997). The combinations of EDTA with nisin or with lauric acid or EDTA-lauric acid-nisin inhibited the growth of *E. coli* (Fig. 17.4b) while EDTA with lauric acid or EDTA-lauric acid-nisin also effectively inhibited *Salmonella enteritidis* (not shown). *Listeria monocytogenes* (Fig. 17.4a) was completely eliminated when exposed to films containing any combination of biocides that included lauric acid. The application of thermally compacted soy films containing 2.5% pure nisin (4%, wt/wt) to *L. monocytogenes* in a liquid medium suppressed cell numbers 1 log CFU/mL after 2 h; however, cell numbers increased to 10^8/mL after 24 and 48 h at 22°C (Dawson *et al.,* 2002). Films containing lauric acid (8%) and nisin completely eliminated detectable cells from a 10^6/mL culture after 8-h exposure to the liquid medium (22°C). Refrigerated bologna exposed to control films increased by 0.5 log from 10^6 after 21 days at 4°C (Fig. 17.5) while nisin films reduced cell numbers on turkey bologna from 10^6 to 10^5 /mL after 21 days as did films containing both nisin and lauric acid. Films with lauric acid alone reduced *L. monocytogenes* culture from 10^6 /mL

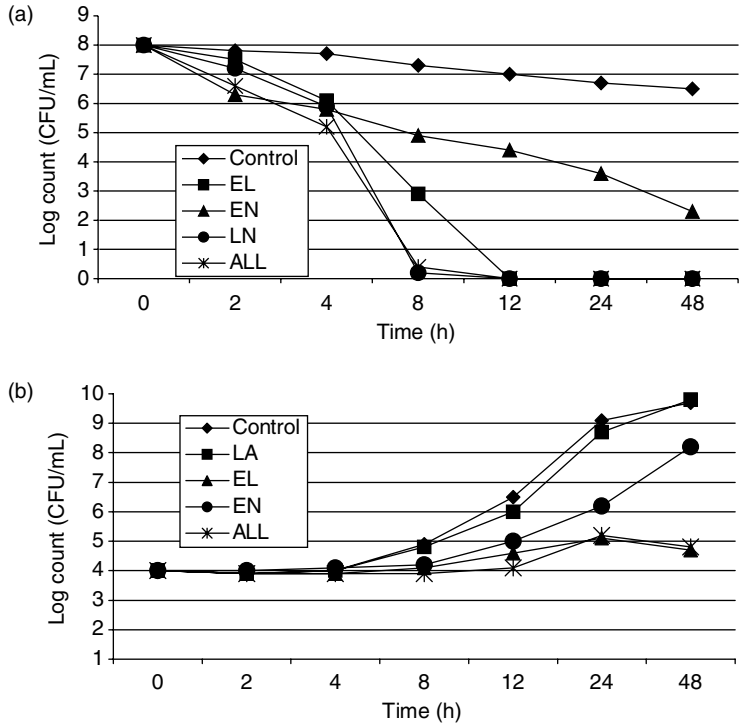

Fig. 17.4 Effects of lauric acid (LA), EDTA–lauric acid (EL), EDTA–nisin (EN), lauric acid–nisin (LN), and EDTA–lauric acid–nisin (ALL) in corn zein films on (a) *L. monocytogenes* and (b) *E. coli* (n = 6). CFU, colony forming units.

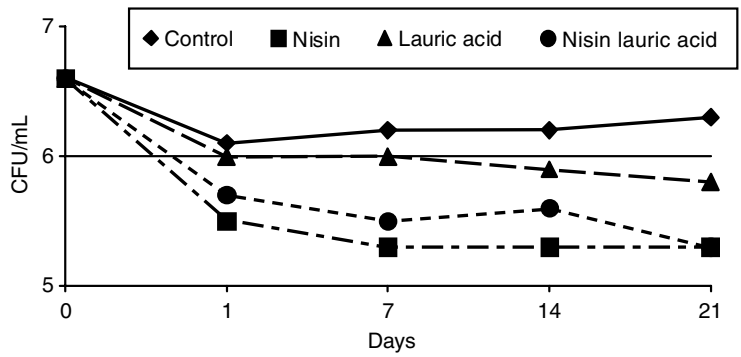

Fig. 17.5 *Listeria monocytogenes* (CFU/mL) on turkey bologna during exposure to biocide-impregnated packaging films at 4°C ± 2°C.

to $<10^2$ /mL after 48 h and by 1 log on turkey bologna after 21 days. McCormick *et al.* (2005) found that when wheat gluten films containing nisin were combined with pasteurization, *L. monocytogenes* populations were significantly reduced (5.7 log reduction) over the 8 weeks of storage. In a separate study, meat color was stabilized by exposing beef surfaces to antioxidant-impregnated corn zein films (Moore *et al.,* 2002). Commercial preparations of butylated hydroxy anisole (BHA), butylated hydroxy toluene (BHT), rosemary extract, and tocopherol were incorporated into zein films and then the films were placed on fresh cut beef surfaces then held in contact with an aerobic over-wrap synthetic film. The meat exposed to any film containing an anti-oxidant retained a redder color longer compared with meat not exposed to the protein-antioxidant film (Fig. 17.6). The BHA-impregnated corn zein film maintained color stability to the greatest extent compared with the other antioxidants tested. Subsequent migration tests revealed that BHA migrated at a faster rate than BHT in water and ethanol simulants, and this may theoretically explain the greater color stability provided by the BHA-treated film.

Nisin was incorporated into a methyl cellulose coating and was found to release slowly from the coating over time (Grower *et al.,* 2002). This is one advantage for biopolymers since many of them are water soluble and can be used as carriers of active components such as antimicrobial agents. Franklin and others (2004) tested a methyl cellulose coating containing four different levels of nisin and one not containing nisin as a control. The hot dogs were inoculated with a 5 log population of

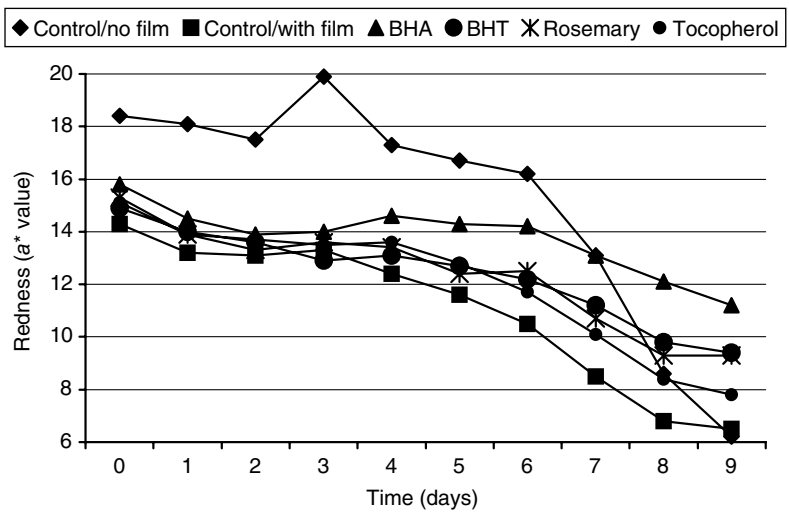

Fig. 17.6 Effect of antioxidant-impregnated corn zein films on the color stability of freshly cut beef tissue. Control/no film, control with no corn zein film; control/with film, control with a corn zein film; BHA, butylated hydroxy anisole added to corn zein; BHT, butylated hydroxy toluene added to corn zein; rosemary, rosemary extract added to corn zein; tocopherol, α-tocopherol added to corn zein.

L. monocytogenes and stored at refrigerated temperature for up to 60 days. It was determined that the nisin-containing methyl cellulose coating completely inhibited *L. monocytogenes* (Figs. 17.7a and 17.7b) for 60 days when 7500 IU/mL nisin was used and for up to 28 days when the coating contained 2500 IU/mL.

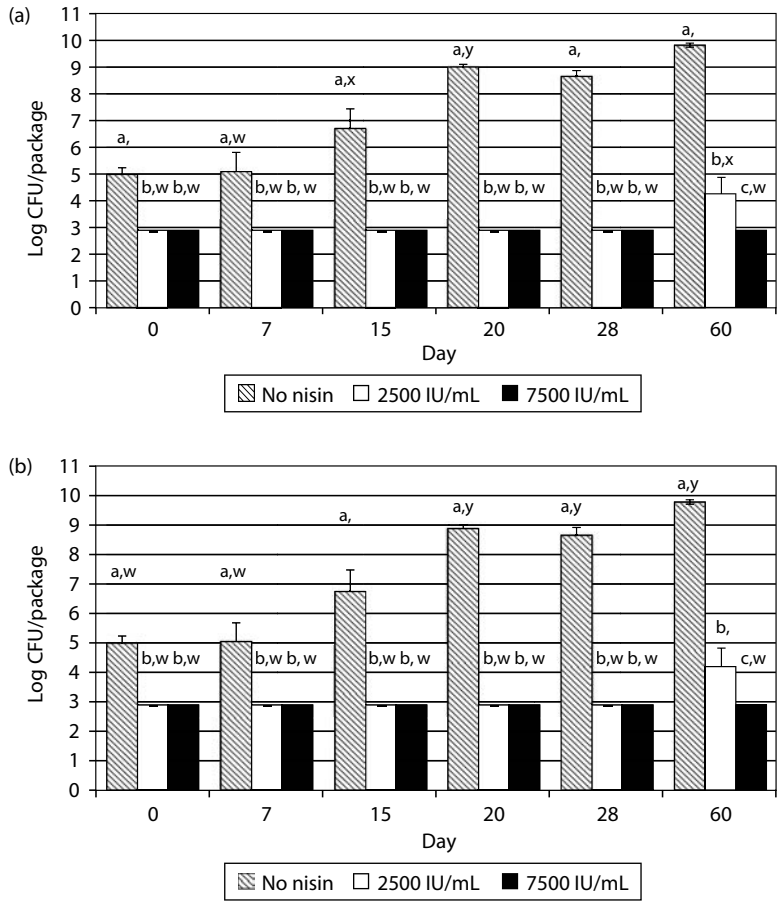

Fig. 17.7 (a) *Listeria monocytogenes* (five-strain cocktail) populations on the surface of hot dogs packaged in film coated with methyl cellulose–hydroxypropyl methyl cellulose solutions containing 7500 or 2500 IU/mL of nisin, or no nisin (control) when enumerated on (a) tryptic soy agar or (b) modified Oxford agar. Populations on hot dogs packaged in film coated with 7500 IU/mL were below the detectable limit (<2.9 log CFU/package) throughout the study. Populations on hot dogs packaged in film coated with 2500 IU/mL were below the detectable limit until day 60. a–c, columns with different letters show significant ($p < 0.05$) differences between nisin treatments (no nisin, 2500 IU/mL and 7500 IU/mL) for each day of storage. w–y, columns with different letters show significant ($p < 0.05$) differences between storage day for each treatment.

17.6 Future trends

Future applications of biopolymer packaging materials are likely to be divided into two groups: (a) coatings that are closely associated with the meat products and which may in fact be consumed with the food; and (2) flexible films/composites that replace current petroleum-based flexible films and containers. Containers produced from potato processing waste are currently being tested as clamshell containers for quick-service restaurants. New or redesigned packaging equipment is being developed to utilize biobased films, for example Polypack in collaboration with Plastic Suppliers, Inc., Earth-First® PLA film, made with Nature Works® PLA resin has developed a series of shrink packaging machines capable of running Earth-First® PLA film. Other advances include the development of blowing agents that can be used for thermoformed packaging based on PP, PS, and PET in applications such as egg cartons, meat trays, fast-food containers. Adeka Palmarole (www.adeka-palmarole.com) has developed a new generation of environmentally friendly blowing agents approved for the manufacture of food-contact PS, PET, or PP foam containers.

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18

Antimicrobial and antioxidant active packaging for meat and poultry

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Abstract: Over the past decades, the security and safety of foods have been major concerns. Increasing consumer demands for ready-to-eat and minimally processed foods containing fewer synthetic additives pose challenges to food technologists as formulating a product of this kind which is safe and has an adequate shelf-life is not straightforward. Developments in food preservation methods are required. There is also increasing interest in the use of packaging made from renewable polymers. Active packaging materials containing natural bioactives and based on renewable resources could help to reduce microbial growth and the oxidative reactions in meat-based products while at the same time, reducing the environmental impact of the packaging. The chapter first outlines meat safety and quality concerns and then describes different approaches to elaborating bioactive packaging materials. Advances in the applications of antioxidant and antimicrobial biopackaging to meat and poultry products are then reviewed. Finally, future trends such as release-on-demand bioactive agents are discussed.

Key words: antioxidant and antimicrobial materials, active biopackaging, preservation of meat products.

18.1 Introduction

Food-borne disease has always topped the list of food safety concerns for most governments around the world (Jones, 2002; Wallace *et al.*, 2000) and meat contaminated with pathogenic bacteria has been implicated in some of the most serious food-borne outbreaks (Sofos, 2008). Aside from meat safety considerations, contamination of meat products by spoilage micro-organisms also leads to significant economic losses (Wolffs and Radstrom, 2006). Another major concern in the meat industry is product sensory quality. Oxidative reactions are a major factor

responsible for reducing the shelf-life of perishable foods, including meat products (Lee *et al.*, 2004), and strategies to reduce oxidative deterioration are required.

Stricter requirements regarding consumer safety are spurring the development of new approaches to and strategies for food preservation in the food and packaging industries (Miltz *et al.*, 2006). Changes in consumer preferences in recent years, such as the growth in the chilled ready-meals market and increase in consumer demand for safe, minimally processed, preservative-free products, have also led to innovations and developments in new packaging technologies, especially active packaging (Llewellyn, 2003). Active packaging is packaging which performs some desired role in food preservation other than providing an inert barrier to external conditions. According to Kerry (2006), the active packaging technologies are anticipated to grow significantly in importance over the next years, due notably to:

- consumer demands for meat and other food products, which are premium quality
- greater demands by retailing outlets for extended product shelf-life
- concerns regarding product authenticity
- growing efforts to reduce unnecessary product/package waste.

Consumers are also increasingly seeking foods containing natural, rather than synthetic additives, so much research has focused on the incorporation of natural compounds such as essential oils from herbs and spices in active packaging. Natural preservatives of this kind are referred to as 'bioactives' in this chapter. Antimicrobial packaging and antioxidant packaging in particular have attracted much attention from the food industry because of their potential as one aspect of preservation strategies for minimally processed food products. A greater emphasis on quality and safety features associated with the addition of bioactive agents in packaging is perhaps one of the next big areas of development in packaging technology (Cha and Manjeet, 2004). Regulation, notably in Europe, is also expected to authorize the use of active and intelligent packaging, provided the packaging can be shown to enhance the safety, quality and shelf-life of the packaged foods (De Jong *et al.*, 2005).

Renewed interest in food packages composed of natural biopolymers has started to emerge in recent years due to concerns about the environment and the requirement to reduce the amount of disposable, non-degradable, petroleum-based conventional packaging materials. Such packaging is referred to as 'biopackaging' in this chapter. Biomass is a naturally abundant source of sustainable biopolymers; in the last few years, increasing environmental awareness has led to growing interest in the development of green compounds with improved performance. Biopolymer films and coatings, which act as a barrier to external elements (bacteria, moisture, oil, gases, volatile organic compounds, etc.), thus protecting food products and extending shelf lives, can be manufactured from pure forms of biological materials such as polysaccharides, proteins, lipids and associated derivatives (Arvanitoyannis, 1999; Petersen *et al.*, 1999; Tharanathan, 2003).

The sections below will not review the whole range of active packaging systems for meat. Instead, they focus on meat product preservation using bioactive biopackaging for meat and poultry preservation. This chapter starts by addressing the question of meat safety and quality concerns. Then, it surveys the range of antimicrobial packaging elaboration processes and this is then followed by a discussion of advances in applications of active biopackaging to preserve meat products. As a conclusion, directions for future research or development are suggested.

18.2 Meat safety and quality concerns

The following sections consider in detail the potential problems in terms of health hazards in packaged meat products, particularly as they are stored for more extended periods.

18.2.1 Meat and food-borne disease

The actual incidence of food-borne disease is unknown, even in countries with fairly sophisticated monitoring systems, because the number of cases is severely under-reported. Recent estimates from the Center for Disease Control and Prevention (CDC) in the United States suggest that there are 76 million illnesses, 325 000 hospitalizations and 5000 deaths each year from food-borne disease. Surprisingly, the actual pathogen responsible for the illness is identified in less than 20% of all cases. As a result, known pathogens account for an estimated 14 million illnesses, 60 000 hospitalizations and 1800 deaths (Table 18.1).

The reported number of food-borne zoonoses and intoxications is still increasing and the frequency of reported outbreaks has also grown, in part due to the success of new detection technologies and better epidemiological surveillance programmes (Aymerich *et al.*, 2008). According to Jones (2002), the increased incidence of all types of viral, bacterial and parasitic infections is also due to changes in consumer consumption patterns. People, in many Western nations, buy more pre-prepared, pre-packaged foods, demand out-of-season and exotic foods from all around the

Table 18.1 Impact of food-borne disease, estimates from the Center for Disease Control and Prevention (CDC) in the United States

Estimates from food-borne disease	Number of cases per year
Total illnesses	76 million
Identified pathogenic micro-organism	*14 million*
Total hospitalizations	325 000
Identified pathogenic micro-organism	*60 000*
Total deaths	5000
Identified pathogenic micro-organism	*1800*

Source: Jones (2002).

Table 18.2 Some factors affecting the incidence of food-borne disease

	Microbial disease outbreaks (%)
Food service establishments	77
Homes	20
Food processing plants	3

Source: Jones (2002).

globe, utilize new technologies such as modified atmosphere, demand food with less salt and fat, and use food services and delis more often. Coupled with these changes, consumers also desire fewer additives that might slow microbial growth.

Another factor increasing the incidence of food-borne disease is an increase in vulnerable populations: the very young, the very old, the chronically ill and the immuno-compromised. The lack of knowledge about food preparation and storage also plays an important role. Results of a CDC study traced 77% of the microbial disease outbreaks to food service establishments, 20% to homes and 3% to food-processing plants (Table 18.2). As a result, while increased food safety programs such as HACCP (Hazard Analysis Critical Control Point) have obviously had a positive impact in food processing plants, there needs to be a plan to reduce problems in food service and an education program to inform consumers about safe food handling and preparation in the home (Jones, 2002).

Salmonella, *Listeria* and *Toxoplasma* account for 75% of the cases of food-related diseases and are responsible for 1500 deaths each year, in the United States. *Campylobacter jejuni*, *Staphylococcus aureus*, *Clostridium perfringens* and *botulinum* and pathogenic strains of *Escherichia coli* (e.g., enterohemorrhagic strain), as well as some parasites, are also problems and are in the news often for their potential to cause very large or lethal outbreaks **(**Jones, 2002). There are some other minor microbial hazards that have to be taken into account – for example, *Yersinia enteroliticca* bacteria-forming biogenic amines, and also molds producing mycotoxins (Aymerich *et al.*, 2006**)**. Salmonellosis and listeriosis represent 31% and 28% of total food-related deaths, respectively. These figures contrast remarkably when compared to food-related deaths associated with microbial pathogens such as *Campylobacter*, *E. coli* and *S. aureus* which represent 5%, 4.3% and 0.8% of fatalities (Aymerich *et al*., 2006; Mead *et al*., 1999). It seems that *Listeria monocytogenes* can be one of the most challenging pathogens to control with its tendency to hide in the food-processing environment and threaten by post-processing contamination. This pathogen can cause severe illness. The human population most at risk from this disease is primarily pregnant women, newborn babies and adults with weakened immune systems.

In addition, it is important to note that sometimes the acute effects of food-borne disease do not end in two or three days. Several significant food-borne pathogens are capable of triggering chronic disease, and even permanent tissue or organ destruction, probably via immune mechanisms. Arthritis, inflammatory bowel disease, haemolytic uremic syndrome and possibly several autoimmune disorders can be triggered by food-borne pathogens or their associated toxins. As

a result, research is needed to more fully understand the mechanisms by which the immune system is inappropriately activated by these common food-borne disease-causing agents (Jones, 2002).

According to Ahn *et al.* (2006), meat is one of the major sources of pathogens that cause food-borne illness in humans. Bacterial enteric pathogens contribute significantly to government's concerns about food safety and food-producing animals (cattle, pigs, chickens and turkeys) are seen as the major sources for many of these pathogens. Meat products are, in particular, a major source *of L. monocytogenes*. It seems that this strain is the pathogen of concern in ready-to-eat meat and poultry products (Gomes *et al.*, 2009). Opportunity for contamination with pathogens exists at every stage in the food chain. Intervention strategies for pathogens in meat can be divided into pre-harvest reduction of micro-organisms in livestock and post-harvest decontamination of carcasses and meat. Post-harvest interventions are traditionally used to decontaminate meat. They involve various physical and chemical methods, or a combination of these methods.

18.2.2 Oxidation in meat and antioxidant agents

The chemical composition of fresh meat is as follows: 60–85% water, 8–23% protein, 2–15% lipids, 0.5–1.5% saccharides and about 1% inorganic substances. These quantities change significantly depending on the type, age, sex, level of fattening and part of the animal carcass (Palka, 2002**)**. The largest fluctuations are observed in the contents of water and lipids (Table 18.3). A variety of unsaturated fatty acids (FAs) occur naturally in large quantities. The most abundant monoenic acid in animal fats is oleic acid (C18:1), such as in beef tallow and lard (Table 18.4). The geometry of double bonds, as well as the number of double bonds, determines the reactivity of unsaturated FAs. A *trans*-linkage produces less irregularity in the

Table 18.3 Chemical composition of meat

Product	Water (%)	Lipids (%)	Crude Protein (N × 6,25 (%))
Beef, lean	72	7	21
Pork, lean	72	7	20
Veal	75	4	20
Lamb	72	7	20
Chicken:Light meatDark meat	7576	25	2320

Source: Adapted from Palka (2002).

Table 18.4 Distribution of fatty acid in some animal fats

	C14:0	C16:0	C18:0	C18:1	C18:2	C18:3
Beef tallow	3	26	22	42	2	0,2
Chicken	1	22	10	41	20	2
Lard	1,3	25	16	46	9	0,3

Source: Adapted from Chu and Hwang (2002).

straight-chain structure; thus the *trans*-FAs usually have a higher melting point and are less reactive. Most naturally occurring FAs are *cis* forms; *cis* acids may be converted to *trans*-isomers in the course of processing, involving heat – for example, in the cases of meat-based products (Chu and Hwang, 2002).

The lipid content of some meat products is particularly significant, leading to a particular susceptibility to lipid oxidation. Moreover, as reported by Nabrzyski (2002), because minerals are an integral part of many enzymes, they play an important role in food processing and notably in meat aging. Some heavy metal ions actively catalyze lipid oxidation. Their presence, even in trace amounts, has long been recognized as potentially detrimental, particularly to the shelf-life of fats and fatty foods. They can activate molecular oxygen by producing superoxide, which then, through dismutation and other steps of biochemical changes, turns into hydroxyl free radicals. Hydroxyl radicals can initiate lipid oxidation.

Lipids undergo auto-oxidative degradation during storage. The oxidative stability of lipids in foods depends on several factors, including the degree of unsaturation, the nature of unsaturation (position of double bonds), antioxidant content (synthetic antioxidants, tocopherols, etc.), pro-oxidant content (trace of metals and enzymes) and storage conditions such as exposure to heat, light, oxygen and moisture (Chu and Hwang, 2002). The higher the storage temperature of the lipids, the faster the rate of lipid oxidation. Light, particularly ultraviolet light, also has a significant effect on oxidation rates. Light-induced oxidation of oleic acid occurs about 30 000 times faster than auto-oxidation. For the fat itself, the greater the degree of unsaturation of the FA residues, the higher the rate of oxidation. Lipid oxidation leads to the formation of hydroperoxides, which are very unstable and decompose to form secondary reaction products, such as aldehydes, ketones, alcohols, acids and hydrocarbons, which are described as off-odor and off-flavor substances. During the initial or induction phase, oxidation proceeds at a relatively low rate. Peroxides are formed during this period. After a certain critical amount of oxidation has occurred, the reaction enters a second phase. The samples begin to smell and taste rancid at the beginning or early second phase. As fat oxidation continues, the peroxides decompose to generate volatile and non-volatile secondary products (Chu and Hwang, 2002).

Development of rancidity and warmed-over flavor, a specific defect that occurs in cooked and reheated meat products following short-term refrigerated storage, has been directly linked to auto-oxidation of highly unsaturated membrane-bound phospholipids and to the catalytic properties of non-haeme iron. The secondary oxidation products, mainly aldehydes, are the major contributors to meat flavor deterioration because of their high reactivity and low flavor thresholds. Ketones and alcohols have a high flavor threshold, thus, causing off-flavor less often (Nabrzyski, 2002).

18.3 Active packaging based on biopolymers and natural bioactives

The following sections investigate packaging that does more than simply provide a physical barrier between the food product and the external environment.

18.3.1 Introduction to active packaging

Active food packaging can take several forms (Coma, 2006). Three categories of antimicrobial packaging can be observed Coma (2008), based around:

1. direct incorporation of bioactive agents into the package matrix, leading to a bioactive agent being merely dispersed in the matrix
2. the utilization of inherently bioactive polymers with film-forming properties or a polymer which was chemically modified resulting in bioactive properties
3. the surface modification of packaging – for example, by coating.

To simplify matters, these three categories can be condensed into two primary approaches:

Approach 1: active packaging based on a globally bioactive matrix
Approach 2: active packaging based on a material with a bioactive surface

In the first approach (Fig. 18.1), the bioactive agent is simply associated with the polymeric matrix. The agents may be added, for example, to the extruder when the film or co-extrusion is produced. High temperatures and extruder shear can cause deterioration in the performance of the additives. Another technique is based on the chemical grafting of a bioactive agent onto a polymer involved in the structure of the material. Finally, we can also use an inherently bioactive polymer in the packaging formulation.

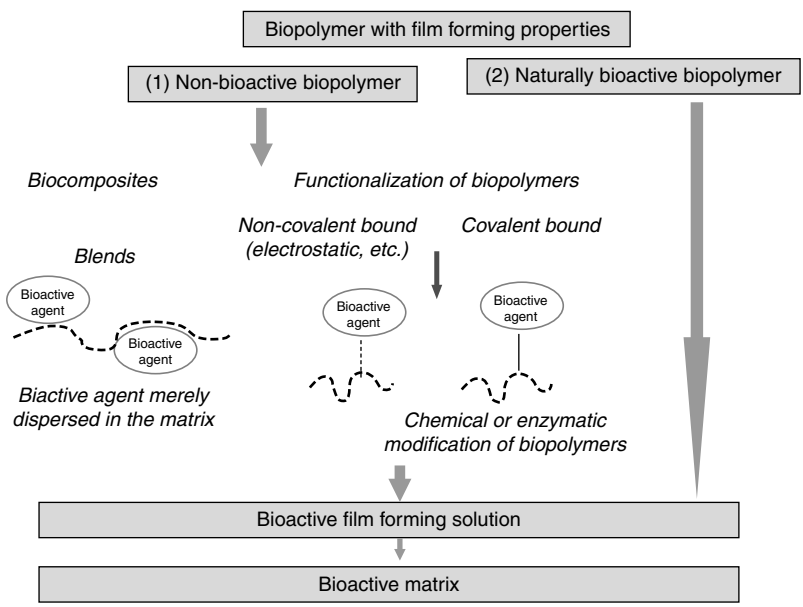

Fig. 18.1 Processes for the elaboration of bioactive matrices.

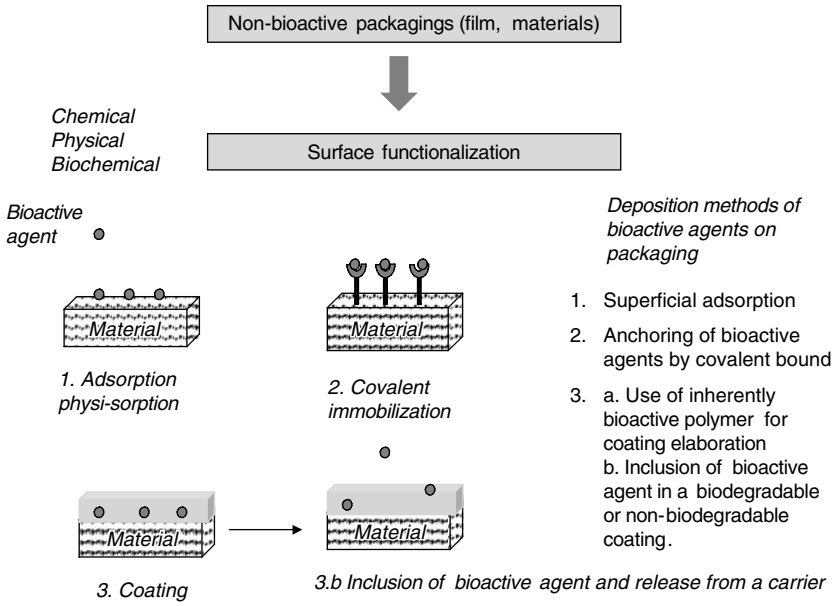

Fig. 18.2 Surface modifications of materials to elaborated bioactive packages.

In approach 2 (Fig. 18.2), (i) superficial adsorption or (ii) superficial grafting by covalent bonding of the bioactive agent to the packaging materials can be employed. Alternatively, the bioactive ingredient can be included in coatings applied to the film surface (another approach to grafting as described above in (iii)). The advantages of applying coatings off-line are the controlled manner of application without the exposure to excessive heat and the ability to apply coatings at a later date, thus minimizing contamination risks.

All of the options described above involve packaging films. An alternative option, which will not be addressed in this chapter, is to include a sachet of an active agent – for example, an antimicrobial agent – within a pack. This has been considered, for example, for the control of pathogens in meat trays (Llewellyn, 2003**)**. Of the techniques mentioned above, the film or coating technique is considered to be the most effective, although more complicated to apply (Cha and Manjeet, 2004).

When designing an active package, issues that are of importance when designing traditional food packages, such as barrier properties to gases and moisture and the mechanical strength required for pack integrity, must still be taken into account. Then the following issues, which are specific to the antimicrobial and antioxidant function of the packages, must principally be considered (Llewellyn, 2003):

- the chemical nature of the bioactive agents and their inhibition mechanism
- physico-chemical characteristics of foods and the organoleptic property of the bioactive agents

- packaging manufacturing processes and their influence on the efficiency of bioactive additives
- storage environments
- migration mechanisms of bioactive agents into foods if needed, and toxicity and regulatory issues
- machinability and processability of the bioactive packaging on the packaging line materials.

Moreover, in the case of antimicrobial matrices, we must also consider the microflora of foods and the physiology of the target micro-organisms.

Bioactive packaging films are generally of two types (Fig. 18.3): films containing a bioactive agent, which migrates to the food surface, and films which are effective without migration. Films in which the agent migrates from the film or coated-film surface are generally more suitable for food preservation, and notably for antimicrobial or antioxidative action. They are effective preservative-release systems, releasing into the pack contents only the amount required to prevent microbial growth or oxidative reactions, thus reducing preservative intake by the consumer. Of course, a sophisticated mechanism is required to ensure that neither too little nor too much preservative is released into the pack, and this represents a major technological challenge for scientists (Lowry, 2007). According to Llewellyn (2003), films or coatings in which the agent remains are in general of more interest in products such as medical gowns, pillows and other medical items,

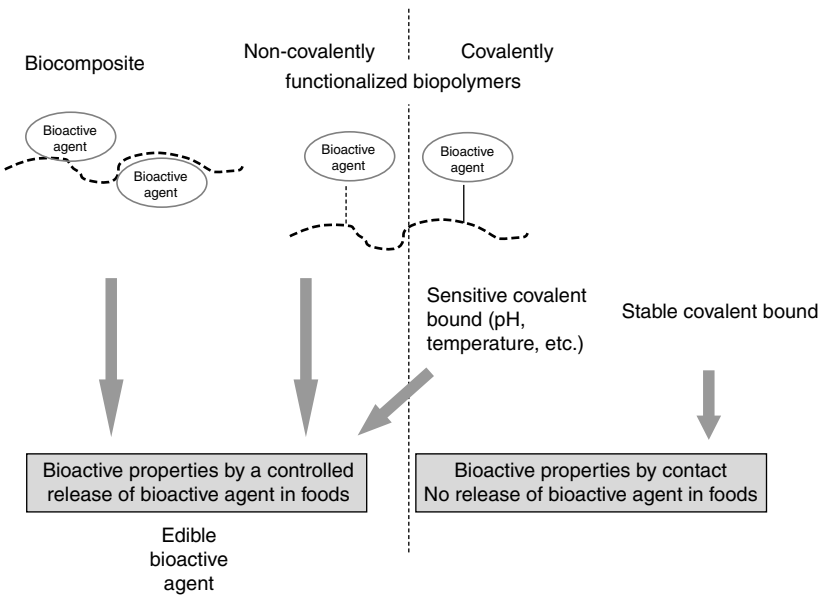

Fig. 18.3 The categories of bioactive packaging materials: bioactive properties with or without the release of bioactive agent.

rather than foods. Nevertheless, materials targeting surface micro-organisms are suitable for use in the packaging of bread, cheese, meat/poultry products and other items in which microbial growth tends to concentrate on the product's surface.

Antimicrobial or antioxidant components incorporated, and intended to be released, into the food (directly or via the headspace) must be approved as food additives and evidence of their effectiveness will need to be produced. Any claim, which is made for the active system also needs to be proven (De Jong *et al*., 2005). Systems in which the antimicrobials do not migrate out of the packaging film into the pack's contents represent a less controversial solution and are far less likely to raise concerns over additive levels in foods (Lowry, 2007). Edible films have also been manufactured and applied to food products. These edible films function as oil, oxygen, moisture and aroma barriers, but can also be used to provide additional nutrients or quality-enhancing ingredients such as antioxidants or antimicrobials (Jung *et al.*, 2006).

18.3.2 Active biopackaging

Numerous active packaging materials for food preservation have been previously described in the literature. For example, some current commercial antimicrobial packaging is based on CO_2, ethanol or chlorine dioxide generators. Other systems, such as Silver zeolite, have also been proposed and are gaining in popularity. The Ethicap sachet (Freund, Japan) can be used as a source of ethanol vapor, in addition; however, in general more development in active packaging using ethanol-generators has taken place in bakery packaging (MAP), than meat packaging, due to the anti-fungal activity. Commercial antioxidant systems can be based on oxygen scavengers (Coma, 2006). This chapter focuses specifically on antimicrobial and antioxidant bioactive biopackaging, however, rather than reviewing the whole range of antimicrobial and antioxidant packaging techniques in use and development today.

Until now, primarily synthetic active agents have been used commercially for the preservation of food products using active packaging. However, because the protection afforded by an antimicrobial compound is the result of a chemical reaction between the preservative and the micro-organisms, it may be expected that these compounds show some reactivity toward food components as well. For example, nitrite, an antimicrobial frequently used in foods, including meat products, has been recognized as a potent inhibitor of micro-organisms, including pathogens such as *C. botulinum*. The active species is nitric oxide. There is some evidence that inhibition of *C. botulinum* outgrowth in nitrite-cured meat products is mainly due to iron binding in such a manner that this is no longer available for outgrowth of Clostridium spores (Ruiter and Voragen, 2002**)**. This strong binding also explains the antioxidative properties of nitrite in these products. Despite all of its desirable effects in processed meat products, however, nitrite is a source of concern due to its role under certain conditions, in the formation of N-nitrosamines at trace quantities (i.e., parts-per-billion levels). Typical volatile N-nitrosamines detected in cured products after heat processing include N-nitrosodimethylamine

and N-nitrosopyrrolidibe, known to be carcinogenic, mutagenic and teratogenic in animals (Tricker and Preussman, 1991). Although the carcinogenicity of N-nitrosamines in humans cannot be tested, epidemiological studies have suggested a possible link to the incidence of various cancers in humans (Pegg and Shahidi, 2006). For this reason, alternatives to nitrite are being sought.

Also, the public tends to believe that the use of synthetic additives, including antimicrobials, may cause side-effects and there is a continuing demand from the consumer point of view, for 'natural' additives (Castellano *et al.*, 2008; Miltz *et al.*, 2006). However, 'natural' does not necessarily mean 'non-toxic' from a health perspective in every case. 'Natural' compounds also need to be processed, purified and possibly then modified in order to remove accompanying substances that have no significance in the final product and to enhance functionality. Generally speaking, purification is more difficult and more complicated for 'natural' additives, as it is also much more problematic to characterize the raw material, which may contain a great many ill-defined compounds whose toxicities are largely unknown. As a result, natural additives must be used with care and efforts must be made to ensure they are safe.

Polymers from renewable resources have been attracting ever-increasing attention over the past two decades, predominantly for two reasons: environmental concerns and the realization that our petroleum resources are finite. In addition, polymers from renewable resources will provide additional income to those involved in agriculture (Yu and Chen, 2009). It is, however, important to note that serious ethical issues surround this practice. For example, in the case of crop-based materials, the development of monocultures of often non-native polymer crops have potential implications for biodiversity, and are associated with competition for agricultural land use (Cruz-Romero and Kerry, 2008). According to the same authors, we have also to take into account the total ecological benefits of such biopackaging, which can be analyzed only by complete life-cycle analysis. Indeed, we have to consider the whole range of impacts, both direct and indirect, including, for example, parameters connected with defined applications or distribution systems. Be that as it may, nature can provide an impressive array of polymers that can be used in fibers, adhesives, coatings, gels, foams, films, thermoplastics and thermoset resins. Modern technologies provide powerful tools to elucidate microstructures at different levels and to understand the relationships between structures and properties. These new levels of understanding bring opportunities to develop new materials for new applications.

18.4 Antimicrobial bioactive biopackaging

Antimicrobials traditionally added to foods include nitrite (mentioned in Section 18.3.2 above) and carboxylic acids (sorbic, benzoic, propionic, etc.). Sorbic acid inactivates several intracellular enzymes (Ruiter and Voragen, 2002**)**. It passes the cell wall in its undissociated form only, which explains its low activity at higher pH values (PKa 4.76 at 25°C). It is mainly active against yeasts, molds and

strictly aerobic bacteria. As in the case of sorbic acid, benzoic acid penetrates the cell wall in undissociated form. As a consequence, it is active at lower pH values only (pKa 4.19 at 25°C). The main cause of its activity is biochemical effects such as the inhibition of oxidative phosphorylation and of enzymes from the citric acid cycle. Benzoic acid is mainly active against yeast and molds, but the growth of micrococci, *E. coli* and many other bacteria is retarded as well (Ruiter and Voragen, 2002**)**.

It is clear that traditional methods of preservation will not totally preclude the presence microbial hazards in foods. Moreover, consumers continue to demand products that are more natural or mildly processed, are safer and more stable, have a longer shelf-life, and do not contain chemical preservatives (Aymerich *et al.*, 2006). Therefore, there is a trend toward the use of a combination of preservation methods to produce safe foods that are attractive to consumers and have an acceptable shelf-life. The combination could include a biopreservation technique, a new post-processing technology (high hydrostatic pressure, irradiation, etc.), and active packaging based on natural active ingredients. All of these methods have received increasing attention in recent years. It is important to note that the use of antimicrobial packaging is not meant to be a substitute for good handling practices, but it should enhance the safety of food as an additional impediment to the growth of pathogenic and/or spoilage micro-organisms (Cooksey, 2005).

A variety of natural antimicrobial molecules, such as bacteriocins, enzymes and chelatants, organic acids – for example, propionic and sorbic acids – and spice extracts (Drosinos *et al.*, 2009), included in different films, have been tested to suppress pathogenic or saprophytic bacterial growth in relation to meat product preservation (Table 18.5). For these systems, contact between the food and the package is obviously necessary and a migration process from the packaging materials to the product is generally expected. Antimicrobial packaging systems for potential applications in fresh meat or processed meats can both inhibit microbial growth on non-sterilized foods and maintain the sterility of pasteurized foods, thus preventing post-contamination (Llewellyn, 2003).

18.4.1 Bacteriocin delivery materials

Bacteriocins are antibacterial peptides produced by lactic acid bacteria. These agents are generally heat-stable, apparently hypoallergenic and readily degraded by proteolytic enzymes in the human intestinal tract. The incorporation of bacteriocins into packaging materials to control spoilage and pathogenic bacteria has been an area of active research for some time. Inhibition of the growth of *L. monocytogenes* and other pathogenic strains potentially found in meat products has been studied in some depth (Coma *et al.*, 2001).

In MAP-packed and refrigerated sliced cooked ham, nisin-adsorbed films were able to reduce *Listeria innocua* counts by 1.5 log CFU/g after 12 days (starting inocula of 3.105 CFU/g) and *S. aureus* was reduced by 2.8 log CFU/g (starting inocula of 3.105 CFU/g (Scannell *et al.*, 2000). Natrajan and Sheldon (2000a, 2000b) reported the efficacy of films containing nisin and varying concentrations

Table 18.5 Selection of bioactive compounds directly incorporated into the packaging for potential applications of meat preservation

Active component	Biopolymer carrier	Food – Target strain	References
Bacteriocin			
Enterocin[a]	Cellulose, meat batter		Huga *et al.* (2002)
Nisin[b]	Cellulose films	Ham	Scannell *et al.* (2000)
	Agar/polysaccharide–based films	Poultry skin	Natrajan and Sheldon (2000a, 2000b)
	HPMC	Culture media – *Listeria monocytogenes*	Sebti and Coma (2002)
	Zein coating	Frankfurters	Lungu and Johnson (2005)
Lacticin[b]	Cellulose film	Ham	Ming *et al.* (1997)
	Cellulose-based inserts	Slice cooked ham	Scannell *et al.* (2000)
Pediocin[b]	CelluloseCellulose casings	Cooked meatsFrankfuter sausages	Ming *et al.* (1997) Huga *et al.* (2002)
Essential oils			
Spanish oregano[a]	Alginate-based films	Ham	Oussalah *et al.* (2007)
Chinese cinnamon[a]	Chitosan-based films	Bologna	Chi *et al.* (2006)
OreganoOregano/pimento[a]	Milk protein-based films	Bologna	Oussalah *et al.* (2004)
Cilantro oil[a]	Gelatin	Whole beef muscle Ham	Gill *et al.* (2002)
Enzymes			
Glucose oxidase[b]	Alginate	Fish	Field *et al.* (1986)
Organic acids			
Lactic acid[b]	Alginate	Beef muscle	Siragusa and Dickson (1992)
Propionic acid[b]	Chitosan	Cooked ham	Ouattara *et al.* (2000)
Others			
Tocopherol[b]		Beef	Moore *et al.* (2000)

[a] Antimicrobial agents which have never been used in packaging applications. Some of them have just been discovered.
[b] Antimicrobial agents which have been recently used in packaging applications.

of citric acid, EDTA and tween 80 against *S. Typhimurium* in fresh boiled drumstick skin samples packed with protein and polysaccharide-based (calcium alginate and agar) films. When nisin was included in calcium alginate gels, *Salmonella* counts decreased by 1.98–3.01 log units after 72 h at 4°C. When nisin was included in agar films at 500 μg/mL, *Salmonella* counts were reduced by 1.8 log cycles (1.25% agar gel) and 4.6 log cycles (0.75% agar gels), respectively, after 72–96 h at 4°C.

Ming *et al.* (1997) studied the activity of another bacteriocin, pediocin, coated onto cellulose casings (9.30 mg/cm^2). It completely inhibited the growth of *L. monocytogenes* in beef, ham and turkey breasts during 12 weeks of storage at 4°C. Compared with non-treated samples, *L. monocytogenes* counts decreased by almost 2 log units in ham and beef, and by almost 3 log units in turkey breasts (Ming *et al.*, 1997). In another example, the bacteriocins enterocin A, sakacin K and two commercial biopreservatives based on nisin and pediocin, applied through cellulose casing in sausages, were able to delay the outgrowth of *L. monocytogenes* artificially contaminated at 1.7 log CFU/g, during six days of storage at 3.5°C. Enterocins and pediocin extended the antimicrobial effect until the 14th day of storage (Huga *et al.*, 2002). Also reported in the literature are the uses of nisin, pediocin AcH and enterocins A and B in meat and meat products (Aymerich *et al*., 2000; Gomes *et al*., 2009; Mangalassary *et al*., 2007).

Aasen *et al.* (2003) have demonstrated the influence of different critical factors on the efficiency of bacteriocins (i.e., nisin and sakarin) and the required dosage in foods. More than 80% of the added bacteriocin is adsorbed onto the muscle protein, but the activity of the protein-bound bacteriocin still requires assessment. Proteolytic activity causes degradation of sakacin P, and probably other bacteriocins in foods that have not been heat-treated, but the losses can be compensated for by increased dosages. Moreover, they are susceptible to adsorption onto food macromolecules and fat may inactivate the bacteriocin in food According to Roller *et al.* (2002), although bacteriocins have the potential to protect some foods from spoilage, their application in raw or processed meat products is limited because binding with meat particles and fat may cause loss of activity. Mauriello *et al.* (2005) reported that low pH favored the migration of the active compound from the film, and low temperature delayed the release of nisin from the film. They concluded that the release of nisin from the plastic film was unpredictable, but temperature- and pH-dependent.

18.4.2 Essential oil extract delivery materials

Antimicrobial agents may either migrate to the food through diffusion and partitioning or be released through evaporation in the headspace. Both of these mechanisms constitute potential applications of essential oils. The majority of studies on the use of essential oils in active packaging have focused on their application via edible protein-based coatings. Oussalah *et al.* (2007) demonstrated that, after five days of storage, alginate-based films containing oregano or cinnamon essential oils were particularly effective against the growth of *S. Typhimurium*. During the

same period, meat inoculated with *E. coli* O157:H7 and coated with films treated with 2% $CaCl_2$ had significantly fewer bacteria when oregano-based films were used than when cinnamon- and savory-based films were used. In another study, Oussalah *et al.* (2004) applied milk protein-based edible films containing 1% (w/v) oregano, 1% (w/v) pimento or a mixture of 1% oregano-pimento (1:1), on beef muscle slices to control the growth of pathogenic bacteria. Films containing oregano were the most effective against *E. coli* O157:H7 or *Pseudomonas* spp. For instance, a 1.12 log reduction of *E. coli* O157:H7 level was noted in samples coated with oregano-based film.

18.4.3 Chitosan

Other natural agents could be used in biopackaging to increase the food safety. Chitosan, a biopolymer that is produced by deacetylation of chitin, which is found in the exoskeleton of crustaceans, has antimicrobial activity. Studies have investigated its use both as the antimicrobial agent and in combination with other substances with antimicrobial activity. Chitosan-based matrices were developed to decrease the surface microbial contamination of some meat-based products, producing a killing-contact system, which is not based on the release of active agents. Chitosan had been reported to significantly inhibit various meat spoilage strains and pathogen micro-organisms (Coma, 2008). Chitosan-based films were also used as matrices allowing a slow release of active agents, since chitosan can be associated with antimicrobial polyphenolic substances (Coma *et al.*, 2010; Portes *et al.*, 2009). In this study, two tetrahydrocurcuminoids (Fig. 18.4), THC1 (5-hydroxy-1,7-bis(4-hydroxy-3-methoxyphenyl)hept-4-en-3-one) (prepared from natural curcumin extracted from turmeric roots (*Curcuma longa* L.)) and THC2 (5-hydroxy-1,7-bis(4-hydroxy-3,5-dimethoxyphenyl)hept-4-en-3-one) were synthesized and incorporated into a chitosan-based film. The antimicrobial properties of tetrahydrocurcuminoids against several Gram-positive bacteria (Venkateswarlu *et al.*, 2000) and several fungal strains, has attracted interest because these compounds may be able to offer protection against *L. monocytogenes* and some mycotoxinogen fungi. Anti-fungal and anti-mycotoxinogenic substances are of interest to decrease the growth of undesirable molds that produce mycotoxins – highly toxic secondary metabolites – which can be observed, for example, on some sausages. These polyphenolic compounds showed a very promising efficiency in

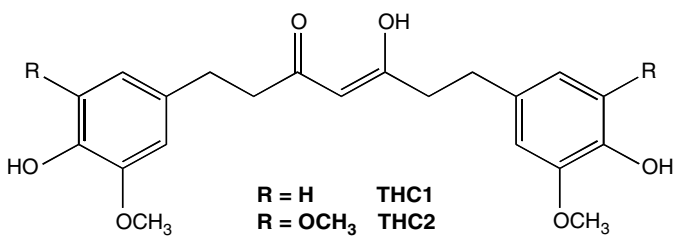

Fig. 18.4 THC1 and THC2 Tetrahydrocurcuminoïds studied by Portes *et al*. (2009).

the reduction of fungal growth and production of mycotoxins. Moreover, due to interaction between chitosan and THCs, a slow release of active compounds was observed (Coma *et al*., 2010).

To improve the preservation of vacuum-packaged processed meats during refrigerated storage, Ouattara *et al.* (2000) prepared antimicrobial films by incorporating acetic or propionic acid into a chitosan matrix, with or without addition of cinnamaldehyde. This type of biopackaging was applied onto bologna, regular cooked ham, or pastrami. Interestingly, differences between acetic and propionic acids with respect to their patterns of release from chitosan films were found. The chitosan films applied to processed meats were found to retain more of the incorporated acetic acid during storage when they contained lauric acid than when they did not. The authors suggested that hydrophobic molecules associated to a hydrophilic matrix can effectively reduce the acid diffusion. The growth of Enterobacteriaceae and *Serratia liquefaciens* was delayed or completely inhibited as a result of film application and strongest inhibition was observed with films containing cinnamaldehyde, as a result of its greater antimicrobial activity under the used conditions.

18.5 Antioxidant bioactive biopackaging

The addition of antioxidants is the most commonly used method of retarding lipid oxidation in fat. Antioxidants can be defined as 'substances that, when present in low concentrations compared to those of an oxidizable substrate, significantly delay or inhibit oxidation of this substrate' (Ruiter and Voragen, 2002). Some of the more popular synthetic antioxidants used are phenolic compounds – for example, butylated hydroxy-anisole (BHA), butylated hydroxy-toluene (BHT), mono-*tert*-butyl-hydroquinone (TBHQ) and propyl gallate (PG) (Chu and Hwang, 2002**)**. As reported by Ruiter and Voragen (2002), of these, TBHQ is by far the most potent antioxidant.

Many of the antioxidants present in food have the function of terminating chain reactions. A variety of compounds such as phenols, aromatic amines and conjugates can function as chain-breaking antioxidants. They react with the chain-propagating radical species, which results in the formation of radical species incapable of extracting hydrogen atoms from unsaturated lipids. The radical may rapidly combine with other radicals or, if a polyphenolic structure is present (e.g., gallic acid ester), it is either disproportionated to its original state or leads to a quinoid form. As mentioned previously, the presence of metals in fats greatly accelerates the oxidation process. Inactivation of the catalysis effect of these metals can be achieved by the use of a sequestering agent – for example, citric acid or EDTA (Chu and Hwang, 2002**)**. In the presence of ADP-chelated iron and traces of copper, oxygen radicals are generated in the sarcoplasmic reticulum of muscle food. Muscle contains notable amounts of iron, a known pro-oxidant, and trace amounts of copper, which can lead to an acceleration of the peroxidative reaction. Iron is a part of the active site of lipoxygenase, which may participate in lipid oxidation (Nabrzyski, 2002). Polyphosphates like

sodium tri-polyphosphate are excellent metal chelators and inhibitors against lipid oxidation. However, when added to raw meat, they are ineffective due to rapid hydrolysis to monophosphate by endogenous phosphatase enzyme (Lee *et al.*, 1998).Reductants such as ascorbic acid, which decrease the local concentration of oxygen, are also able to decrease the formation of peroxy radicals (Ruiter and Voragen, 2002**)**.

Dietary antioxidant treatments (i.e., the inclusion of antioxidants in animal feed) have been shown to stabilize lipids in membranes and reduce the extent of lipid oxidation in meat during storage, but antioxidant effects in meat can differ between muscle types (Ahn *et al.*, 2006; Morrissey *et al.*, 1997). This could be due to the fatty acid composition and endogenous pro-oxidative or antioxidative constituents, which are factors influencing lipid oxidation in raw meat products. Other factors can have an impact on meat oxidation, such as processing and storage conditions. Cooked meats are generally highly susceptible to oxidative rancidity. During cooking, lipids can generate volatile compounds that oxidize, producing off-odors and flavors. Moreover, fat oxidation can be initiated by bacteria. It can be suppressed by the addition of preservatives such as benzoic or sorbic acids (Ruiter and Voragen, 2002**)**. As a result, the use of additives having antimicrobial properties could have synergistic effects in killing micro-organisms and decreasing oxidation in meat. Finally, lipid oxidation can also be delayed or reduced by other means. One method is to reduce the concentration of oxygen in the fat by packing the products under vacuum or nitrogen (Chu and Hwang, 2002**)**. Packaging materials with antioxidant properties could be particularly efficient (Nabrzyski, 2002).

The commercial use of natural antioxidants such as rosemary oleoresin by the meat industry is growing because of consumer demands for natural products (producers such as Origanox by SCIOS – Auckland, New Zealand; Sabinsa Corp., East Windsor, USA). Different antioxidant agents, such as rosemary extract, tocopherol, ascorbic acid and different plant extracts may be successfully included in bio-based films, to decrease oxidative reactions in meat products (Table 18.6). Chitosan-based microcapsules (Kosaraju *et al*., 2006; Parize *et al.*, 2008) and chitosan-based films (Özmeric *et al.*, 2000), as well as other materials such as cellulose and starch, can be used as carriers for the antioxidants. Mathew and Abraham (2008) successfully incorporated ferulic acid into starch-chitosan blend films, and reported intermolecular interactions between the different components. Such ferulic acid-based films result in a reduced formation of lipid peroxide. According to Oussalah *et al.* (2004), oregano/milk protein-based edible films have potential to stabilize lipid oxidation in beef muscle samples. Different films were studied −1% (w/v) oregano, 1% (w/v) pimento or a mixture of 1% oregano-pimento (1:1) – and pimento-based films presented the highest antioxidant activity. Spizzirri *et al*. (2009) used gallic acid and catechin with gelatin as a carrier. The gelatin-conjugates demonstrated a good antioxidant activity, and this study confirmed that antioxidant moieties covalently bound to a natural polymer allowed for the introduction into the macromolecule, particular features for specific industrial applications such as active food packaging. Coatings exhibiting

Table 18.6 Selection of bio-based antioxidant compounds studied for meat preservation applications

Active component	Potential meat product	References
Rosemary extract[a]	Meat, pork sausage	Sebranek *et al.* (2005)
Fenugreek, rosemary and vitamin E[a]	Raw and diced cooked poultry breast meat	Armitage *et al.* (2002)
Tocopherols[a]	Mechanically deboned turkey meat	Mielnik *et al.* (2003)
Ascorbic acid[a]	Mechanically deboned turkey meat	Mielnik *et al.* (2003)
Carex distachya extracts (polyphenols)[b]	General food uses	Fiorentino *et al.* (2008)
Cinnamoyl derivatives[b]	General food uses	Fiorentino *et al.* (2008)
Ascorbic acid, cinnamon, cloves[b]	Meat, beef and pork	Jayathilakan *et al.* (2007)
Oriental non-culinary/ nutraceutical herb extracts[b]	Turkey breast rolls, Raw and cooked meat	Lee and Ahn (2005) Han and Rhee (2004)
Plum concentrates and powder[b]	Pre-cooked roast beef Turkey breast rolls	Nuñez de Gonzalez *et al.* (2008)
Extracts from several culinary herbs (sage, basil, thyme or ginger)[b]	Fresh chicken minced meat, and fresh and cooked pork patties	El-Alim *et al.* (1999)
Mediterranean plant[b]	General foods	Pacifico *et al.* (2008)
Carob fruit extracts (condensed tannins)[b]	Cooked pork	Bastida *et al.* (2009)

[a] Antioxidative agents which have been recently used in packaging applications.
[b] Antioxidative agents which have never been used in packaging applications. Some of them have just been discovered.

both antimicrobial and antioxidative properties appear very attractive and have already been developed by Lee *et al*. (2004) from a combination of nisin and α-tocopherol in a vinyl acetate-ethylene copolymer binder. In the same context, Portes *et al.* (2009) elaborated environmentally friendly films exhibiting both antioxidative and antibacterial properties from chitosan and curcumin derivatives. The resulting tetrahydrocurcuminoid-chitosan films exhibited a high free-radical scavenging activity against 2,2-diphenyl-1-picrylhydrazyl (DPPH) in methanol. Antioxidative properties of films resulted from a progressive release of curcumin derivatives, due to interactions between chitosan and antioxidative agents. The molecular nature of these interactions was ascertained using glucosamine, but the exact nature of the complex remains unelucidated. Chitosan retained its antimicrobial properties against the growth of listerial and Samonella strains when associated with antioxidative agents. Moreover, it was shown that chitosan dissolved in an aqueous solution exhibited antioxidative properties (Huang *et al.*, 2006; Kim and Thomas, 2007; Park *et al.*, 2004; Yen *et al.*, 2008), and bio-based packaging could be developed from this aminopolysaccharide to create new matrices exhibiting antioxidative properties, connected, for example, to a release of low molecular weight fragments in food.

18.6 Future trends

New antimicrobial and antioxidant packaging materials are continually being developed. One of the challenges in this area is the development of new bioactive non-toxic substances from natural resources. Another challenge is the development of new processes to release bioactive substances in foods. Two aspects related to the practical application of antimicrobials in general, and natural antimicrobials in particular, are hampering their wider use:

- interactions of these compounds with components in the food matrix and food ingredients – for example, proteins, fats, saturated fatty acids or NaCl, may affect their efficacy;
- emergence of food-borne micro-organisms, such as *Salmonella*, *Campylobacter,* Shiga toxin-producing *E. coli*, *L. monocytogenes* and *Y. enterocolitica*, that display a dramatically increased resistance to many effective antimicrobials, including naturally derived ones (Miltz *et al.*, 2006).

These reasons have prompted an urgent search for new, effective, natural antimicrobial agents.

According to Miltz *et al.* (2006), a potential solution to these problems may be found in some of the antimicrobial peptides (AMPs) that have been isolated from animals and plants during the past two decades. AMPs comprise a group of molecules that are important ubiquitous defense components, controlling cell proliferation and invading pathogens. They show a broad spectrum of bactericidal, fungicidal and antiviral activities. They can be isolated from various natural sources, such as plants, insects, amphibians, crustaceans and marine organisms, as well as from mammals. Although the precise mechanism of action of AMPs is not fully understood, results so far are nonetheless consistent with the hypothesis that antimicrobial activity is not mediated by interaction with a chiral centre and, consequently, AMPs can prevent the emergence of resistant strains. It is supposed that AMPs kill the target micro-organisms by destabilizing, through lipid displacements, the ordered structure of the cell membrane, via either a pore-forming 'barrel stave' mechanism or a non-pore-forming 'carpet-like' mechanism, leading to cell permeabilization.

Other new biocides of natural origin such as N-alkyl-β-D-glucopyranosylamines have shown interesting bioactive properties on pathogen strains encountered on foodstuffs. Muhizi *et al*. (2009a, 2009b) elaborated new bioactive agents, for example, β-D-glucopyranosylamine, *N*-ethyl-β-D-glucopyranosylamine, *N*-butyl-β-D-glucopyranosylamine, *N*-hexyl-β-D-glucopyranosylamine, *N*-octyl-β-D-glucopyranosylamine, *N*-dodecyl-β-D-glucopyranosylamine, *N*-(2-hydroxyethyl)-β-D-glucopyranosylamine, *N*,*N*-di(2-hydroxyethyl)-β-D-glucopyranosylamine and *N*,*N*-diethyl-β-D-glucopyranosylamine. An improvement of both antifungal and antibacterial activity with the increase of alkyl chain length was observed. *N*-dodecyl-β-D-glucopyranosylamine completely inhibited bacteria growth at 0.025×10^{-4} mole mL^{-1} and 0.05×10^{-4} mole mL^{-1} for *L. innocua* and *S. Typhimurium*, respectively.

It seems that bioactive packaging materials based on released bioactive compounds are more efficient than bioactive systems without any agent liberation. However, we have to note that the migration could be difficult to model and to plan. The major properties of functional barriers have been identified, but diffusion and migration of active components through these barriers depends on many more factors than with monolayer materials: conditions (time and temperature) of processing, storage conditions of the empty material and conditions of filling and of storage of the food (Feigenbaum *et al.*, 2005). Mauriello *et al.* (2005) found that low pH favored the migration of the active compound (nisin) from the film, and low temperature delayed the release of the agent. They concluded that the release of nisin from the plastic film was unpredictable, but temperature- and pH-dependent. There is certainly a future for the development of release-on-demand packaging materials.

A preservative-releasing packaging system called the 'BioSwitch' concept has recently been developed and patented (Thijssen *et al*., 2004). This system only releases its preservative on command, which means that a preservative will be released from the packaging material if bacterial growth occurs, thus inhibiting growth of the emerging bacteria. These particles were used to absorb lysozyme, a natural antimicrobial compound isolated from chicken eggs. To enable absorption of lysozyme, a starch gel with suitable porosity was developed by varying the degree of cross-linking and introducing negatively charged groups (De Jong *et al.*, 2005). The evaluation of 'BioSwitch' involved examining whether lysozyme could be released *in vitro* by adding amylase to a suspension of starch-based particles. Under normal conditions, the particles do not release any lysozyme – creating a so-called no-release situation. On addition of amylase, a rapidly induced release of lysozyme was observed. The advantage of such a system is that the antimicrobial agent only comes into contact with the food matrix when there is microbial growth, so lower levels of preservatives can be used in the food itself. Another advantage is that the preservative can be made highly selective to inhibit spoilage organisms without, for example, inhibiting a desired fermentation process. This system is under development in such a way that it will be legally applicable under the new European legislation for active packaging. The general concept of antimicrobial packaging systems based on liberation-on-demand is shown in Fig. 18.5. This figure illustrates a system that is able to detect changes (external stimulus) in the environment and to respond to them automatically. For instance, the external stimulus may be a change in temperature, pH, humidity, UV or the presence of certain metabolites.

Microgels, which are widely used as drug delivery vehicles, may be the basis for another release-on-demand packaging system. Microgels from natural polymers are attractive for food and biomedical applications because of their biodegradability. There is an increasing demand for effective encapsulation systems consisting of natural polymers in which the active compounds are well protected and can be released at the time and place where they are needed The idea is that if lysozyme-containing starch particles are exposed to an initial microbiologically contaminated environment, microbial enzymes will lead to hydrolysis of

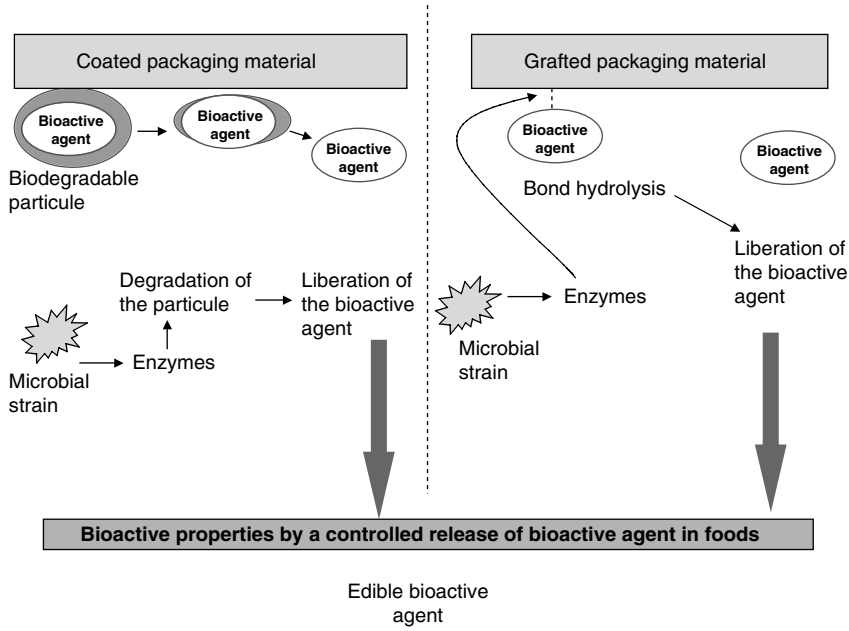

Fig. 18.5 General concept of bioactive material based on a release-on-demand of the active agent.

the starch. As a result, lysozyme is released into the environment where it then inhibits microbial growth (Li *et al.*, 2009).

In addition, interest and activities in nanostructures have increased over recent years. Applications of nanotechnology within the food industry and food packaging material are rather limited compared to other industries, such as the microelectronic or pharmaceutical industries, for example. However, as reported by Weiss *et al.* (2006), nanotechnology has the potential to impact many aspects of food systems – for example, encapsulation and delivery systems that carry, protect and deliver bioactive molecules to their specific site of action. Nanolaminates could be useful for the preparation of active edible coatings or films. A nanolaminate consists of two or more layers of material with nanometer dimensions that are physically or chemically bonded to each other and to which it would be possible to incorporate active functional agents. It is important to note that, in the case of nanolaminated coatings, they could be created entirely from food-grade ingredients by using simple processes such as dipping and washing (Weiss *et al.*, 2006). In addition, the development of nanoparticles, and especially biopolymeric nanoparticles, could be of great interest in the development of on-demand bioactive matrices. These particles may be formed by promoting self-association or aggregation of simple biopolymers or by inducing phase separation in mixed biopolymer systems. Bioactive compounds can be encapsulated in nanoparticles and released in response to specific environmental triggers by altering the solution

conditions to induce complete particle dissolution or change in particle porosity. In addition, incorporation of nanoparticles into a polymer matrix could improve many of its properties, including mechanical bulk, barrier and surface properties. Moreover, from a general point of view, nanocomposites can assist reduction in packaging waste due to their capability to enhance the properties of packaging produced from renewable polymers.

It will be important that any future legislation takes into account that all active and intelligent packaging systems are subject to pre-existing food contact material (FCM) regulations and, consequently, they must not endanger human health (1935/2004/EC). In addition, some of the systems may also be subject to regulations on food additives, biocides, labeling, environment/waste, modified atmosphere, food hygiene, safety, weight and volume control. Releasing systems are, however, allowed to change the composition of the food, provided the released substance is an authorized food additive. Labeling should comply with the food additive Directive and the release or absorption of substances should not mislead the consumer (De Jong *et al*., 2005).

18.7 Conclusion

Future developments in antimicrobial packaging materials will notably be in systems that are active only at a specific time and place – that is, when and where required. The general advantages of the concept are, for example, increased stability and specificity and/or reduction in the chemicals needed. This concept creates interesting possibilities for improving the security of meat-based foods without the use of significant quantities of preservatives. Moreover, if the packaging system is based on renewable resources, such as chitosan, the environmental impact of packaging waste can be reduced.

18.8 References

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19

Edible films for meat, poultry and seafood

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Abstract: In the past decade there has been an increase in the investigation of edible films related to controlling product quality, enhancing sensory properties, improving product safety and increasing the shelf life of meat, poultry and seafood. The focus of this review will be the use of different materials in the manufacture of edible films and the various characteristics of these films upon application to meat, poultry and seafood. New technologies covered include the use of various antimicrobial agents, antioxidants or nutrients that are incorporated into edible films for contact with the surface of meat, poultry and seafood products.

Key words: edible films, antimicrobial agents, antioxidants, nutrients.

19.1 Introduction

Edible films have existed and been used for centuries, with the first reported case being the use of waxes on fruits by the Chinese to minimize water loss. The heightened demands by consumers for quality and freshness of food products, as well as for environmentally friendly packaging, have given rise to the increased development of edible films. Edible films are a thin layer of material coated or wrapped around a food product to act as a barrier to the surrounding environment. In addition, edible films can serve as carriers of natural or chemical antimicrobial agents, antioxidants, enzymes, vitamins or minerals.

Edible films and coatings have received considerable attention in recent years because of their alternative potential to minimize or replace the use of certain synthetic films, which could minimize packaging waste and reduce environmental pollution. The materials used for the preparation of edible films or coatings

Table 19.1 Composition of various types of edible films used on the surface of meat, poultry and seafood

Edible film	Composition	Food product	References
Apple film wraps	3% high methoxyl pectin, golden delicious apple pure and 12% glycerin	Chicken breast, ham	Ravishankar *et al*. (2009)
Milk protein-based film	Calcium caseinate (93% w/w protein, whey protein isolate (93% w/w protein), 5% glycerol (w/v), 0.25% carboxymethyl cellulose (w/v)	Beef muscle slices	Oussalah *et al*. (2004)
Starch-alginate-based film	Starch (2.5% w/v), alginate (1% w/v), ethanol (2:1), glycerin (40% w/w of the total solution), stearic acid (20% of total solution) and lecithin (30% w/w of fatty acid)	Ground beef patties	Wu *et al*. (2001)
Whey protein isolate coating	Whey protein isolate (10%), and glycerol (4.5%)	Turkey frankfurter	Gadang *et al*. (2008)
Chitosan coating	Chitosan (from crab 10 g/L deionized water), 10 g acetic acid, glycerol (1 mL/g of chitosan)	Herring and Atlantic cod	Jeon *et al*. (2002)
Gelatin-based film	Gelatin (from pigskin 4% w/v), sorbitol and glycerol (15% of the gelatin each)	Cold-smoked sardines	Gomez-Estaca (2006)
Pectin-based coating	Pectin (3 g/99 mL distilled water), polyethylene glycol (1 mL)	Pork patty	Kang *et al*. (2007)
Agar-based film	*Gelidium corneum* (1.5% powder *Gelidium corneum*) and 1.5 g glycerol	Sausages	Ku *et al*. (2008)
Whey protein coating	Whey protein (5% w/v), sorbitol (2.5% w/v), $CaCl_2$ (0.125% w/v) and carboxymethyl cellulose (0.25% w/v)	Low-fat sausage	Shon and Chin (2008)
Sodium caseinate film	Sodium caseinate (7.5 g protein/100 g) and glycerol (ratio of 0.32 w/v)	Sliced turkey meat	Caprioli and O'Sullivan (2009)

for meat, poultry or seafood products can be classified into three categories: hydrocolloids, lipids and composites (Table 19.1). Suitable hydrocolloids include proteins, cellulose derivatives, alginate, pectin, starch and other polysaccharides. Suitable lipids include fatty acids, acylglycerol and waxes. Composites contain both hydrocolloid and lipid components (Donhowe and Fennema, 1994).

19.2 Edible film materials

The following sections look at the various types of edible films that are available, as well as the factors to be borne in mind when selecting the most appropriate material in a particular case.

19.2.1 Hydrocolloidal materials

Hydrocolloids are hydrophilic polymers of vegetable, animal, microbial or synthetic origin that generally contain many hydroxyl groups and may be polyelectrolytes (e.g., alginate, carrageenan, carboxymethyl cellulose, gum arabic, pectin and xanthan gum). Hydrocolloid-based films can be used in applications where the control of water-vapor migration is not the primary objective. Hydrocolloids possess good barrier properties to oxygen, carbon dioxide and lipids. Most of these films also have desirable mechanical properties, making them useful for improving the structural integrity of fragile products. Water solubility of polysaccharide films is advantageous in situations where the film will be consumed with a product that is heated prior to consumption, such as with fried foods. During heating, the hydrocolloid film or coating would dissolve and, ideally, would not alter the sensory properties of food. Application of main hydrocolloid-based films and coatings on meat, poultry and seafood products is discussed below.

- **Starch**: Starch, composed of amylose and amylopectin, is primarily derived from corn (maize), wheat, potatoes, tapioca or rice. Genetic modification of starch crops has recently led to the development of starches with improved and targeted functionality (Copeland *et al*., 2009). Starch-based films can exhibit physical characteristics similar to plastic films, such as odorless, tasteless, colorless, non-toxic, biologically absorbable, semi-permeable to carbon dioxide and resistance to passage of oxygen (Cutter, 2006). High amylose starch films were made commercially available under the trade name of Ediflex (American Maize Products Company, Hammond, IN) in the late 1960s. These films were primarily intended for use on frozen foods including frozen meat, poultry and fish (Gennadios *et al*. 1997).
- **Alginate**: Alginates are mainly derived from brown seaweed, and possess good film-forming properties that make them particularly useful in food applications. Desirable properties attributed to alginate films include moisture retention, reduction in shrink, improved product texture, juiciness, color and odor of treated muscle foods (Cutter, 2006).
- **Carrageenan**: Carrageenan is a collective term for polysaccharides extracted from certain species of red seaweed of the family *Rhodophycae*. Carrageenan edible films and coatings have been used in various fields of food industry such as application on fresh and frozen meat, poultry, fish and shrimp to prevent superficial dehydration, as well as sausage-casings (Ninomiya *et al*., 1997; Petersen, 2000).
- **Cellulose ethers**: Cellulose is a non-digestible component of plant cell walls. In the manufacture of edible films, cellulose-based films tend to be water soluble, resistant to fats and oils, tough and flexible (Cutter, 2006). Cellulose casings also are widely used by the meat industry in the manufacture of ready-to-eat meat and poultry products, including frankfurters, sausages, bologna and other small-diameter meat products subject to thermal processing (Cutter, 2006).
  Plant cellulose has to undergo a harsh chemical treatment to remove lignin, hemicellulose and pectins. This treatment severely impairs the material

characteristics of plant cellulose. Bacterial strains of Acetobacterxylinum and *A. pasteurianus* are able to produce an almost pure form of cellulose with chemical and physical structure identical to the cellulose formed in plants (Brown, 1996), which represents a potential material for edible films and coating. Bacterial cellulose has been used in the food industry for applications such as low-calorie desserts, salads and fabricated food (Cheng *et al*., 2009).

- **Chitosan**: Chitosan is an edible and biodegradable polymer derived from chitin. Chitosan is the second most abundant natural and non-toxic polymer in nature after cellulose, and is mainly made from crustacean shells. Some desirable properties of chitosan are that it forms films without the addition of additives, exhibits good oxygen and carbon dioxide permeability, as well as excellent mechanical properties (Suyatma *et al*., 2004). Chitosan showed strong antimicrobial activity against bacteria, yeasts and molds (Vartiainen *et al*., 2004), therefore, it can extend the shelf life of food because of the antimicrobial effect, as suggested by related research on fish soup (Fernandez-Saiz *et al*., 2010) and fresh fillets of Atlantic cod (Gadusmorhua), herring (Clupeaharengus) and bonito fish (Sardasarda) (Alak *et al*., 2010; Jeon *et al*., 2002). Chitosan films prepared with oregano essential oil were applied to bologna slices and suggested as an antimicrobial packaging material for processed meat (Chi *et al*., 2006). Gelatin-chitosan films incorporated with essential oils were tested on fish extracts; results suggested that they can be used as antimicrobial agents for fish preservation (Gómez-Estaca *et al*., 2010).
- **Proteins**: Animal and plant proteins, such as collagen, gelatin, milk proteins, wheat gluten, soy protein, corn zein and peanut protein, have been processed into edible films (Gennadios *et al*., 1997). Protein-based films adhere well to hydrophilic surfaces, provide barriers for oxygen and carbon dioxide, but do not resist water diffusion, in a similar manner to polysaccharide films (Cutter, 2006). Several studies have been conducted to overcome the poor water-vapor barrier properties of protein-based edible films (Kokoszka *et al*., 2010; Liu *et al*., 2006; Min *et al*., 2009; Monedero *et al*., 2009). These studies showed that by adding a lipid component to polysaccharide- and protein-based films, the barrier and physical properties were enhanced. Protein-based film had been widely used on meat, poultry and seafood products (Cutter, 2006; Ustunol, 2009).

19.2.2 Lipid materials

Lipid-based edible films (waxes or fat-based oils) were developed specifically to limit moisture migration within food. These hydrophobic substances are effective barriers against moisture migration because of their apolar nature. Chemical properties of the molecules, such as presence of polar components, hydrocarbon chain length and degree of unsaturation or acetylation, affect barrier efficiency. For components having a common chemical nature, an increasing chain length modifies barrier properties because molecular polarity decreases, and this does not favor the water solubility of the film (McHugh and Krochta, 1994a, 1994b). Films and coatings made from lipids alone, however, lack structural integrity and are brittle.

19.2.3 Composite materials

Composite films can be formulated to take advantage of the positive attributes that lipid and hydrocolloids components possess, thereby lessening the negative attributes of each. Therefore, biopolymer composites can modify film properties and create desirable film structures for specific applications. For example, when a barrier to water vapor is desired, the lipid component can serve to address this function, while the hydrocolloid component provides the necessary durability. A composite film can exist as a bilayer, in which one layer is a hydrocolloid and the other lipid, or as a conglomerate, where the hydrocolloid and lipid components are interspersed throughout the film.

19.2.4 Selecting an edible film material

The selection of edible packaging for application will primarily depend on the specific characteristics of the food product that requires containment, preservation and appropriate storage. Collagen films and sausage-casings probably constitute the most successful commercial form of applied edible films for meat products. In addition to collagen, other proteins such as casein, whey proteins, soy protein, wheat gluten, corn zein and egg albumin have also been investigated in the production of edible films and coatings for meat and seafood applications (Ben and Kurth, 1995; Sathivel and Kramer, 2010). Such films are good barriers to gases such as oxygen and carbon dioxide, and adhere well to hydrophilic surfaces. Various edible polysaccharide films and coatings, such as starch and its derivatives, alginates, carrageenans, cellulose ethers and pectin also have been used to extend shelf life of meat and poultry products by delaying dehydration, oxidative rancidity and surface browning (Kester and Fennema, 1986). The ability of some polysaccharides (e.g., methylcellulose) to form thermally induced gelatinous coatings has also made them desirable for reducing oil absorption during frying (Nisperos-Carriedo, 1994).

A single-component film generally has either good barrier or good mechanical properties, but typically not both. Therefore, in formation of composite films and coatings, two or more components are combined to improve mechanical properties, gas exchange and adherence to surfaces and/or moisture barrier properties (Baldwin *et al*., 1995). They may be applied as emulsions or bilayers. Plasticizers, such as glycerol, polyethylene glycol or sorbitol, may be added to modify film mechanical properties and provide increased flexibility (Ben and Kurth, 1995). The modified caseinate-lipid composite films have been reported to reduce the drip loss and keep meat juicier (Ben and Kurth, 1995).

19.2.5 Producing edible films

To increase the film-forming capabilities of edible films plasticizers are often added. Plasticizers used in edible films must have a low molecular weight with a high boiling point and be compatible with the biopolymers used in the edible films (Cagri *et al*., 2004). Plasticizers are also used to control the physical properties

of edible films. To increase flexibility and decrease brittleness of edible films plasticizers such as sorbitol, glycerol, mannitol, sucrose and polyethylene glycol can be used. Plasticizers can decrease protein interactions and increase intermolecular spacing in protein-based edible films. The concentration of plasticizer can affect the film-forming properties of protein-based edible films. The mechanical strength, barrier properties and elasticity decrease when high levels of plasticizers are used in protein-based edible films (Cagri *et al*., 2004).

Edible films can also contain covalent cross-linking agents that improve water resistance, cohesiveness, rigidity, mechanical strength and barrier properties. Irradiation, enzymes and UV light will increase cross-linking of the biopolymers in protein-based edible films (Cagri *et al*., 2004).

Procedures used for the successful production of edible films can include solvent casting, thermal gelation solidification of melt and extrusion. Hydrocolloid-based edible films (agar, alginate, carrageenan, cellulose derivatives, chitosan, gum, pectin, starch, gelatin, whey protein) can be formed by solvent casting. During this procedure the edible materials, plasticizer and cross-linking agents are dissolved into a solvent solution, cast in a thin layer, allowed to dry then peeled away from the pouring surface. During the drying process the solvent evaporates and the biopolymers align themselves to form a film.

Another step involved in some protein film manufacture is heating the solution before casting. Heating of protein solutions causes the biopolymers to denature, gel or coagulate and precipitate. During denaturation of proteins the intramolecular and intermolecular disulfide bonds are cleaved and reduced to sulfhydryl groups (Cagri *et al*., 2004). Once the solution is cast the disulfide bonds link the polypeptide chains to form the film.

The use of extrusion technology for edible films would increase their commercial potential and be more attractive to the food industry because the equipment is commercially available (Hernandez-Izquierdo and Krochta, 2008). Extrusion is the process of continuously introducing raw materials into a hopper then pushed through a die of a desired shape by a screw (Hernandez-Izquierdo and Krochta, 2008). Extrusion of edible film casings from collagen for use on sausages, hot dogs, bologna, salami and smoked ham has been practiced for over 50 years in the food industry. The cost of producing edible coatings by extrusion is significantly lower compared to the natural gut casings. Several researchers have investigated the use of extrusion for producing edible films composed of starch, pectin and protein (Ha and Padua, 2001; Pommet *et al*., 2003; Silva *et al*., 2010; Zhu *et al*., 2010).

19.3 Antimicrobial edible films

Edible films or coatings containing antimicrobial agents can be used to control pathogenic or spoilage microorganisms on the surface of raw or cooked meat, poultry or seafood products. Edible films can serve as carriers for a wide range of food additives, including antimicrobials, which can reduce microbial growth at

meat, poultry and seafood surfaces to improve product safety and extend product shelf life. The primary advantage of antimicrobial edible films and coatings is that inhibitory agents in these films can be specifically targeted to post-processing contaminants on the food surface.

There are several categories of antimicrobials that can be potentially incorporated into edible films and coatings, such as: organic acids (acetic, benzoic, lactic, propionic, sorbic); fatty acid esters (glycerylmonolaurate); polypeptides (lysozyme, peroxidase, lactoferrin, nisin); plant essential oils (cinnamon, oregano, lemongrass); and nitrites and sulfites (Franssen and Krochta, 2003). Antifungal compounds, organic acids and potassium sorbate, as well as the bacteriocin nisin, were reported to be more effective in reducing levels of foodborne microorganisms when immobilized or incorporated into edible gels (i.e., starch, carrageenan, waxes, cellulose ethers or alginate) and applied to meat surfaces than when these agents were used alone (Cutter, 2006; Cutter and Summer, 2002). Plant essential oils are outstanding alternatives to chemical preservatives because their use in foods meets consumer demands for minimally processed natural products (Burt, 2004).

Edible films with no added antimicrobial agent have a slight effect on the bacterial populations on the surface of meat, poultry or seafood products. For example, calcium alginate edible films coated on lamb or beef cuts reduced bacterial counts on the surface of these meat products (Lazarus *et al*., 1976; Williams *et al*., 1978). Beef lion strips dipped into a calcium alginate edible film had significantly lower bacterial counts, about 1 log cycle, compared to the non-coated beef cuts over 7 days at 5°C (Williams *et al*., 1978). In addition, lamb carcasses sprayed with a calcium alginate edible film had significantly lower total surface counts between day 5 and 7 at 2°C than the control non-coated carcasses, while the plastic-wrapped lamb carcasses had higher counts than the control and calcium alginate treatment (Lazarus *et al*., 1976).

Edible films have also shown promise in enhancing the microbial safety and extending the shelf life and quality of eggs (Biladeau and Keener, 2009; Caner, 2005; Kim *et al*., 2008; Panuwat *et al*., 2010; Xie *et al*., 2002). Xie *et al*. (2002) evaluated the mechanical and bacterial barrier properties of washed (tap water, sodium carbonate, Na_2CO_3, sodium hypochlorite, NaOCl) non-coated eggs and eggs coated with soy protein isolate, whey protein isolate, carboxyl methylcellulose or wheat gluten. All coated eggshells showed greater puncture strength than those of non-coated eggs. The film-coated eggs reduced post-wash bacterial penetration as measured by a dye penetration method. Whey protein isolate coatings completely inhibited blue dye penetration on average. Their results suggest that whey protein isolate coatings can be used to reduce breakage of eggshell and egg microbial contamination.

Spices and herbs have antimicrobial activity against a wide range of microorganisms because they are rich in phenolic compounds, such as flavonoids and phenolic acid. Studies have shown that when spices and/or herbs are added to edible coatings bacterial counts can be reduced. Edible apple wrap films containing 1.5% carvacrol or cinnamaldehyde were used on raw chicken to determine

if it controlled the natural spoilage bacteria. After 72 h at 4°C the unwrapped chicken had 2.8×10^3 CFU/g whereas the apple wrap with antimicrobial agent had non-detectable levels (Ravishankar *et al*., 2009). Milk protein-based edible films containing 1.0% oregano applied to whole beef muscle reduced *E. coli* O157:H7 and *Pseudomonas* spp. by 1 log CFU/g from controls after 7 days of storage at 4°C (Oussalah *et al*., 2004). Thyme oil and cinnamaldehyde added to soy or whey edible films coated onto pre-cooked shrimp increased the shelf life by 12 days (Ouattara *et al*., 2001). Cinnamaldehyde with acetic acid or propionic acid added to chitosan films applied on processed meats inhibited or delayed the growth of spoilage bacteria (Ouattara *et al*., 2000).

Edible films containing nisin show promise in the control of foodborne pathogens on the surface of ready-to-eat meat, poultry and seafood. Nisin, a hydrophobic protein that has antimicrobial activity against gram-positive bacteria (Klaenhammer, 1993; Ralph *et al*., 1995), has 'generally recognized as safe' (GRAS) status for use in soft cheese products (Ralph *et al*., 1995). Edible films containing nisin are more effective when an additional food grade additive is added to the film solution. Corn zein films containing nisin, lauric acid and EDTA (sodium Ethylenediaminetetraacetic acid) were very effective in reducing *L. monocytogenes* when the bacterial cells were directly inoculated onto the surface of the films. After a 24 h exposure to the film *L. monocytogenes* was reduce by 8 log cycles compared to the films without nisin (Hoffman *et al*., 2001). Janes *et al*. (2002) investigated the inhibition of nisin added to zein films coated onto ready-to-eat chicken against *L. monocytogenes*. This study found that *L. monocytogenes* was reduced to a non-detectable level on the surface of refrigerated ready-to-eat chicken coated with ethanol zein film containing nisin.

A major concern of the meat, poultry and seafood industry is the contamination of raw products with Gram-negative foodborne pathogens such as *E. coli* O157:H7, *Salmonella* and *Campylobacter* species. A possible solution for controlling these foodborne pathogens is edible films containing antimicrobial agents such as nisin. In order for edible films containing nisin to inhibit Gram-negative bacteria, a chelating agent such as EDTA needs to be added to the film solution (Shefet *et al*., 1995; Stevens *et al*., 1991). The mechanism which nisin uses to destroy bacteria is the formation of pores in the bacterial plasma membrane (Abee *et al*., 1994). This results in the loss of magnesium and other cations, which in turn reduces the proton motive force leading to lysis of the bacterial cells. Gram-negative bacteria have an outer membrane that protects them from the bacterial cidal action of nisin. Chelating agents have the ability to bind up magnesium and calcium in the outer membrane of Gram-negative bacteria that causes the membrane lipids to become unstable and which makes the membrane more permeable to nisin. Several studies have shown that the combination of nisin and EDTA in edible films will control Gram-negative species on the surface of meat, poultry and seafood (Bennett *et al*., 2006; Fang and Tsai, 2003; Fei *et al*., 2009; Natrajan and Sheldon, 2000a).

By combining organic acid, nisin and plant extracts into edible films the antimicrobial activity of edible films can be increased against foodborne pathogens

when applied onto the surface of meat, poultry and seafood. Gadang *et al*. (2008) combined nisin, grape seed extract, malic acid and EDTA into whey protein isolate film coatings and applied them to the surface of turkey frankfurters to determine the effect against *L. monocytogenes*, *E. coli* O157:H7 and *Salmonella typhimurium*. After 28 days at 4°C *L. mononcytogenes* and *E. coli* O157:H7 had been reduced 2 log CFU/g from controls whereas *S. typhimurium* was reduced to non-detectable levels.

Chitosan is a biopolymer derived by deacetylation of chitin (poly-ß-(1→4) *N*-acetyl-D-glucosamine. It is a major component of the cell walls of fungi and shells of crustaceans such a crab, shrimp and crawfish (No *et al*., 2002). Chitosan has been reviewed for antimicrobial application in the biomedical and food industries (Knorr, 1984; Muzzarell, 1977). Chitosan as an antimicrobial edible film is a good choice due to its film-forming properties (Darmadji and Izumimoto, 1994). No *et al*. (2002) found that chitosan generally showed a stronger bactericidal effect against Gram-positive bacteria than Gram-negative bacteria. In addition, chitosan films have been shown to have antimicrobial activity against mold (Chien and Chou, 2006). Chitosan-based edible films and film coatings have been shown to be effective in controlling the growth of *L. monocytogenes* on the surface of ready-to-eat beef, salmon and shrimp (Beverly *et al*., 2008; Jiang *et al*., 2011; Roller and Covill, 2000).

19.3.1 Factors that influence the effectiveness of antimicrobial edible films

The controlled release of antimicrobial agents from edible film are very important considerations to address in order that antimicrobial films and coatings function efficiently and effectively. Release of antimicrobial substances from edible films is dependent on many factors, including electrostatic interactions between the antimicrobial agent and polymer chains, ionic osmosis and structural changes induced by the presence of the antimicrobial and environmental conditions. Diffusion of antimicrobials through the edible film is also influenced by film (type, manufacturing procedures), food (pH, water activity), hydrophilic characteristics and storage conditions (temperature, relative humidity, duration) (Dawson *et al*., 1996; Janes *et al*., 2002; Ko *et al*., 2001; Natrajan and Sheldon, 2000b; Padgett *et al*., 1998; Rossi-Marquez *et al*., 2009).

The type of edible film (hydrophobicity/hydrophilicity) and the antimicrobial agent used to control foodborne pathogens needs to be considered with regard to the surface of raw meat, poultry and seafood. Ko *et al*. (2001) determined the effects of hydrophobicity/hydrophilicity of edible films against *Listeria monocytogenes* strain V7 by various nisin concentrations (4.0–160 IU/film disk) and pH values ranging from 2.0 to 8.0 and the mechanical properties and water-vapor permeability of films prepared with or without nisin. Using nisin, edible films with higher hydrophobicity values of 280–450 units under an acidic environment exerted a greater inhibitory effect against *L. monocytogenes*.

Temperature also affects the activity of certain antimicrobial compound in edible films against foodborne pathogens. Janes *et al*. (2002) showed that the

most effective storage temperature for reduction of *L. monocytogenes* by nisin in zein films on the surface of ready-to-eat chicken was 4°C. It is well known that lower temperatures cause sub-lethal damage to *L. monocytogenes* cells (Abee *et al*., 1994; De Martinis *et al*., 1997; Dykes, 1999; Dykes and Withers, 1999; Montville *et al*., 1995; TerSteeg *et al*., 1999). Damage to the cell wall of *Listeria monocytogenes*, when exposed to low temperatures, would make the cytoplasmic membrane more accessible to nisin molecules, thus increasing the ability of the bacteriocin to form pores in the membrane which results in bacterial death due to depletion of the proton motive force and leakage of ions (Montville *et al*., 1995).

Furthermore, the concentration of proteins used in edible films can affect the ability of antimicrobial compounds to kill foodborne pathogens on the surface of meat, poultry and seafood products. Natrajan and Sheldon (2000b) found that a lower concentration of agar gel with nisin and 50 mM EDTA was more effective in reducing *Salmonella typhimurium* on the surface of broiler drumstick skins. After 96 h exposure at 4°C, *Salmonella typhimurium*, on the surface of the broiler drumstick skins, was reduced 4.6 log cycles whereas a higher agar concentration of 1.25% only reduced *S. typhimurium* by 1.8 log cycles. The tighter cross-linking of the higher agar concentration could have prevented the migration of nisin to the surface of the broiler drumstick, thus preventing contact with the pathogen.

How films are prepared and manufactured also greatly influences the reduction of foodborne pathogens by the antimicrobial agents. Padgett *et al*. (1998) found soy protein or zein films containing lysozyme or nisin prepared by casting were more effective in reducing *Lactobacillus plantarum* or *Escherichia coli* than when they were heat pressed. Corn zein films prepared by casting with a combination of lysozyme (66 mg/g of film) and 30 mM EDTA produced zones of inhibition against *E. coli* whereas the same corn zein film formulation heat pressed had no zones of inhibition. Furthermore, the minimum concentration of nisin required to retain its activity in heat-pressed zein films against *Lactobacillus plantarum* in the zone of inhibition tests was relatively high at 0.6% whereas in heat-pressed soy films a lower concentration of nisin 0.01% was needed (Dawson *et al*., 1996).

Another factor to consider when formulating edible films with antimicrobial agents is what affects the antimicrobial compound will have on the mechanical and physical properties of edible films. For example, nisin incorporation into edible films increases the thickness and lowers the puncture strength, while tensile strength and water-vapor permeability are not affected (Ko *et al*., 2001; Rossi-Marquez *et al*., 2009).

19.4 Edible films containing antioxidants and other nutrients

There are many potential benefits in using edible films as carriers of other additives (e.g., flavors, antioxidants, coloring agents, vitamins, probiotics and nutraceuticals) which justify continued research into their use in the field of active packaging. Antioxidants are the most extensively investigated nutrients other than antimicrobials.

Antioxidants increase the stability of food components, especially polyunsaturated lipids, and maintain nutritional value and color by preventing oxidative rancidity, degradation and discoloration. Acids such as citric and ascorbic acid, or phenolic compounds such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tertiary butylated hydroxyquinone (TBHQ), propyl gallate and tocopherols act as antioxidants. Antioxidants obtained from natural plant sources are more potent and safe. Carnosol extracted from rosemary leaves showed greater antioxidant activity than BHA or BHT (Wu *et al*., 1982). Aazza *et al*. (2011) evaluated antioxidant and anti-acetylcholinesterase activities of some commercial essential oils, and showed that *Thymus vulgaris*, with the major compounds of phenolic monoterpenes thymol and carvacrol, has the best antioxidant activity. Miguel (2010) gives a brief review about antioxidant ability of essential oils isolated from aromatic plants, which shows that the antioxidant activities of the essential oils have been well documented; nevertheless their further application could be hampered due to the chemical variability of the oils.

Incorporation of antioxidants such as garlic or ascorbic acids into carrageenan coatings has been reported to extend shelf life of poultry products (Ustunol, 2009). Antioxidants were incorporated into a mixture of lard and tallow coating containing lactic acid-fatty acid triacylglycerol, which was used to coat freeze-dried and fresh meats, including beef steaks, pork chops and beef cubes; thiobarbituric acid levels were significantly reduced in these coated meats (Ustunol, 2009). Pork chops treated with alginate-starch coatings containing tocopherol were reported to be juicier and less susceptible to lipid oxidation, compared to the untreated controls (Hargens-Madsen *et al*., 1995). It has been reported that turkey breasts wrapped in corn zein films containing BHA had a lower hexanal content than samples packaged in polyvinylidene chloride (PVDC) (Herald *et al*., 1996). A study by Oussalah *et al*. (2004) evaluated the antioxidant properties of milk protein-based edible films containing oregano, pimiento and oregano-pimiento mix and showed that pimiento-containing films provided the highest antioxidant activity on beef muscle slices. In addition, oregano-based films were also able to inhibit lipid oxidation in beef muscle samples.

Armitage *et al*. (2002) evaluated the antioxidant activities of egg albumen coatings with natural antioxidants fenugreek, rosemary and vitamin E in diced raw and diced cooked poultry breast meat. Coatings with added antioxidant showed most effect against lipid oxidation in both raw and cooked samples. The gelatin-based edible films enriched with the oregano or rosemary extracts could lower lipid oxidation levels of cold-smoked sardines (Gómez-Estaca *et al*., 2006). Chitosan coatings incorporating fish oil significantly reduced thiobarbituric acid reactive substances (TBARS) values, inhibited growth of total and psychrotrophic bacteria, and enhanced the total lipid and omega-3 fatty acid contents in lingcod fish fillets during cold and frozen storage (Duan *et al*., 2010).

Integration of nutritional or nutraceutical ingredients into edible films or food coatings is another developing field of interest. Nutraceuticals are chemicals found as natural components of foods or other ingestible forms that have been determined to be beneficial to the human body in preventing or treating one or

more diseases or improving physiological performance. Calcium and vitamin E are important nutraceuticals as they play significant roles in the human body in preventing certain diseases (Elliott, 1988; Pszczola, 1998). Few studies have evaluated the feasibility and functionality of calcium (Gluconal Cal) or vitamin E incorporated with milk protein-based, chitosan-based edible films or xanthan gum coatings, and their applications for fruit and vegetable products (Han *et al*., 2004; Hernández-Muñoz *et al*., 2006; Mei *et al*., 2002; Mei and Zhoa, 2003; Park and Zhao, 2004), and no research has been done for meat, poultry and seafood products, as far we know.

Incorporation of probiotics into functional edible films and coatings has been scarcely studied. Tapia *et al*. (2007) developed probiotic edible films for coating fresh-cut fruits. In this work, fresh-cut apples and papayas were successfully coated with alginate or gel film-forming solutions containing viable bifidobacteria, which demonstrated the feasibility of alginate- and gellan-based edible coatings to carry and support viable probiotics on fresh-cut fruit, and potentially opens new possibilities to develop probiotic films and coating products for meat, poultry and seafood products.

19.5 Conclusion

With recurring recalls of meat, poultry and seafood products due to contamination with foodborne pathogens there is a clear need to develop additional methods to prevent the economic loss and possible deaths that can occur from foodborne infections. Edible films with antimicrobial agents show promise in the control of foodborne pathogens on the surface of these products because microbial contamination primarily occurs due to post-processing handling (Coma, 2008). Furthermore, antimicrobial agents added to edible films are more efficient and can control the foodborne pathogens for a more extended amount of time as compared to the antimicrobial agent alone on the surface of meat, poultry and seafood products (Cutter, 2006). Several intrinsic (pH, hydrophobicity, concentration of polymers and composition of the film-forming agents) and extrinsic factors (temperature and humidity) need to be considered during formulation of edible films that contain antimicrobial agents for them to be effective at controlling foodborne pathogens and maintaining their mechanical and physical properties.

Edible films containing flavors, antioxidants, coloring agents, vitamins, probiotics or nutraceuticals have the potential to extend the food shelf life and improve the quality and health of consumers. However, only the antioxidants have been extensively investigated. Research has shown that antioxidants, such as garlic and ascorbic acid, BHA, BHT and some essential oils could extend the shelf life of meat, poultry and seafood products. The antioxidant property of essential oil has gained attention because consumers prefer a more naturally occurring addictive. Integrating nutraceutical ingredients and probiotics, such as calcium, vitamin E or bifidobacteria, into edible films has only been studied in fruit and vegetable

products. These active ingredients could have an application in edible films for meat, poultry and seafood products and more intensive and systematic work is needed to investigate their actual health benefits for consumers.

With greater understanding of edible film properties, edible films containing natural or chemical antimicrobial agents, antioxidants, enzymes, vitamins, or minerals could be effectively developed for meat, poultry and seafood products to improve food quality.

19.6 References

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20

Application of smart packaging systems for conventionally packaged muscle-based food products

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Abstract: Food packaging is indispensable in the world in which we live today and is probably the greatest of all of the technologies available to us in delivering food preservation and product shelf life. This review serves to highlight how smart packaging technologies have been used, are being used or have the potential of being used to further enhance the safety and physicochemical properties of packaged muscle-based food products; or provide in- or on-pack indication as to the safety and quality of muscle-based food products; or provide new and more clever means by which consumers can interact with muscle-based food products in a manner that delivers enhanced convenience and safety beyond that provided by the conventional primary food packaging system.

Key words: muscle-based foods, smart packaging, active, intelligent, scavengers, absorbers, emitters, indicators, sensors, biosensors, RFID (radio frequency identification), antimicrobial, antioxidant.

20.1 Introduction

There is no official definition of smart packaging but most would agree that it is packaging that goes beyond the use of simple packaging materials combined with traditional printed features, such as alphanumerics, graphics and simple bar-codes (Kerry and Butler, 2008). This relatively new form of packaging has been classified in many ways – 'active', 'intelligent', 'diagnostic', 'functional' and 'enhanced'; however, this author prefers to use the term 'smart' as it is a more encompassing term and one which is sympathetic to the great number of technologies that are covered under these other more specific headings. Additionally, the term 'smart' provides the scope for other yet undeveloped technologies to be included in the future under this banner term with ease.

In the hierarchical order of packaging, we have primary, secondary and tertiary packaging, which relate to sales, collation/handling and transport of goods, respectively. Smart packaging technologies can be applied in different ways and for different reasons to all three of these forms of packaging, yet no packaging level has been described for smart packaging entities. In our own packaging group in UCC, we describe all of the packaging materials and formats utilized in primary, secondary and tertiary packaging as 'first-level packaging', while smart packaging is described as 'second-level packaging'. For the purposes of this review, this is how these forms of packaging will be described.

It is important to point out at this juncture that this review builds on relatively recent reviews on the use of smart packaging technologies as applied specifically to conventionally packaged muscle-based foods (meat, poultry and seafood) and include reports by Kerry *et al*. (2006), Coma (2008), Hogan and Kerry (2008), O'Grady and Kerry (2008), Pacquit *et al*. (2008) and is supported in this book by Chapter 6, presented by McMillin and Belcher.

The fundamental aspects of all packaging materials is that, in an economical manner, they must contain, protect, preserve, inform (throughout the entire distribution process from point of manufacture to point of consumer usage) and provide convenience (at many different levels) while acknowledging the constraints placed upon their usage from both legal and environmental perspectives. As these fundamental principles apply to all forms of packaging materials and systems, it follows that, irrespective of the specific level at which the packaging is industrially applied, all must conform to these same principles (Cruz-Romero and Kerry, 2008).

20.1.1 First-level packaging for application to muscle-based food products

First-level packaging for fresh meat, poultry and seafood is carried out to avoid contamination, delay microbial, chemical and biochemical spoilage, permit some enzymatic activity to improve tenderness (as in the case of fresh meat), reduce weight loss and visually present the muscle-based product to the consumer in a format which enhances overall product appearance and meets consumer desires and expectations. When considering processed muscle-based products, factors such as dehydration, lipid oxidation, discoloration and loss of aroma must be taken into account (Mondry, 1996). Many muscle-based packaging systems currently exist within the retailing environment, each with different attributes and applications.

The packaging systems used for muscle-based food products range from overwrapping for short-term chilled storage and/or retail display, to a diversity of specified modified atmosphere packaging (MAP) systems for longer-term chilled storage and/or display, to vacuum packaging applications, bulk-gas flushing or MAP systems using 100% carbon dioxide for long-term chilled storage. Due to the diversity of product characteristics associated with meat, poultry and seafood and basic packaging demands and applications, any packaging technologies offering to deliver more product and quality control in an economic and diverse

manner would be favourably welcomed. This is what second-level packaging exists to do.

20.1.2 Second-level packaging for application to muscle-based food products

As outlined above, 'smart packaging' is a broad term encompassing a range of relatively new packaging concepts, most of which can be placed in one of the two principle categories; active-packaging and intelligent packaging.

Active packaging refers to the incorporation of certain additives into packaging systems (whether loose within the pack, attached to the inside of packaging materials or incorporated within the packaging materials themselves) with the aim of maintaining or extending product quality and shelf life. Packaging may be termed 'active' when it performs some desired role in food preservation other than providing an inert barrier to external conditions (Hutton, 2003). Active packaging 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' (Ahvenainen, 2003: 5–21). The development of a whole range of active packaging systems, some of which may have applications in both new and existing food products, is fairly new. Active packaging includes additives or 'freshness enhancers' that can participate in a host of packaging applications and, by so doing, enhance the preservation function of the primary packaging system (Table 20.1).

Intelligent packaging is packaging that in some way senses some properties of the food it encloses or the environment in which it is kept and which is able to inform the manufacturer, retailer and consumer of the state of these properties. Although distinctly different from the concept of active packaging, features of intelligent packaging can be used to check the effectiveness and integrity of active packaging systems (Hutton, 2003). Intelligent packaging has been defined as packaging 'systems which monitor the condition of packaged foods to give information about the quality of the packaged food during transport and storage' (Ahvenainen, 2003: 5–21). Smart packaging devices, which may be an integral component or inherent property of a foodstuff's packaging, can be used to monitor a plethora of food pack attributes (Table 20.2).

Table 20.1 Examples of active packaging applications for use within the food industry

Absorbing/scavenging properties – oxygen, carbon dioxide, moisture, ethylene, flavours, taints, UV light
Releasing/emitting properties – ethanol, carbon dioxide, antioxidants, preservatives, sulphur dioxide, flavours, pesticides
Removing properties – catalysing food component removal: lactose, cholesterol
Temperature control – insulating materials, self-heating and self-cooling packaging, microwave susceptors and modifiers, temperature-sensitive packaging
Microbial and quality control – UV and surface-treated packaging materials

Table 20.2 Examples of intelligent packaging applications for use within the food industry

Tamper evidence and pack integrity – breach of pack containment
Indicators of product safety/quality – time–temperature indicators (TTIs), gas-sensing devices, microbial growth, pathogen detection
Traceability/anti-theft devices – radio frequency identification (RFID) labels, tags, chips
Product authenticity – holographic images, logos, hidden design print elements, RFID

From the outline descriptions of the numerous active and intelligent packaging technologies currently in existence, only a limited number are currently relevant to meat, poultry and seafood packaging applications. However, research developments within the area of smart packaging are progressing rapidly and potential applications are likely. Therefore, the purpose of this review is to examine the smart packaging systems that have been, or are currently being used for muscle-based product application, and assess new and developing systems that may have potential for commercial use with such products into the future.

20.2 Packaging technologies for gas and moisture control

The following sections discuss the various methods used to control the atmosphere surrounding the food product in such a way as to contribute to the extension of its shelf life.

20.2.1 Oxygen scavengers

High levels of oxygen present in food packages may facilitate microbial growth, off-flavour and off-odour development, colour changes and nutritional losses, thereby causing significant reduction in the shelf life of foods. Therefore control of oxygen levels in food packages is important to limit the rate of such deteriorative and spoilage reactions in foods. Oxygen-absorbing systems provide an alternative to vacuum and gas flushing technologies as a means of improving product quality and shelf life (Ozdemir and Floros, 2004). Although oxygen-sensitive foods can be packaged accordingly using MAP or vacuum packaging, such techniques do not always facilitate complete removal of oxygen. Oxygen which permeates through the packaging film or is trapped within the muscle tissue, within the product or between product pieces or slices cannot be removed by these techniques. Using an oxygen scavenger, which absorbs the residual oxygen after packaging, quality changes in oxygen-sensitive foods can often be minimized (Vermeiren *et al*., 1999). Existing oxygen-scavenging technologies utilize one or more of the following concepts: iron powder oxidation, ascorbic acid oxidation, photosensitive dye oxidation, enzymatic oxidation (e.g., glucose oxidase and alcohol oxidase), unsaturated fatty acids (e.g., oleic or linolenic acid) rice extract or immobilized yeast on a solid substrate (Floros *et al*., 1997). More comprehensive information and details relating to oxygen scavengers can be obtained

from other reviews (Floros *et al*., 1997; Vermeiren *et al*., 1999). Structurally, the oxygen-scavenging component of a package can take the form of a sachet, label, film (incorporation of scavenging agent into the packaging film), card, closure liner or concentrate (Suppakul *et al*., 2003).

The majority of currently commercially available oxygen scavengers are based on the principle of iron oxidation (Smith *et al*., 1990):

$$Fe \rightarrow Fe^{2+} + 2e^{-}$$
$$\tfrac{1}{2}\, O_2 + H_2O + 2e^{-} \rightarrow 2OH^{-}$$
$$Fe^{2+} + 2OH^{-} \rightarrow Fe(OH)_2$$
$$Fe(OH)_2 + \tfrac{1}{4}\, O_2 + \tfrac{1}{2}\, H_2O \rightarrow Fe(OH)_3$$

Comprehensive details on a variety of commercially available oxygen scavengers are presented by Suppakul *et al*. (2003). Ageless® (Mitsubishi Gas Chemical Co., Japan) is the most common oxygen-scavenging system based on iron oxidation. The sachets are designed to reduce oxygen levels to less than 1%. Additional examples of oxygen-absorbing sachets include ATCO® (Emco Packaging Systems, UK; Standa Industrie, France), FreshPax® (Multisorb Technologies Inc., USA) and Oxysorb® (Pillsbury Co., USA).

The scientific literature contains a number of references to studies which examine the influence of oxygen-scavenger sachets on fresh beef discoloration. Gill and McGinnis (1995) performed an oxygen-absorption kinetics study with a commercial oxygen scavenger (FreshPax™ 200R) and reported that discoloration could be prevented in ground beef if large numbers of scavengers were used in each pack to bring residual oxygen to < 10 ppm within 2 h at a storage temperature of −1.5°C. The inclusion of oxygen scavengers (Ageless® SS200) in master packs flushed with 50% CO_2:50% N_2 significantly improved the colour stability of *M. longissimus dorsi* and *M. psoas major*, relative to controls (Allen *et al*., 1996). Tewari *et al*. (2001) examined the effect of two commercial oxygen scavengers (Ageless® FX-100 and FreshPax® R-2000) in conjunction with controlled atmosphere packaging (CAP) on the discoloration of *M. psoas major* in master packs filled with nitrogen and stored at 1 ± 0.5°C. Steaks packaged without oxygen scavengers had more discoloration and significantly higher proportions of metmyoglobin when compared to steaks packaged with oxygen scavengers. Prevention of metmyoglobin formation was influenced by the number but not the type of oxygen scavenger employed.

Payne *et al*. (1998) examined the effect of vacuum CAP with carbon dioxide, packs flushed with carbon dioxide, packs flushed with carbon dioxide and containing Ageless™ (Z50) oxygen scavengers and packages containing oxygen scavengers alone on the drip loss, microbial and sensorial properties of *M. longissimus lumborum* stored for up to 20 weeks at −1.5°C. Beef in packs flushed with carbon dioxide and flushed containing the oxygen scavenger had lower drip loss than the standard CAP system. The packages flushed with carbon dioxide and those containing the oxygen scavenger alone gave the best results, depending on the storage shelf life required.

In addition to fresh beef, oxygen-scavenging technology has also been applied to pork (Doherty and Allen, 1998) and pork products, where, Martínez *et al*. (2006) reported that fresh pork sausages stored in 20% CO_2:80% N_2 plus an oxygen scavenger (Ageless® FX-40) for up to 20 days at 2 ± 1°C had reduced psychrotrophic aerobe counts and an extended shelf life in terms of colour and lipid stability. Smith *et al*. (1995) described the successful usage of Ageless oxygen scavengers in minimizing chemical and microbial spoilage of seafood products at retail level, which included dried seaweed, dried salmon jerky, dried sardines, dried shark's fin, dried rose mackerel, dried cod, dried squid, fresh yellow tail, sliced salmon, dried/smoked salmon, dried octopus leg, dried bonito, salmon roe, dried squid/vinegar/soybean sauce and sea urchin. It could be argued that, because of the unique composition that certain seafood products possess – that is, unique pigmentation, high levels of polyunsaturated fats and high initial microbiological loadings – such products are the most suitable in terms of utilizing oxygen-scavenging technologies within first-level packaging systems to minimize the effects of oxygen on these components.

Oxygen-scavenging labels are widely used commercially as oxygen scavengers in pre-packed cooked meat products. Emco Packaging Systems, specialists in active and intelligent packaging, are a UK manufacturer and distributor for ATCO® DE 10S self-adhesive oxygen-absorbing labels. Emco supply ATCO® labels for use in pre-packed sliced cooked meats, especially hams, to meat processors in Ireland, throughout the UK and in Europe. While labels used in sliced cooked meat packages scavenge between 10 and 20 ccs of oxygen, Emco have recently launched larger oxygen-scavenging labels onto the market (ATCO® 100 OS and 200 OS), which scavenge between 100 and 200 ccs oxygen, for use in larger-capacity packaging applications.

An alternative to sachets involves the incorporation of the oxygen scavenger into the packaging structure itself. This minimizes negative consumer responses and offers a potential economic advantage through increased outputs. It also eliminates the risk of accidental rupture of the sachets and inadvertent consumption of their contents (Suppakul *et al*., 2003).

Cryovac® 0S2000™ polymer-based oxygen-scavenging film has been developed by Cryovac Div., Sealed Air Corporation, of the United States. This UV light-activated oxygen-scavenging film, which structurally is composed of an oxygen-scavenger layer extruded into a multilayer film, can reduce headspace oxygen levels from 1% to ppm levels in 4–10 days, comparable with oxygen-scavenging sachets. The OS2000™ scavenging films have applications in a wide variety of food products including dried or smoked meat products and processed meats (Butler, 2002). A similar UV light-activated oxygen-scavenging polymer, $ZERO_2$™, developed by CSIRO, Div. of Food Science Australia in collaboration with VisyPak Food Packaging, Visy Industries, Australia, forms a layer in a multilayer package structure and has many applications, including reduced discoloration of sliced meats.

One rather novel oxygen-scavenger system was documented by Altieri *et al*. (2004). These researchers manufactured oxygen-scavenger films using aerobic microorganisms as the 'active compound'. They entrapped the microorganisms *K. varians* DSM 20033 and *P. subpelliculosa* in a polymeric film of either

hydroxyethyl cellulose (HEC) or polyvinyl alcohol (PVOH). These researchers found that the desiccated film could be stored over a period of 20 days without any appreciable decrease in microbial viability. These films were able to remove oxygen from the vial-active space; however, the authors suggested that the best efficiency of oxygen absorption might be achieved by using the film as an active coating for high-humidity foods.

20.2.2 Carbon dioxide emitters and scavengers

The function of carbon dioxide within a packaging environment is to suppress microbial growth. Therefore, a carbon dioxide-generating system can be viewed as a technique complementary to oxygen scavenging (Suppakul *et al*., 2003). Since the permeability of carbon dioxide is three to five times higher than that of oxygen in most plastic films, it must be continuously produced to maintain the desired concentration within the package (Ozdemir and Floros, 2004). High carbon dioxide levels (10–80%) are desirable for foods such as meat, poultry and seafood in order to inhibit surface microbial growth and extend shelf life. Removal of oxygen from the package creates a partial vacuum which may result in the collapse of flexible packaging. Additionally, when a package is flushed with a mixture of gases, including carbon dioxide, the carbon dioxide dissolves in the product creating a partial vacuum. In such cases, the simultaneous release of carbon dioxide from inserted sachets which consume oxygen is desirable. Such systems are based on either ferrous carbonate or a mixture of ascorbic acid and sodium bicarbonate (Rooney, 1995). Examples of commercially available dual-action combined carbon dioxide generators/oxygen scavengers are Ageless® G (Mitsubishi Gas Chemical Co, Japan) and FreshPax® M (Multisorb Technologies Inc, USA). Sivertsvik (1999) showed that by combining various approaches to MAP with oxygen-absorbing and carbon dioxide-releasing forms of active packaging the microbiological quality of salmon fillets were superior to other packaging approaches investigated which omitted the use of intelligent packaging devices.

Carbon dioxide-emitting sachets or labels can also be used alone. The Verifrais™ package, manufactured by SARL Codimer (Paris, France) has been used to extend the shelf life of fresh meats and fish. This innovative package consists of a standard MAP tray but has a perforated false bottom under which a porous sachet containing sodium bicarbonate/ascorbate is positioned. When juice exudates from the packaged meat drips onto the sachet, carbon dioxide is emitted, thus replacing any carbon dioxide absorbed by the meat and preventing package collapse. The product CO_2 Fresh Pads, patented by CO_2 Technologies, functions in a similar manner and has been positioned in the market to be used for meat, poultry and seafood products. Like moisture-absorbing pads (which will be described later), the drip or moisture loss from these muscle foods is absorbed into the pads whereupon the moisture reacts with citric acid and sodium bicarbonate contained within the pads, consequently resulting in the generation of carbon dioxide which is contributed to the internal atmosphere of the package, thereby enhancing product preservation.

The inhibition of spoilage bacteria utilizing active packaging technology may reduce bacterial competition and thus permit growth and toxin production

by non-proteolytic *C. botulinum* or the growth of other pathogenic bacteria (Sivertsvik, 2003). Lövenklev *et al*. (2004) reported that while a high concentration of carbon dioxide decreased the growth rate of non-proteolytic *C. botulinum* type B, the expression and production of toxin was greatly increased, which means the risk of botulism may also be increased, instead of reduced, if used in MAP systems. Research into the risks associated with the use of carbon dioxide in packaging systems is necessary.

Carbon dioxide absorbers (sachets) consisting of either calcium hydroxide and sodium hydroxide, or potassium hydroxide, calcium oxide and silica gel may be used to remove carbon dioxide during storage in order to prevent gas-generated pressure building up and bursting of the package. Possible applications include their use in packs of dehydrated poultry products and beef jerkey (Ahvenainen, 2003), as well as in fermented or roasted foods (Lee *et al*., 2001). CO_2 scavengers can be composed either of a physical absorbent such as Zeolite or an active carbon powder, or of a chemical absorbent such as calcium hydroxide, sodium carbonate, magnesium hydroxide, etc. (Charles *et al*., 2006). One such example is the use of CO_2 absorbers in the form of Zeolite and active carbon to control the pressure build-up and volume expansion of *kimchi* (fermented foods often containing meat and fish) packages due to CO_2 production during fermentation (Lee *et al*., 2001).

20.2.3 Moisture control

The main purpose of liquid water control is to lower the water activity of the product, thereby suppressing microbial growth (Vermeiren *et al*., 1999). Temperature cycling of high water activity foods has led to the use of plastics with an anti-fog additive that lowers the interfacial tension between the condensate and the film. This contributes to the transparency of the film and enables the customer to clearly see the packaged food (Rooney, 1995), although it does not affect the amount of liquid water present inside the package. Several companies manufacture drip-absorbent sheets or pads such as Cryovac® Dri-Loc® (Sealed Air Corporation, USA), Thermarite® or Peaksorb® (Australia), Toppan™ (Japan) and Fresh-R-Pax™ (Maxwell Chase Technologies, LLC, USA) for liquid control in high water activity foods such as meat, poultry and seafood. These systems consist of a super-absorbent polymer located between two layers of a micro porous or non-woven polymer. This material, which is supplied as sheets of various sizes, is used as a drip-absorbing pad which can typically be found in tray formatted (overwrap and MAP) fresh muscle-food products, including beef steaks, premium beef roasts, pork loin chops, lamb chops, lamb leg cuts, poultry pieces, fresh chickens, turkeys and ducks, fish cutlets, fish darnes and skinless fish fillets. The format and dimension of the pad for application is determined by the size and weight of the product to be placed in the tray and on the anticipated drip loss emanating from specific product types.

A novel approach to extending the shelf life of fresh fish is presented in the commercial form called ‘Pitchit films’, which have been developed by the Showa Denko Company in Tokyo, Japan. Pitchit films form a kind of pillow pack which

contains propylene glycol held between layers of polyvinyl alcohol (PVA). PVA is traditionally used in laminate constructions for its excellent gas-barrier properties; however, this can only be achieved when sandwiched between two packaging layers that protect it from water, as this limits its performance. While PVA is permeable to water, it is impermeable to propylene glycol. Consequently, when this film is wrapped around muscle foods, the propylene glycol absorbs free water from the product surface through the PVA film, thereby preventing spoilage microorganisms from proliferating and extending product shelf life. The use of such films reduced microbial counts in seafood products (Labuza and Breene, 1989) and enhanced colour characteristics in a range of muscle foods (Arakawa *et al*., 1990).

20.3 Antimicrobial packaging

Food spoilage is any change which renders a food product unfit for human consumption (Hayes, 1985). Food spoilage is an important global issue, with some figures suggesting that 25% of all the world's food supply is lost through microbial spoilage alone (Huis In't Veld, 1996). In developed countries the majority of spoilage can be attributed to microbial activity, usually by psychrotrophic microorganisms, yeasts and moulds. This may present itself as visible growth (slime, colonies), as textural changes (degradation of polymers) or as off-odours and off-flavours (Gram *et al*., 2002). The type of organism involved in spoilage of a food or beverage product depends greatly on the characteristics of the product as a substrate base and on processing, preservation and storage conditions. The degree of proliferation of the spoilage organisms present depends on a number of physical properties of the food. These include intrinsic parameters such as water activity (a_w), pH and redox potential, and on extrinsic environmental factors parameters such as storage temperature, humidity and the surrounding atmosphere. Spoilage is most rapid in proteinaceous foods such as meat, poultry, fish, milk and some other dairy products, as these products are highly nutritious, possess a neutral or slightly acid pH and a high a_w (Huis In't Veld, 1996).

Microbial contamination and subsequent growth reduces the shelf life of foods and increases the risk of food borne illness. Traditional methods of preserving foods from the effect of microbial growth include thermal processing, drying, freezing, refrigeration, irradiation, MAP and addition of antimicrobial agents or salts. However, some of these techniques cannot be applied to food products such as fresh meats (Quintavalla and Vicini, 2002). Antimicrobial packaging is a promising form of active packaging, especially for meat products. Since microbial contamination of meat products occurs primarily at the surface, due to post-processing handling, attempts have been made to improve safety and to delay spoilage by the use of antibacterial sprays or dips. Limitations of such antibacterials include neutralization of compounds on contact with the meat surface or diffusion of compounds from the surface into the meat mass. Incorporation of bactericidal agents into meat formulations may result in partial inactivation of

the active compounds by meat constituents and therefore exert a limited effect on surface microflora (Quintavalla and Vicini, 2002). Antimicrobial food packaging materials have to extend the lag phase and reduce the growth phase of microorganisms in order to extend shelf life and to maintain product quality and safety (Han, 2000). Comprehensive reviews on antimicrobial food packaging have been published by Appendini and Hotchkiss (2002) and Suppakul *et al*. (2003). To confer antimicrobial activity, antimicrobial agents may be coated, incorporated, immobilized, or surface modified onto package materials (Suppakul *et al*., 2003). A comprehensive list of antimicrobial agents for use in antimicrobial films, containers and utensils is presented in a review by Suppakul *et al*. (2003). The classes of antimicrobials listed range from acid anhydride to alcohol, bacteriocins, chelators, enzymes, organic acids and polysaccharides. Many antimicrobial members derived from these ingredient classes have been evaluated for their antimicrobial properties in various film structures, synthetic polymers and edible films.

Bacteriocins are bacterial proteinaceous products which are produced by a variety of bacteria to inhibit the growth of closely related species (Farkas-Himsley, 1980). A number of these have been incorporated into packaging systems with a view to inhibiting microbial growth. In one study, Enterocin 416K1, a bacteriocin produced by *Enterococcus casseliflavus* IM 416K1, was entrapped in an organic-inorganic hybrid coating applied to a LDPE (low-density polyethylene) film (Iseppi *et al*., 2008) and its anti-listerial activity was evaluated. Coating was achieved by spin-coating followed by thermal deposition using a poly(ethylene)-block-poly(ethylene glycol) (PE-PEG) co-polymer. The study demonstrated that the activated coatings significantly inhibited the growth of *Listeria monocytogenes* in artificially contaminated food samples (frankfurters and fresh cheeses) at both room and refrigeration temperatures. Nisin is another bacteriocin which has also been shown to exhibit antimicrobial properties (Cao-Hoang *et al*., 2010; Nguyen *et al*., 2008). Cao-Hoang *et al*. (2010) produced nisin-containing sodium caseinate films, produced by a standard casting protocol (Kristo *et al*., 2008), which showed an ability to inhibit the growth of *Listeria innocua* in inoculated soft cheese by direct surface contact.

Lysozyme is one of the most frequently used biopreservatives in antimicrobial packaging (Quintavalla and Vicini, 2002). This is an enzyme which shows antimicrobial activity mainly on Gram-positive bacteria by splitting the bonds between *N*-acetylmuramic acid and *N*-acetylglucosamine of the peptidoglycan in their cell walls (Mecitoglu *et al*., 2006). Lysozyme has been well studied as an active packaging constituent (Gemili *et al*., 2009; Mendes De Souza *et al*., 2010; Mecitoglu *et al*., 2006). Mecitoglu *et al*. (2006) incorporated lyophilized lysozyme into an edible corn zein film and demonstrated that these films had an inhibitory effect on different bacteria including *Bacillus subtilis* and *Lactobacillus plantarum.* Lysozyme has also been combined with other antimicrobial agents in packaging systems. In one study a mixture of lysozyme and nisin at a ratio of 3:1 (w/w) was applied to pork loins that were then stored in vacuum packages at 2°C for up to 6 weeks. The mixture was shown to be effective in controlling the growth of lactic acid bacteria, lactic acid bacteria able to grow in the presence of

acetate and *Brochothrix thermosphacta* (Nattress and Baker, 2003). In another study a lysozyme and nisin were again combined and applied to the surface of ready-to-eat turkey bologna, which was then pasteurized, vacuum-packaged and refrigerated. The antimicrobial combination significantly reduced the recovery and growth of *Listeria monocytogenes* post-pasteurization (Mangalassary *et al*., 2008). Other enzymes with potential antimicrobial activity like glucose oxidase have been studied (Field *et al*., 1986; Labuza and Breene, 1989; Padgett *et al*., 1998), but less extensively.

The organic acids sorbic acid, p-aminobenzoic acid, lactic acid and acetic acid have a long history as generally recognized as safe (GRAS) food preservatives and there is a growing demand for natural preservatives such as these in the food industry (Burt, 2004). When used in combination with lactic and/or acetic acid, sorbic acid can inhibit the growth of *Listeria monocytogenes*, *Salmonella typhimurium* and *E. coli* O157:H7 in many low-acid foods, including cold-pack cheese, bologna and beaker sausage (Cagri *et al*., 2001), while p-aminobenzoic acid has been reported to exhibit significant inhibitory activity against *L. monocytogenes*, *E. coli* and *Salmonella enteritidis* (Richards, 1995). Edible whey protein isolate films incorporating sorbic acid and p-aminobenzoic acid have shown inhibitory action towards of *Salmonella typhimurium*, *L. monocytogenes* and *E. coli* O157:H7 when placed in direct contact with inoculated culture (Cagri *et al*., 2001). A study was also undertaken to evaluate antimicrobial films prepared by incorporating acetic or propionic acid into a chitosan matrix, with or without addition of lauric acid or the essential oil, cinnamaldehyde (Ouattara *et al*., 2000). These films were directly applied to bologna, regular cooked ham or pastrami. Propionic acid was released from the chitosan matrix at a faster rate than acetic acid and the addition of lauric acid, but not cinnamaldehyde, to the chitosan matrix reduced the release of acetic acid. However, lactic acid bacteria were not affected by the antimicrobial films under study, the growth of Enterobacteriaceae and *Serratia liquefaciens* was delayed or completely inhibited as a result of film application. Strongest inhibition was observed on drier surfaces (bologna), onto which acid release was slower, and with films containing cinnamaldehyde, as a result of its greater antimicrobial activity under these conditions. One novel study combined MAP packaging and organic acid incorporation on the preservation of fresh salmon (Schirmer *et al*., 2009). The salmon was packed with a small amount of 100% CO_2 (gas/product ratio 0.2/1.0 v/v) and a brine solution containing various combinations of citric acid (3% w/w, pH 5), acetic acid (1% w/w, pH 5) and cinnamaldehyde (200 μg mL^{-1}). CO_2, acetic acid and citric acid alone each inhibited the growth of total bacterial counts, lactic acid bacteria, sulphur-reducing bacteria and Enterobacteriaceae, but effects were enhanced in combination. It was found that the combination of CO_2 and organic acids completely inhibited bacterial growth during 14 days of storage at 4°C both in inoculation experiments and in experiments on salmon with natural background flora. The addition of cinnamaldehyde did not influence bacterial growth.

Many *in vitro* studies have demonstrated antibacterial activity of essential oils (EOs) against *L. monocytogenes*, *S. typhimurium*, *E. coli* O157:H7, *Shigella*

dysenteria, *Bacillus cereus* and *S. aureus* at levels between 0.2 and 10 μL mL^{-1} . A number of EO components have been identified as effective antibacterials – for example, carvacrol, thymol, eugenol, perillaldehyde, cinnamaldehyde and cinnamic acid, having minimum inhibitory concentrations (MICs) of 0.05–5 μL mL^{-1} *in vitro*. A higher concentration is needed to achieve the same effect in foods (Burt, 2004). Direct application of eugenol, coriander, clove, oregano and thyme oils have been found to be effective at levels of 5–20 μL g^{-1} in inhibiting *L. monocytogenes*, *Aeromonas hydrophila* and autochthonous spoilage flora in meat products (Burt, 2004). Essential oils have also been used as antimicrobials in fish products, with a high fat content appearing to reduce effectiveness. For example, oregano oil at 0.5 μL g^{-1} is more effective against the spoilage organism *Photobacterium phosphoreum* on cod fillets than on salmon, which is a fatty fish (Mejlholm and Dalgaard, 2002). One study was conducted to evaluate the combined effect of low-dose gamma irradiation and essential oil coating on the shelf life of pre-cooked shrimp (Ouattara *et al*., 2001). Antimicrobial coatings were obtained by incorporating various concentrations of thyme oil and *trans*-cinnamaldehyde in coating formulations prepared from soy or whey protein isolates. Coated shrimps were stored at 4 ± 1°C under aerobic conditions. Results showed that gamma irradiation and coating treatments had synergistic effects in reducing the aerobic plate counts and *Pseudomonas putida* numbers resulting in at least a 12-day extension of shelf life. However, an issue commonly associated with essential oils emerged. Incorporation of 1.8% essential oils in the coating solutions significantly decreased the sensory acceptability of the products.

Interest in biopolymers has increased greatly in recent years, owing to their renewable biodegradable nature and their natural derivation. Some polymers are inherently antimicrobial and have been used in films and coatings. Polylactic acid (PLA), made primarily from renewable agricultural (i.e., corn) sources, is one such polymer (Cutter, 2006). PLA polymers are composed of chains of lactic acid and exhibit high tensile strength, are resistant to oil-based products, sealable at low temperatures and can act as flavour and odour barriers for foods (Cutter, 2006). Several studies have shown the antimicrobial effect of PLA. Mustapha *et al*. (2002) demonstrated the effect of PLA alone or in combination with lactic acid or nisin against *E. coli* O157:H7 in vacuum-packaged, irradiated, raw meat. However, these authors noted here that the inhibitory action was not significantly greater than lactic acid alone. Other studies also identified the antimicrobial action PLA, but also showed limitations. Chellappa (1997) examined the effect of PLA for reducing pathogens on raw meat. *E. coli* O157:H7, *L. monocytogenes*, *S. typhimurium* or *Yersinia enterocolitica* associated with lean beef surfaces were treated with PLA, lactic acid or sterile water. PLA treatments at pH of 3.0 resulted in significant reductions of *E. coli* O157:H7; however, *E. coli* O157:H7 was not inhibited when PLA was applied at pH 5.0, 6.0 and 7.0 (Chellappa, 1997). These limitations suggest PLA may be best utilized in active packaging systems through combination with other active molecules like nisin, as above, or by blending with other biopolymers with complementary properties like chitosan (Suyatma *et al*., 2004).

Chitosan is a linear polysaccharide consisting of (1,4)-linked 2-amino-deoxy-β-d-glucan, is a deacetylated derivative of chitin, which is the second most abundant polysaccharide found in nature after cellulose. Chitosan has been found to be non-toxic, biodegradable, biofunctional, biocompatible in addition to having antimicrobial characteristics (Dutta *et al*., 2009). Edible film coatings of chitosan alone or in combination with another biopolymer, sodium caseinate, have been applied to salami samples (Moreira *et al*., 2011). Chitosan and sodium caseinate/chitosan films exerted a significant bactericidal action on mesophilic, psychrotrophic bacteria, as well as a reduction in yeast and mould counts in the three samples. Greater bactericidal properties were observed in the caseinate/chitosan than in the chitosan alone. This can be attributed to the greater film-forming and thermoplastic properties of sodium caseinate – that is, the polymers work synergistically. To harness its antimicrobial properties and overcome its weak film-forming properties chitosan has also been cross-linked with existing packaging polymers. One study developed a novel antimicrobial coating based on chitosan and poly(vinyl alcohol) (PVA) and evaluated its effect on minimally processed tomato (Tripathi *et al*., 2009). Films were prepared by blending chitosan and PVA with glutaraldehyde as a cross-linker. Fourier-transform infrared spectroscopy (FTIR) results showed that a molecular miscibility between PVA and chitosan was achieved. The microbiological screening demonstrated the antimicrobial activity of the film against *E. coli*, *S. aureus* and *B. subtilis*. Chitosan-based films have also been experimented with in the packaging of meat, fish and other food products (Dutta *et al*., 2009; Izumimoto, 1994; Jongrittiporn *et al*., 2001). A number of other biopolymers have been investigated in relation to antimicrobial packaging. Among these is the seaweed-derived alginate which possesses good film-forming properties. Alginate films have demonstrated an ability to reduce microbial counts; however, films have often been found to be unacceptable from a sensory point of view owing to the bitterness imparted by the calcium chloride required to set them (Dang *et al*., 2009).

Other sourced antimicrobials such as triclosan, silver zeolites and fungi have been incorporated directly into polymers (Quintavalla and Vicini, 2002). Silver-substituted zeolites are the most widely used of these. Sodium ions present in zeolites are substituted by silver ions, which are antimicrobial against a wide range of bacteria and moulds. These substituted zeolites are incorporated into polymers like polyethylene, polypropylene, nylon and butadiene styrene at levels of 1–3% (Appendini and Hotchkiss, 2002).

Examples of commercial antimicrobial materials in the form of concentrates (e.g., AgION™, AgION Technologies LLC, USA) extracts (Nisaplin® (Nisin), Integrated Ingredients, USA) and films (Microgard™ Rhone-Poulenc, USA) have been presented. Antimicrobial packages have had relatively few commercial successes outside of Japan where Ag-substituted Zeolite is the most common antimicrobial agent incorporated into plastics; commercial examples of these zeolites include AgION, Zeomic®, Apacider®, Bactekiller and Novaron.

Ag-ions inhibit a range of metabolic enzymes and have strong antimicrobial activity (Vermeiren *et al*., 1999). Antimicrobial films can be classified into two

types: those that contain an antimicrobial agent which migrates to the surface of the food and those which are effective against surface growth of microorganisms without migration.

20.3.1 Coating of films with antimicrobial agents

Coating of films with antimicrobial agents can result in effective antimicrobial activity. Natrajan and Sheldon (2000) carried out a study to evaluate the potential use of packaging materials as delivery vehicles for carrying and transferring nisin-containing formulations onto the surfaces of fresh poultry products. The efficacy of nisin coated (100 μg/mL) polymeric films of varying hydrophobicities (polyvinyl chloride (PVC), linear low-density polyethylene (LLDPE) and nylon in inhibiting *S. typhimurium* on fresh broiler drumstick skin was evaluated. It was concluded that packaging films coated with nisin were effective in reducing *S. typhimurium* on the surface of fresh broiler skin and drumsticks.

20.3.2 Incorporation of antimicrobial agents

The direct incorporation of antimicrobial additives in packaging films is a convenient means by which antimicrobial activity can be achieved. Ouattara *et al*. (2000) carried out a study to assess the inhibition of surface spoilage bacteria in processed meats following the application of antimicrobial films prepared with chitosan. Antimicrobial films were prepared by incorporating acetic or propionic acid into a chitosan matrix, with or without addition of lauric acid or cinnamaldehyde, and were applied onto bologna, regular cooked ham or pastrami. During the storage period, packages were opened and the amount of antimicrobial agents remaining in the chitosan matrix was measured. Propionic acid was released from the matrix must faster than acetic acid. Addition of lauric acid, but not cinnamaldehyde, to the chitosan matrix reduced the release of acetic acid and the release was more limited onto bologna than onto ham or pastrami. Lactic acid bacteria were unaffected by the antimicrobial films studied whereas growth of *Enterobacteriaceae* and *Serratia liquefaciens* (surface-inoculated onto the meat products) was delayed or completely inhibited as a result of film application. The strongest inhibition was observed on drier surfaces (bologna), onto which acid release was slower, and with films containing cinnamaldehyde, as a result of its greater antimicrobial activity under these conditions. Vermeiren *et al*. (2002) reported that a 1.0% triclosan film had a strong antimicrobial effect *in vitro* simulated vacuum-packaged conditions against the psychrotrophic food pathogen *L. monocytogenes*. However, the triclosan film did not effectively reduce spoilage bacteria and growth of *L. monocytogenes* on refrigerated vacuum-packaged chicken breasts stored at 7°C.

Ha *et al*. (2001) examined the effect of grapefruit seed extract (GFSE), a natural antimicrobial agent, incorporated (0.5% or 1% concentration) by co-extrusion or a solution-coating process in multilayered polyethylene (PE) films, on the microbial status and quality (colour (L, a, b), TBARS and pH) of fresh minced beef.

The antimicrobial activity of the fabricated multilayer films was also evaluated using an agar plate diffusion method. It was reported that coating the PE film with GFSE with the aid of a polyamide binder resulted in greater antimicrobial activity compared to GFSE incorporation by co-extrusion. Using the agar diffusion test, the co-extruded film with 1% w/w GFSE showed antimicrobial activity against *M. flavus* only, whereas a film coated with 1% GFSE showed activity against several microorganisms such as *E. coli*, *S. aureus* and *B. subtilis*. Both types of GFSE-incorporated multilayer PE films reduced the growth of aerobic and coliform bacteria in minced beef wrapped with film and stored for up to 18 days at 3°C, relative to controls. The film coated with a higher concentration (1%) of GFSE had a more pronounced effect in inhibiting bacterial growth compared to the other films tested. GFSE-coated films were better than co-extruded films in preserving the chemical quality (TBARS) of packaged beef. Beef colour was unaffected by packaging treatment. The level of GFSE employed (0.5% and 1%) did not differ significantly in terms of film efficacy for preservation of beef quality.

There is a growing interest in edible coatings due to factors such as environmental concerns, the need for new storage techniques and opportunities for creating new markets for under-utilized agricultural commodities with film-forming properties (Quintavalla and Vicini, 2002). Edible coatings and films prepared from polysaccharides, proteins and lipids have a variety of advantages such as biodegradability, edibility, biocompatibility, aesthetic appearance and barrier properties against oxygen and physical stress. Advantages of using edible coatings and films on meat and meat products have been discussed by Gennadios *et al*. (1997). Edible coatings could:

- help alleviate the problem of moisture loss during storage of fresh or frozen meats
- hold juices of fresh meat and poultry cuts when packed in retail plastic trays
- reduce the rate of rancidity caused by lipid oxidation and myoglobin oxidation
- reduce the load of spoilage and pathogenic microorganisms on the surface of coated meats
- restrict volatile flavour loss and foreign odour pick-up.

As an application of active packaging, edible coatings carrying antioxidants or antimicrobials can be used for the direct treatment of meat surfaces. In the case of edible films and coatings, selection of the incorporated active ingredient is limited to edible compounds, therefore edibility and safety is important. Siragusa and Dickinson (1993) demonstrated that alginate coatings containing organic acids were marginally effective on beef carcasses, reducing levels of *L. monocytogenes, S. typhimurium* and *E. coli* 0157:H7 by 1.80, 2.11 and 0.74 log cycles, respectively. Complete inhibition of *L. monocytogenes* on ham, turkey breast and beef was achieved using pediocin or nisin fixed on a cellulose casing (Ming *et al*., 1997). Commercial application of this technology is described in a US Patent (5 573 797) assigned to a manufacturer of cellulose food casings (Viskase

Co. Inc., USA). The package is a film, such as a polymer film or a regenerated cellulose film, containing heat-resistant *Pediococcus*-derived bacteriocins in synergistic combination with a chelating agent to inhibit or kill *L. monocytogenes* on contact with food (Katz, 1999).

20.3.3 Immobilization

Some antimicrobial packaging systems utilize covalently immobilized antimicrobial substances which suppress microbial growth. Scannell *et al*. (2000) investigated the immobilization of bacteriocins nisin and lacticin 3147 to packaging materials. The plastic film (PE/polyamide (70:30) formed a stable bond with nisin, in contrast to lacticin 3147, and maintained activity for a 3-month period both at room temperature and under refrigerated storage conditions. The antimicrobial packaging reduced the population of lactic acid bacteria in ham stored in MAP (60% N_2:40% CO_2), thereby extending product shelf life. Nisin-adsorbed bioactive inserts reduced the level of *L. innocua* and *S. aureus* in hams.

20.3.4 Other naturally derived antimicrobial agents used in smart packaging applications

The use of naturally derived antimicrobial agents is important as they represent a lower perceived risk to the consumer (Nicholson, 1998). Skandamis and Nychas (2002) studied the combined effect of volatiles of oregano essential oil and modified atmosphere conditions (40% CO_2:30% O_2:30% N_2, 100% CO_2, 80% CO_2, vacuum-packaged and aerobic storage) on the sensory, microbiological and physiochemical attributes of fresh beef stored at 5°C and 15°C. Filter paper containing absorbed essential oil was placed in the packages but not in direct contact with the beef samples. The shelf life of beef samples followed the order: aerobic storage < vacuum packaged < 40% CO_2:30% O_2:30% N_2 < 80% CO_2:20% air < 100% CO_2. Longer shelf life was observed in samples supplemented with the volatile compounds of oregano essential oil.

Ethanol is another example of a naturally derived antimicrobial agent. The incorporation of ethanol in films and sachets for slow release and ethanol vapour generation within food packs has led to the development of commercial products like Ethicap, Antimold 102, Negamold (Freund Industrial), Oitech (Nippon Kayaku), ET Pack (Ueno Seiyaku) and Ageless type SE (Mitsubishi Gas Chemical) and many of these systems have been used in the packaging of semi-moist and dried fish products (Day, 2003, 2008).

20.4 Other applications of smart/active technologies

The sections below look at the different and various ways in which these smart/active technologies can be applied and also consider potential future developments in the field.

20.4.1 Antioxidative packaging

Oxidation is a major mechanism of food deterioration. Lipid oxidation is associated with the development of rancidity and loss in nutritive value of meat products (Maqsood and Benjakul, 2010). This is a particular problem in the packaging of fresh products containing high fat levels, particularly if the fat in question is polyunsaturated in natural form or composition.

While many studies have been reported on the benefits of applying antioxidants directly to muscle-based food products in terms of extending chemical shelf-life stability, little attention has focused on applying antioxidants to packaging materials as an alternative means of exerting the same controlling action on food products, but in a much more indirect and non-contributing manner.

While the practice has been limited, antioxidants have been incorporated into active packing systems. Huang and Weng (1998) investigated the effects of wrapping fish fillets and fish oil in butylated hydroxytoluene (BHT)-incorporated polyethylene-based films. These authors showed that lipid oxidation was reduced in both products by the presence of this synthetic antioxidant compared to non-packaged controls. Active films containing the natural antioxidant oregano were tested for their ability to extend the shelf life of beef steaks through the inhibition of lipid oxidation (Camo *et al*., 2011). The active films were prepared according to an innovative procedure protected by European Patent 1477519-A1 (Garcés *et al*., 2003) based on covering a polypropylene film with a layer of varnish containing the oregano extract. The display life of beef samples with at least 1% oregano exhibited a significant increase in display life from 14 to 23 days and showed a reduction in 2-thiobarbituric acid reactive substances (TBARS) indices. However, at oregano concentrations greater than 4%, a sensory-based unacceptability of the samples emerged. In another study active packaging film containing antioxidants derived from barley husks achieved a reduction in lipid damage during frozen storage of Atlantic cod. The fish samples were packaged in low-density polyethylene film coated with the barley husk extract. After 6 months, oxidation levels in the control sample were approximately 30–50% higher than in samples packaged in film containing antioxidants (Marato *et al*., 2012). This shows the potential of active forms of antioxidants in maintaining the sensory qualities and nutritional value of muscle-based food products.

20.4.2 Flavour/odour adsorbers

During the storage of fresh and processed muscle-based food products, irregular production of off-odours and off-flavours can occur; I describe this phenomenon as ‘compartmentalized odour’. Such odours provide, in many cases, the false impression that the food product in question is putrid and inedible, leading to it being discarded by the consumer. Compartmentalized odour generation is complex and odours generated may be comprised of volatile components derived from the degradation of amino acids, fatty acids, aldehydes, etc. from the muscle product, combining with packaging gases and other volatiles derived from the packaging materials. Packaged seafood products, in particular, and poultry to a lesser degree,

appear to suffer the most from compartmentalized odour generation, with hydrogen sulphide (H_2S) being present and associated with both poultry and seafood, and trimethylamine (TMA) being present and associated with seafood.

Franzetti *et al*. (2001) investigated the effects of using an odour-removing plastic packaging foam tray for various fish species: sole, hake and cuttlefish held under MAP conditions. The odour-removing tray adsorbed TMA to a significant degree compared to the controls. Vermeiren *et al*. (1999) described research conducted in Japan using polymers internally lined with acidic groups to strategically degrade the presence of amines on the surfaces of food products.

Flavour/odour adsorbers may have potential in active-packaging technology for muscle foods. The Anico Co. (Japan) manufactured polymeric bags called Anico bags containing ferrous salt and an organic acid, which could be either citric or ascorbic acid and were claimed to oxidize amines and other oxidizable odour-causing compounds (Rooney, 1995). A number of companies have developed odour-adsorbing technologies which are specific to this function or are combined with other active technologies; Multisorb Technologies (USA) commercially produced odour-adsorbing sachets called MINIPAX1 and STRIPPAX1, United Desiccants (USA) produced a packaging system that combined silica gel and activated carbon for both moisture absorption and odour adsorption in a product called 2-in-1, DuPont (USA) produced an odour and taste control (OTC) technology for aldehyde removal. While available and well described, few of these odour absorbers have been commercially trialled for use with muscle-based foods.

Adsorber systems employ mechanisms such as cellulose triacetate, acetylated paper, citric acid, ferrous salt/ascorbate and activated carbon/clays/zeolites. Mr Rodney Abbott reported that a Swedish company, EKA Noble, in cooperation with a Dutch company, Akzo, developed a range of synthetic aluminosilicate zeolites, which they claim absorb odorous gases within their highly porous structure. Their BHM™ powder can be incorporated into packaging materials, especially those that are paper-based; apparently odorous aldehydes are adsorbed in the pore interstices of the powder (www.pira.co.uk/admin/_private/TechnicalArticles/00123.pdf). Similar applications exist for various flavour-emitting polymers (Ahvenainen, 2003); however, few of these are relevant or applicable to meat, fish or seafood.

20.4.3 Miscellaneous potential future applications of other smart/active technologies

In addition to smart packaging techniques described earlier, additional active technologies, applicable to other foodstuffs (Ahvenainen, 2003), may have potential applications in muscle-based food products. For example, self-heating aluminium or steel cans and containers, currently used by coffee manufacturers (Nescafé, 'hot when you want it'), may have applications in the production of ready meals containing various muscle-based foods. Since consumer demand for ready-to-eat convenience meals is constantly increasing, packaging of ready meals in self-heating active packaging is an important future application. Similarly, self-cooling

technologies may hold some interest for usage with similar products in specific regions of the globe; however, major developments with the technology are still required. Microwave susceptors consist of aluminium or stainless steel deposited on substrates such as polyester films or paperboard and serve to dry, crisp and ultimately brown microwave food. Modifiers for microwave heating consist of a series of antenna structures which alter the way microwaves arrive at food, thereby resulting in even heating, surface browning and crisping (Ahvenainen, 2003). Incorporation of such susceptors or modifiers into muscle-based product packages is an additional future application for active packaging of meat, poultry and fish-based products.

20.5 Sensors for smart packaging

Many smart or intelligent packaging concepts involve the use of sensors and indicators. For the purposes of clarity these two areas will be discussed separately, although such a distinction is somewhat arbitrary and some overlap is unavoidable. The use of these systems is generally envisaged in terms of incorporation into established packaging techniques such as MAP and vacuum packaging.

MAP is an extremely important packaging technique used extensively for the distribution, storage and display of meat products in markets with a controlled cold distribution chain (Sivertsvik *et al*., 2002). MAP works by replacement of the air surrounding a meat product with formulated gas mixtures, thereby extending shelf life and quality. The most important (non-inert) gases in MAP products are oxygen and carbon dioxide and their headspace partial pressures serve as useful indicators of the quality status of a meat product. The profiles of oxygen and carbon dioxide can change over time and are influenced by product type, respiration, packaging material, pack size, volume ratios, storage conditions, package integrity, etc. A number of analytical techniques are available to monitor gas phases in the MAP products. Instrumental techniques such as GC and GC/MS require breakage of package integrity and are time-consuming and expensive. Portable headspace oxygen and/or carbon dioxide gas analysers use 'minimally destructive' techniques (packages can be resealed) but tend not to be applicable to real-time, on-line control of packaging processes or large-scale usage. An optical sensor approach offers a realistic alternative to such conventional methods (Peterson *et al*., 1984).

A sensor is defined as a device used to detect, locate or quantify energy or matter, giving a signal for the detection or measurement of a physical or chemical property to which the device responds (Kress-Rogers, 1998a). To qualify as a sensor, a device must be able to provide continuous output of a signal. 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. The transducer is a device capable of transforming the energy carrying the physical or chemical information about the sample into a useful analytical signal.

Research and development of sensor technology has, until recently, been largely concentrated in biomedical and environmental applications (Demas *et al*., 1999). The specifications of such sensors are, however, quite different from those required for food packaging applications. The development of improved methods to determine food quality such as freshness, microbial spoilage, oxidative rancidity or oxygen and/or heat induced deterioration is extremely important to food manufacturers. In order to maximize the quality and safety of foodstuffs, a prediction of shelf life based on standard quality control procedures is normally undertaken. Replacement of such time-consuming and expensive quality measurements with rapid, reliable and inexpensive alternatives has led to greater efforts being made to identify and measure chemical or physical indicators of food quality. The possibility of developing a sensor for rapid quantification of such an indicator is known as the marker approach (Kress-Rogers, 2001). Determination of indicator headspace gases provides a means by which the quality of a meat product and the integrity of the packaging in which it is held can be established rapidly and inexpensively. One means of doing so is through the production of intelligent packaging incorporating gas-sensor technology.

Chemical-sensor and biosensor technology has developed rapidly in recent years. The main types of transducers with potential use in meat-packaging systems include electrical, optical, thermal or chemical signal domains. Sensors can be applied as the determinant of a primary measurable variable or, using the marker concept, as the determinant of another physical, chemical or biological variable (Kress-Rogers, 1998a). In the case of headspace gas sensing, accurate measurements are desirable as indicators of meat, poultry and seafood product quality. Recent developments in sensor technology have narrowed the gap between the theoretical and the commercially viable, and, although practical uses of sensors in the meat industry remain very limited, significant practical steps towards more widespread use have been made (Kerry and Papkovsky, 2002). High development and production costs, strict industry specifications, safety considerations and relatively limited demand (in comparison with the biomedical sector), from industry and consumer alike, have proved the main obstacles to commercial use. Very few systems to date have been able to match exacting industry standards required for successful application. However, developments in materials science, continuous automation processes, signal processing and process control, along with transfer of technology from the biomedical, environmental and chemical sectors all lead towards the likelihood of more universal adoption of sensor technology in food packaging. Greater pressure on food manufacturers to guarantee safety, quality and traceability is also likely to promote the establishment of commercial sensor technology in food packaging.

20.5.1 Gas sensors

Gas sensors are devices that respond reversibly and quantitatively to the presence of a gaseous analyte by changing the physical parameters of the sensor; they are monitored by an external device. Systems presently available for gas

detection include amperometric oxygen sensors, potentiometric carbon dioxide sensors, metal oxide semiconductor field effect transistors, organic conducting polymers and piezoelectric crystal sensors (Kress-Rogers, 1998b). Conventional systems for oxygen sensors based on electrochemical methods have a number of limitations (Trettnak *et al*., 1995). These include factors such as consumption of analyte (oxygen), cross-sensitivity to carbon dioxide, hydrogen sulphide and fouling of sensor membranes (Gnaiger and Fortsner, 1983). Such systems also involve destructive analysis of packages.

In recent years, a number of instruments and materials for optical oxygen-sensing have been described (Papkovsky *et al*., 1995; Thompson and Lakowicz, 1993; Trettnak *et al*., 1995). Such sensors are usually comprised of a solid-state material, which operates on the principle of luminescence quenching or absorbance changes caused by direct contact with the analyte. Such systems provide a non-invasive technique for gas analysis through translucent materials and as such are potentially suitable for intelligent packaging applications. The solid-state sensor is inert and does not consume analyte or undergo other chemical reactions (Wolfbeis, 1991). Optochemical sensors have the potential to enhance quality control systems through detection of product deterioration or microbial contamination by sensing gas analytes such as hydrogen sulphide, carbon dioxide and amines (Wolfbeis and List, 1995).

Approaches to optochemical sensing have included (a) a fluorescence-based system using a pH-sensitive indicator (Wolfbeis *et al*., 1988), (b) absorption-based colorimetric sensing realized through a visual indicator (Mills *et al*., 1992) and (c) an energy transfer approach using phase fluorimetric detection (Neurater *et al*., 1999). The latter allows for the possibility of combining oxygen and carbon dioxide measurements in a single sensor through compatibility with previously developed oxygen-sensing technology. Most carbon dioxide sensors, however, have been developed for biomedical applications and the potential use of existing carbon dioxide sensors for food packaging applications is still somewhat distant (Kerry and Papkovsky, 2002).

Fluorescence-based oxygen sensors

Flourescence-based oxygen sensors represent the most advanced and promising systems to date for remote measurement of headspace gases in packaged meat products. Reiniger *et al*. (1996) first introduced the concept of using luminescent dyes quenched by oxygen as non-destructive indicators in food packaging applications. A number of oxygen-sensing prototypes have been developed and are expected to appear in large-scale commercial applications in the near future. These sensors can be produced cheaply, are disposable and when used in conjunction with accurate instrumentation provide rapid determination of oxygen concentration (Kerry and Papkovsky, 2002).

The active component of a fluorescence-based oxygen sensor normally consists of a long-delay fluorescent or phosphorescent dye encapsulated in a solid polymer matrix. The dye-polymer coating is applied as a thin film coating on a suitable solid support (Wolfbeis, 1991). Molecular oxygen, present in the packaging

headspace, penetrates the sensitive coating through simple diffusion and quenches luminescence by a dynamic – that is, collisional mechanism. Oxygen is quantified by measuring changes in luminescence parameters from the oxygen-sensing element in contact with the gas or liquid sample, using a pre-determined calibration. The process is reversible and clean: neither the dye nor oxygen is consumed in the photochemical reactions involved, no by-products are generated and the whole cycle can be repeated.

Materials for oxygen sensors must meet strict sensitivity and working performance requirements if they are to prove suitable for commercial intelligent packaging applications. They must also have fluorescent characteristics suited to the construction of simple measuring devices. Fluorescence and phosphorescence dyes with lifetimes in the microsecond range are best suited to oxygen sensing in food packaging. Other necessary features include suitable intensity, well-resolved excitation and emission long wave bands and good photostability characteristics of the indicator dye. Such features allow sensor compatibility with simple and inexpensive optoelectronic measuring devices (LEDs, photodiodes etc.), minimize interference by scattering and sample fluorescence and allow long-term operation without recalibration (Papkovsky, 1995). Materials using fluorescent complexes of ruthenium, phosphorescent palladium(II)- and platinum(II)-porphyrin complexes and related structures have shown promise as oxygen sensors (Papkovsky *et al*., 1991, 1995; Wolfbeis, 1991). Subsequent work on phosphorescent complexes of porphyrin-ketones elucidated favourable sensing properties such as high stability, water insolubility, non-volatility and low toxicity (Papkovsky *et al*., 1995).

The combination of indicator dye and the encapsulating polymer medium in which oxygen quenching occurs determines the sensitivity and effective working range of such sensors. For the purposes of food packaging applications, dyes with relatively long emission lifetimes (~ 40–500 μs) such as Pt-porphyrins combined with polystyrene as polymer matrix appear to offer greatest potential (Papkovsky *et al*., 2000; Wolfbeis, 1991). Sensors on microporous support materials (Papkovsky *et al*., 1998) also provide a number of unique features for special sensing applications, including those applicable to food packaging systems. Other polymers with good gas-barrier properties such as polyamide, polyethylene terephthalate and PVC are not suitable for oxygen sensing as oxygen quenching is slow in such media (Comyn, 1985). The use of plasticized polymers is also unsuitable due to toxicity concerns associated with potential plasticizer migration.

Sensor fabrication involves a simple process of dissolution of lipophilic indicator dye and appropriate polymer support in an organic solvent. This cocktail is applied to a solid substrate such as a polyester film or glass and allowed dry to produce a fluorescent film coating or spot. A number of coating techniques that lend themselves to large-scale, continuous production (casting, dipping, spin-coating, drop dispensing and spraying) offer possibilities for commercial production. Relatively high concentrations of indicator dye are used to obtain high fluorescence signals. Sensors, normally 1–2 cm in diameter, are coloured (due to the dye) and are readily visible on different support materials.

Oxygen-sensor active elements can be manufactured on a large scale using relatively inexpensive materials and equipment. They are robust, suitable for long-term/continuous monitoring and can be disposed of easily. Such materials have been successfully used in a variety of non-food applications. In order to ensure successful commercial uptake in food packaging a number of practical criteria must be considered:

- *Working range*: Most oxygen sensors work effectively within two orders of oxygen concentration (and in some cases more). Most of the sensors described work within the range from 0 to100 kPa of oxygen, or at least 0–21 kPa (0–21%) with detection limits of 0.01–0.1 kPa (where, in simple terms, kPa corresponds to percentage oxygen pressure (at room temperature and ambient air pressure)). Such working ranges are, in general suitable for many meat-packaging applications and MA packaging in particular.
- *Temperature dependence*: Sensors for food packaging applications are required to operate over a wide temperature range (~ − 20°C to + 30°C). A lack of systematic and comparative data exists on the behaviour of oxygen sensors over such wide temperature ranges with few studies having addressed this issue (Papkovsky *et al*., 2000). Further research is required to ensure the effectiveness of such systems under all meat storage and distribution conditions.
- *Response*: The use of thin film coatings for the sensing material results in low diffusion barrier properties and very fast sensor responses to changes in oxygen concentration – in some case as low as tenths of milliseconds (Kolle *et al*., 1997). This feature is important for real-time, on-line quality control of large-volume throughput of packages. Such rapid screening allows for immediate identification of improperly sealed units and their removal.
- *Stability*: Sensors incorporated into meat packages are required to remain operable and reliable from the point of packaging to the point of opening. In the case of chill storage of meat products this can be up to several weeks duration. Exposure to light, including UV/retail display lighting can cause gradual photobleaching of certain dyes or ageing of polymers. In the case of phase fluorimetric oxygen sensors this is not important but can be problematical for other sensor types.
- *Intrinsic toxicity*: Sensor materials – that is, dyes, polymers, residual solvents and additives – are the main cause for concern in terms of potential toxicity issues. In general, the total quantity required to produce a single pack sensor is normally less than 1 mg, of which the encapsulating polymer represents > 95%. The amount of dye per sensor usually varies to within a few micrograms. For most organic dyes, such quantities are far below established toxicity levels. It is advisable that solvents normally used in the food industry be used in sensor manufacture in order to avoid dangers associated with residual solvents. O'Riordan *et al*. (2005) examined the migration of active components of two metalloporphyrin and one ruthenium dye-based oxygen sensors and established their stability, safety and suitability for large-scale use in food packaging applications.

Other recent publications on the suitability of fluorescence-based oxygen sensors have provided much useful data on their effectiveness in meat packaging applications. Fitzgerald *et al*. (2001) examined the potential of platinum-based disposable oxygen sensors as a quality control instrument for vacuum-packed raw and cooked meat and MA-packed sliced ham. Direct contact of sensors on the foods provided accurate oxygen profiles over time and correlated well with conventional (i.e., destructive) headspace analysis. Smiddy *et al*. (2002c) used oxygen sensors to examine the effects of residual oxygen concentration on lipid oxidation in both anaerobically packaged MA and vacuum-packed cooked chicken and in raw and cooked beef (Smiddy *et al*., 2002a). These studies further demonstrated the suitability of such sensors to measure non-destructively oxygen levels in commercially used meat packaging and their potential as predictors of quality in processed muscle foods. Papkovsky *et al*. (2002) used oxygen sensors to measure oxygen content in the headspace of four commercial sliced ham products. Accurate measurements were made under ambient light conditions, in direct contact with the product and under conditions of significant temperature variation. Although the sensor demonstrated minor changes in calibration as a result of direct physical contact with the meat surface over a prolonged period, these effects were minimized through optimization of the sensor material. It is unlikely, in any case, that the presence of sensors in direct contact with a meat product would be acceptable to either producer or consumer. O'Mahony *et al*. (2004) used fluorescent oxygen sensors printed directly onto the packaging material of *sous vide* beef lasagne. A clear correlation between oxygen profiles, microbial growth and lipid oxidation was established.

Fluorescent oxygen sensors are also useful in detecting the substantial fraction of commercial anaerobically MA- or vacuum-packed meat products containing elevated levels of oxygen (Papkovsky *et al*., 2002; Smiddy *et al*., 2002b).

The development of oxygen sensors outlined above is indicative of the move towards commercialization of indicator-type smart packaging. The result, given the viable outcome of future research initiatives, may ultimately see the incorporation of sensors in every meat pack produced. Such a scenario would mean the production of millions of sensors and thousands of measurement devices at different points in the production and distribution chain. It has been estimated that, in today's terms, each sensor should cost less than one cent to produce (Kerry and Papkovsky, 2002) and impact minimally on packaged meat production costs.

OxySense® (http://www.oxysense.com) is the first commercially available fluorescence-quenching sensor system for measurement of headspace or dissolved oxygen in transparent or semi-transparent, sealed packages. The system uses an oxygen sensor (O_2xyDot™) placed in the package before filling and is non-destructive, rapid (measurements take less than 5 sec) and able to withstand pasteurization temperatures without loss of sensitivity. Two new analytical techniques, the GreenLight™ system for rapid enumeration of total viable counts (TVC) in food homogenates and the Optech™ system for non-destructive sensing of residual O_2 in package headspaces are all based on fluorescence-type oxygen

sensing and have been developed through research conducted within UCC and commercialized by LUXEL Biosciences (Ireland) and MOCON (USA).

20.5.2 Biosensors

Other approaches to freshness indication, which may be more likely to find commercial application in smart meat, poultry and seafood packaging systems are those based on recently developed biosensor technologies.

Biosensors are compact analytical devices that detect, record and transmit information pertaining to biological reactions (Yam *et al*., 2005). 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. Smart or intelligent packaging systems incorporating biosensors have the potential for extreme specificity and reliability. Market analysis of pathogen detection and safety systems for the food packaging industry suggests that biosensors offer considerable promise for future growth (Alocilja and Radke, 2003).

The majority of available biosensor technology is not yet capable of commercial realization in the food sector. However, a significant number of commercially available biosensor and/or indicator systems have been developed over the past two decades; FreshTag (Cox Recorders, USA), FreshQ (Food Quality Sensor International Inc., USA), CO_2 detectors (Sealed Air, USA), Transia test strips (Transia GmbH, Germany), Freshness Guard Indicator (UPM Raflatac, Finland), It's Fresh™ (It's Fresh Inc., USA), ToxinGuard™ (Toxin Alert Inc., Canada) and Food Sentinel SystemTM (Sira Technologies, USA) (Smolander, 2008). The more recently developed ToxinGuard™ is a visual diagnostic system that incorporates antibodies in a polyethylene-based plastic packaging capable of detecting *Salmonella sp*., *Campylobacter sp*., *E. coli 0517* and *Listeria sp*. (Bodenhammer, 2002; Bodenhammer *et al*., 2004). Of recent development also is the Food Sentinel System™ (SIRA Technologies, California, USA), which is a biosensor system capable of continuous detection of contamination through immunological reactions occurring in part of a barcode. The barcode is rendered unreadable by the presence of contaminating bacteria. Such systems give some insight into products likely to become more popular in the years to come.

20.6 Indicators for smart packaging

An indicator may be defined as a substance that indicates the presence or absence of another substance or the degree of reaction between two or more substances by means of a characteristic change, especially in colour. In contrast with sensors, indicators do not comprise receptor and transducer components and communicate information through direct visual change (see commercial examples provided above).

20.6.1 Integrity indicators

An alternative approach to such package-destructive techniques is the use of non-invasive indicator systems as part of an MA package. Such systems usually provide qualitative or semi-quantitative information through visual colorimetric changes or through comparison with standard references. The majority of indicators have been developed for package integrity testing, an essential requirement for the maintenance of quality and safety standards in packaging of muscle or muscle-based products. The most common cause of integrity damage in flexible plastic packages is associated with leaking seals (Hurme, 2003). Permanent attachment of a leak indicator or sensor (i.e., visual or optochemical) to a package appears to hold most promise in ensuring package integrity throughout the production and distribution chain. A number of studies on package integrity in MAP meat products (Ahvenainen *et al*., 1997; Eilamo *et al*., 1995; Randell *et al*., 1995) have established critical leak sizes and associated quality deterioration. Although a number of destructive manual methods are available for package integrity and leak testing, such tests are laborious and can test only limited numbers of packs (Hurme, 2003). Available non-destructive detection systems (which include a number of stimulus-response techniques) have other disadvantages such as the need for specialized equipment, slow sampling time and an inability to detect leakages that are penetrable by pathogens (Hurme and Ahvenainen, 1998; Stauffer, 1988).

Much work on the development of integrity detection for packaged foods has focused on visual oxygen indicators in MAP foods (as opposed to those oxygen sensors previously discussed, which are also applicable to integrity testing). With the exception of high oxygen content MA packaging of fresh meat (primarily to enhance colour) many foods are packaged in low (0–2%) oxygen atmospheres. In such cases, leaks normally result in a significant increase in oxygen concentration. Many visual oxygen indicators, consisting mainly of redox dyes, have been patented (Davies and Gardner, 1996; Krumhar and Karel, 1992; Mattila-Sandholm *et al*., 1995; Yoshikawa *et al*., 1987). Such devices have been tested as leak indicators in MA-packaged minced steaks and minced-meat pizzas, respectively, and reported as reliable (Ahvenainen *et al*., 1997; Eilamo *et al*., 1995). Disadvantages of such devices include high sensitivity (~ 0.1% oxygen concentration required for colour change means indicators are susceptible to residual oxygen in MA packs) and reversibility (undesirable where increased oxygen due to a leak is consumed during subsequent microbial growth). Few of these devices have been taken up commercially. One indicator system, specifically designed for MAP foods, contains, in addition to an oxygen-sensitive dye, an oxygen-absorbing component and exemplifies active and intelligent packaging in a single system (Mattila-Sandholmand *et al*., 1998).

A number of companies have produced oxygen indicators, the main application of which has been for the confirmation of proper functioning of oxygen absorbers (an active packaging function). Trade names of such devices include Ageless Eye®, Vitalon®, and Samso-Checker® (Smolander *et al*., 1997).

A visual carbon dioxide indicator system consisting of calcium hydroxide (carbon dioxide absorber) and a redox indicator dye incorporated in polypropylene resin was described by Hong and Park (2000) and may be applicable to certain meat-packaging applications.

20.6.2 Freshness indicators

The information provided by intelligent packaging systems on the quality of meat products may be either indirect (e.g., changes in packaging oxygen concentration may imply quality deterioration through established correlation) or direct. Freshness indicators provide direct product quality information resulting from microbial growth or chemical changes within a food product. Microbiological quality may be determined through reactions between indicators included within the package and microbial growth metabolites (Smolander, 2003). As yet the number of practical concepts of intelligent package indicators for freshness detection is very limited. Despite this, considerable potential exists for the development of freshness indicators based on established knowledge of quality-indicating metabolites. The chemical detection of spoilage of foods (Dainty, 1996) and the chemical changes in meat during storage (Nychas *et al*., 1998) provide the focus for which freshness indicators may be developed, based on target metabolites associated with microbiologically induced deterioration. Using the marker concept in this manner may result in the more widespread commercial development of freshness indicators for meat products in the not too distant future.

The formation of different potential indicator metabolites in meat products is dependent on the product type, associated spoilage flora, storage conditions and packaging system. A number of marker metabolites associated with muscle-food products exist upon which indicator development may be based.

- Changes in the concentration of organic acids such as n-butyrate, L-lactic acid, D-lactate and acetic acid during storage offer potential as indicator metabolites for a number of meat products (Shu *et al*., 1993). Colour-based pH indicators offer potential for use as indicators of these microbial metabolites.
- Ethanol, like lactic acid and acetic acid, is an important indicator of fermentative metabolism of lactic acid bacteria. Randell *et al*. (1995) reported an increase in the ethanol concentration of anaerobically MA-packaged marinated chicken as a function of storage time.
- Volatile compounds such as TMA, dimethylamine (DHA) and nitrogen (collectively described as TVB-N) or the biogenic amines such as histamine, hypoxanthine, putrescine, tyramine and cadaverine have been implicated as indicators of muscle-based product decomposition (Kaniou *et al*., 2001; Okuma *et al*., 2000; Rokka *et al*., 2004; Taoukis *et al*., 1999). Given toxicological concerns associated with these compounds and their lack of impact on sensory quality, the development of effective amine indicators would be of benefit. Detection systems described by Miller *et al*. (1999) and Loughran and Diamond (2000) provide potential for commercial development. In 1999,

COX Technologies (USA), launched FreshTag® colour-change indicator labels that react to volatile amines produced during storage of fish or and other seafoods. The ownership of this interesting technology moved from COX Technologies to Sensitech in 2004 and then to Carrier Corporation, none of which has assisted the further development or commercialization of the technology. Research on a sensor technology similar to the FreshTag® technology is currently under development by the Adaptive Sensor Group in Dublin City University (Pacquit *et al*., 2008).

- Carbon dioxide produced during microbial growth can in many instances be indicative of quality deterioration. In MA-packaged meat products containing high carbon dioxide concentration (typically 20–80%), indication of microbial growth by changes in carbon dioxide content is problematical, although application of pH dye indicators may hold promise in other meat packaging systems.
- Hydrogen sulphide, a breakdown product of cysteine, with intense off-flavours and low threshold levels is produced during the spoilage of meat, poultry and seafood by a number of bacterial species. It forms a green pigment, sulphmyocin, when bound to myoglobin and this pigment formed the basis for the development of an agarose-immobilized, myoglobin-based freshness indicator in unmarinated broiler pieces (Smolander *et al*., 2002). The indicator was not affected by the presence of nitrogen or carbon dioxide and offers potential.

A variety of different types of freshness indicators have been described (Smolander, 2003, 2008), the majority of which are based on indicator colour change in response to microbial metabolites produced during spoilage. Freshness indicators based on broad-spectrum colour changes have a number of disadvantages which need to be resolved before widespread commercial uptake is likely. A lack of specificity means that colour changes indicating contamination can occur in products free from any significant sensory or microbiological quality deterioration. The presence of certain target metabolites is not necessarily an indication of poor quality. More exact correlations appear necessary between target metabolite, product type and organoleptic quality and safety. The possibilities of false negatives are likely to dissuade producers from adopting indicators unless specific indication of actual spoilage can be guaranteed.

20.6.3 Time–temperature indicators (TTIs)

A time–temperature indicator or integrator (TTI) may be defined as a device used to show a measurable, time–temperature-dependent change that reflects the full or partial temperature history of a food product to which it is attached (Taoukis and Labuza, 1989). Operation of TTIs is based on mechanical, chemical, electrochemical, enzymatic or microbiological change, usually expressed as a visible response in the form of a mechanical deformation, colour development or colour movement (Taoukis and Labuza, 2003). The visible response thus gives a cumulative indication of the storage temperature to which the TTI has been exposed.

TTIs may be classified as either partial-history or full-history indicators, depending on their response mechanism. Partial-history indicators do not respond unless a temperature threshold has been exceeded and indicate that a product has been exposed to a temperature sufficient to cause a change in product quality or safety. Full-history TTIs give a continuous temperature-dependent response throughout a product's history and constitute the main focus of interest for research and commercial exploitation.

Essentially, TTIs are small tags or labels that keep track of time–temperature histories to which a perishable product is exposed from the point of manufacture to the retail outlet or end-consumer (Fu and Labuza, 1995). Their use in meat, poultry and seafood products, where monitoring of the cold distribution chain, microbial safety and quality are of paramount importance, offers enormous potential.

The basic requirement of an effective TTI is to indicate clear, continuous, irreversible reaction to changes in temperature. Ideally, TTIs should also be low cost, small, reliable, easily integrated into food packaging, have a long pre- and post-activation shelf life and be unaffected by ambient conditions other than temperature. TTIs should also be flexible to a range of temperatures, robust, pose no toxicological or safety hazard and convey information in a clear manner.

A large number of TTI types have been developed and patented, the principles and applications of which have been reviewed previously (Fu and Labuza, 1995; Selman, 1995;Taoukis, 2008; Taoukis and Labuza, 2003). TTIs currently commercially available include a number of diffusion, enzymatic and polymer-based systems, all of which offer potential for usage in meat, poultry and seafood products.

Diffusion-based TTIs

The 3M Monitor Mark® (3M Company, St Paul, Minnesota, USA) is an indicator dependent on the diffusion of a coloured fatty acid ester along a porous wick made of high-quality blotting paper. The measurable response is the distance of the advancing diffusion front from the origin. The useful range of temperatures and the response life of the TTI are determined by the type and concentration of ester.

Another diffusion-based TTI, Fresh-Check®, produced by the same company incorporates a viscoelastic material that migrates into a diffusively light-reflective porous matrix at a temperature-dependent rate. This causes a progressive change in the light transmissivity of the porous matrix and provides a visual response.

The TT Sensor® (Avery Dennison Corporation, USA), again based on diffusion-reaction, allows for the diffusion of a polar compound between two polymer layers and the change in its concentration causes the colour change of a fluorescent indicator from yellow to bright pink (Taoukis, 2008).

Enzymatic TTIs

The CheckPoint® TTI (VITSAB AB, Malmö, Sweden) is based on a colour change induced by a drop in pH resulting from the controlled enzymatic hydrolysis of a lipid substrate. The indicator consists of two separate compartments containing an aqueous solution of lipolytic enzymes and another containing the lipid substrate suspended in an aqueous medium and a pH indicator mix. Different enzyme-substrate

combinations are available to give a variety of response lives and temperature dependencies. Activation of the TTI is brought about by mechanical breakage of a seal separating the two compartments and may be done manually or by on-line automation. Hydrolysis of the substrate causes a drop in pH and a subsequent colour change in the pH indicator from dark green to bright yellow. Visual evaluation of the colour change is made by reference to a five-point colour scale. CheckPoint® labels are the latest TTIs developed by VITSAB, which comprise a label type designed to create a better subjective reading response for users and offer direct application to seafood, poultry and ground beef products. VITSAB, in conjunction with British Airways, has also developed a TTI system (Flight 17 Smart Label) that allows airline personnel to check the status of perishable pre-prepared foods.

The eO® Cryolog (Gentilly, France) adhesive TTI label takes the form of a flower-shaped gel pad which changes from green (good) to red (not good). The colour change is pH induced and caused by microbial growth within the gel itself. The TRACEO® (Cryolog) transparent label is designed for use on refrigerated products and placed over the barcode. The colour of the transparent adhesive label changes from colourless to red when the product is no longer fit for consumption (O'Grady and Kerry, 2008).

Polymer-based TTIs

Lifelines Freshness Monitor® and Fresh-Check TTIs (Lifelines Technology Inc., Morris Plains, New Jersey, USA) are based on temperature-dependent polymerization reactions in which diacetylene crystals polymerize via 1,4 addition polymerization to a highly coloured polymer. Resulting changes in reflectance can be measured by scanning with a laser optic wand. The Fresh-Check® consumer version uses a circular label in which the colour of the inner circle is compared to that of an outer circle in order to establish use-by status.

The OnVu™ TTI labels (Ciba Specialty Chemicals Inc., Switzerland) are based on organic pigments which change colour with time at rates determined by temperature. The TTI label consists of a heart-shaped apple motif containing an inner heart shape. The image is stable until activated by UV light from a LED lamp, which in turn causes the inner heart shape to become deep blue in colour. A filter is then added over the label to prevent it from becoming recharged. The inner blue heart changes to white as a function of both time and temperature. This system can be applied as a label or printed directly onto the package (O'Grady and Kerry, 2008).

Initial expectations on the potential of TTIs to contribute to improved standards in food distribution, quality and safety have not been realized to date. Factors such as cost, reliability and applicability have all been influential in this regard. The cost of TTIs has been estimated at approximately $0.02–$0.20 per unit (Taoukis and Labuza, 2003). Given normal economies of scale, cost-benefit analysis should favour more widespread use of TTIs. Faith in the reliability of TTIs has been undermined somewhat by insufficient supporting data. It appears now that TTI systems have achieved high standards of production and quality assurance and provide reliable and reproducible responses according to BSI specifications (BS 7908, 1999). The most substantial hurdle to extensive commercial TTI use has been the question

of applicability. Generalizations on the relationship between temperature and quality of general food classes have proved insufficient, as even foods of similar type differ markedly in terms of response. For successful application of TTIs to meat, poultry and seafood products, and food products in general, there is a requirement that the TTI response matches the behaviour of the food. While the expectation for a TTI to strictly match the behaviour of a foodstuff over a wide temperature range is unfeasible, a thorough knowledge of the shelf-life loss behaviour of a food system based on accurate kinetic models is essential (Taoukis and Labuza, 2003). Advances in food modelling are now making this possible (Taoukis, 2001).

A number of validation studies have been undertaken in order to establish the usefulness of TTIs in food products (Riva *et al*., 2001; Shimoni *et al*., 2001; Welt *et al*., 2003). Yoon *et al*. (1994) showed a positive correlation between oxidative stability and TTI colour change using a phospholipid/phospholipase-based TTI in frozen pork. Smolander *et al*. (2004) and Vainionpää *et al*. (2004) determined the applicability of VITSAB®, Fresh-Check® and 3M Monitor® TTIs for monitoring the quality of MAP broiler cuts at different temperatures and in comparison with several standard analytical methods respectively. Otwell (1997) also assessed the VITSAB® TTI in MAP salmon.

In all three studies, TTIs were closely correlated with microbiological analyses of spoilage bacteria and, in some cases, were shown to be more effective than certain metabolic quality indices such as spoilage-associated volatiles, biogenic amines and organic acids. Pacquit *et al*. (2008) highlighted the fact that commercial trials have been conducted for seafood products, primarily salmon products, using TT Sensor™, Fresh-Check® and CheckPoint® TTI technologies.

In 1991, a UK survey (MAFF, 1991) indicated that 95% of respondents (n = 511) considered TTIs to be a good idea but indicated that substantial publicity or an educational campaign would be required for general use. It is likely that such attitudes still apply today. Despite predictions for the full commercial realization of TTIs, adoption has been very limited. However, given technological developments in recent years, greater consumer appreciation for the need for food safety monitoring (particularly in muscle-based products) and the growing legislative demand for guaranteed food safety, analysts believe that TTIs will inevitably find widespread commercial application in the food industry. The critical importance of maintaining proper storage temperatures for meat and poultry products throughout the distribution chain means that this sector of the food industry could be a major beneficiary from such a development.

20.7 Radio frequency identification tags (RFID) and potential future applications of other smart/intelligent technologies

RFID technology does not fall into either sensor or indicator classification but rather represents a separate electronic information-based form of intelligent

packaging. RFID uses tags affixed to assets (cattle, containers, pallets etc.) to transmit accurate, real-time information to a user's information system. RFID is one of the many automatic-identification technologies (a group which includes barcodes) and offers a number of potential benefits to the meat production, distribution and retail chain. These include traceability, inventory management, labour-saving costs, security and promotion of quality and safety (Mousavi and Sarhadi, 2002). Prevention of product recalls is also considered an important role of RFID technology (Kumar and Budin, 2006). RFID technology has been available for approximately 40 years, although its broad application in packaging is a relatively recent development.

At its most basic level, an RFID tag contains a tiny transponder and antenna that has a unique number or alphanumerical sequence; the tag responds to signals received from a reader's antenna and transmits its number back to the reader. While the tags are relatively simple, much better inventory information than barcode or human entry systems can be gained through tracking software. RFID tags have the advantage over barcoding in that tags can be embedded within a container or package without adversely affecting the data. RFID tags also provide a non-contact, non-line-of-sight ability to gather real-time data and can penetrate non-metallic materials including bio-matter (Mennecke and Townsend, 2005). RFID tags can hold simple information (such as identification numbers) for tracking or can carry more complex information (with storage capacity at present up to about 1MB) such as temperature and relative humidity data, nutritional information, cooking instructions etc. Read-only and read/write tags are also available depending on the requirements of the application in question.

Tags can be classified according to two types: active tags function with battery power, broadcast a signal to the RFID reader and operate at a distance of up to approximately 50 m. Passive tags have a shorter reading range (up to approximately 5 m) and are powered by the energy supplied by the reader (giving them essentially unlimited life).

Common RFID frequencies range from low (~ 125 KHz) to UHF (850–900 MHz) and microwave frequencies (~ 2.45 GHz). Low frequency tags are cheaper, use less power and are better able to penetrate non-metallic objects. These tags are most appropriate for use with meat products, particularly where the tags might be obscured by the meat itself, and are ideal for close-range scanning of objects with high water content.

The costs of RFID are decreasing rapidly as major companies such as Wal-Mart, 7-Eleven and Marks & Spencers adopt the technology. At present, the cost of passive RFID tags range from approximately $0.50–$1.00. For the technology to be truly competitive analysts estimate that tags must cost less than $0.05 (others below $0.01) (Want, 2004). It is expected that tags will fall to the $0.01 per-tag level in due course (Mennecke and Townsend, 2005). Initiatives to establish formal standards should also serve to reduce further the cost of RFID systems.

RFID is beginning to be used in a number of countries for tracing individual animals (mainly cattle) from birth to the processing plant. The key to individual

animal traceability lies in the ability to transfer animal information sequentially and accurately to sub-parts of the animal during production. RFID-based tracking systems provide an automated method of contributing significantly to that information exchange (Townsend and Mennecke, 2008). At present, individually RFID tagged muscle-based food products are not available to the consumer (to the best of the author's knowledge), although the use of RFID tagging of meat cuts has extended, in one case at least, in the pig-processing industry (http://www.flagshipfoods.co.uk/dalehead) from the individual pig to its primal pieces – that is, hams. Hedgepeth (2005) outlined how RFID tags were being employed on pallets of fish exported from Alaska as a means of verifying origin, storage and transportation. Currently, SINTEF are conducting trials in Norway to monitor the controlled movement of super-chilled lamb from slaughter through to retailed storage using RFID. Although the purpose of these tracking schemes is for quality control, traceability and accountability, it does exemplify the developing use of RFID technology within the muscle food industry. Although the implementation of intelligent packaging of meat, poultry and seafood products using RFID technology is still largely hypothetical, indications suggest it is unlikely to remain so for very much longer.

20.7.1 Miscellaneous potential future applications of other smart/intelligent technologies

Smart cooking is a new cooking innovation combining the cooking capabilities of a convection oven with microwave and grill cooking. The smart cooking process is made possible through the innovation of smart ovens (e.g., Samsung BCE 1197) which have the capacity to read special, on-pack SmartCodes (two-dimensional barcodes). The SmartCode is scanned by the built-in oven scanner and the smart oven converts the code into cooking instructions. Every SmartCode contains a unique set of instructions which provide the smart oven with the correct temperature, microwave power and time to cook the food to perfection and consistently (O'Grady and Kerry, 2008). Marks & Spencer's were one of the first retailing chains to adopt this technology and apply it to a range of muscle-based food products.

With the growing popularity of smart phones, a new phenomenon, associated with a wide range of fast-moving consumer goods, called rapid communication (RC) is starting to become popular. Special RC codes are starting to appear on a wide variety of food packs. Consumers can engage much more with the food products in question by scanning RC codes with their smart phones and whole new levels of communication can take place between the consumer and product and between the consumer and product manufacturer. Clearly, this technology could pose some major advantages for those companies producing meat, poultry and seafood products around areas such as safe handling of product, cooking instructions, presentation of cooking options, recipe ideas, traceability information, animal welfare information and much more.

20.8 Conclusions

The ultimate incentive for deployment of any new technology is cost. The cost effectiveness of smart packaging devices is dependent on the perceived benefits derived from such systems. Producers must ultimately derive benefit from increased profit margins and consumers must derive benefit as 'utility' or satisfaction from economic exchange. Economies of scale suggest that the cost of many active packaging devices (scavengers, absorbers, emitters) or intelligent packaging devices such as oxygen sensors, TTIs or passive RFID tags are not currently or will not be a factor prohibitive to mass commercialization. What little consumer-attitude information that is available seems to be positive towards such packaging concepts (Lähteenmäki and Arvola, 2003).

Changes in consumer preferences have led to innovations and developments in new packaging technologies. Smart packaging which is active in nature is useful for extending the shelf life of fresh, cooked and other meat, poultry and seafood products. Forms of active packaging relevant to muscle foods include oxygen scavengers, carbon dioxide scavengers and emitters, moisture absorption, antimicrobial packaging, antioxidant packaging and odour or flavour removal. Recognition of the benefits of active packaging technologies by the food industry, development of economically viable packaging systems and increased consumer acceptance opens new frontiers for active packaging technology. Commercially, there is widespread use of oxygen scavengers in both fresh and pre-packed cooked sliced meat products. Antimicrobial packaging is gaining interest from researchers and industry due to its potential for providing quality and safety benefits, especially in the area of nanotechnology using nanoparticles and other related materials. Future research in the area of microbial active packaging should focus on naturally derived antimicrobial agents, biopreservatives and biodegradable packaging technologies. The possibility of utilizing additional active packaging technologies, as currently applied to other foodstuffs, for safe and effective storage of meat, poultry and seafood also merits investigation.

In order to address the present imbalance between potential and actualization of intelligent packaging use, a number of research gaps need to be filled. These include further modelling of the interactions between foods and microbes and their metabolites under dynamic storage conditions, better understanding of correlations between spoilage indication and sensory quality, effective incorporation of sensors and indicators into high-volume packaging processes, knowledge on the behaviour of intelligent packaging devices at all points of the storage and distribution chain, issues relating to sensitivity (including over-sensitivity) and reliability. Food manufacturers can ill-afford inaccurate extrapolations based on a limited knowledge base. Nor will they risk commercial investment on unproven technologies.

The potential advantages of smart packaging which is intelligent in nature for muscle-based foods are many and varied. Apart from aspects of quality, safety and distribution already outlined, intelligent packaging offers considerable potential as a marketing tool and the establishment of brand differentiation for meat

products. Assuming intelligent packaging can effectively provide solutions to current producer and consumer problems, it appears likely that intelligent packaging systems for muscle-based food products will become more commercially viable and commonplace in the years to come.

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21

Traceability in the meat, poultry and seafood industries

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Abstract: Traceability is important in maintenance of food safety and quality at each stage of the food supply chain, particularly in identification of contamination sources and routes in meat, poultry and seafood. There are a variety of identification techniques for individual animals, batches of similar sources and for types of products. Each species has unique harvesting, processing, storage and distribution characteristics which influence identification methods and subsequent systems for tracking information to end users and tracing information back to source. Record keeping and data management are key components of a successful traceability system. Radio frequency identification (RFID) is often used for pallet, case and individual package identification and tracing, but bar coding and quick response technologies are being integrated into some production and marketing schemes for inventory control and the conveying of desired information to processors and consumers. Systems using multiple flexible reading and recording technologies and centralized internet data access allow the many different components in the meat, poultry and seafood industries to be linked together.

Key words: traceability, primary packaging, secondary packaging, tertiary packaging, transponders.

21.1 Introduction

The food supply is characterized by increasing concentration into larger entities, and by highly integrated supply chains linking producers and consumers (Opara, 2003). Traceability allows the tracking of the route of an ingredient or product from its source to its final use through an information trail. The primary goal of

animal traceback systems is to provide information on the source of any infection or prohibited additives so that preventive and control measures can be applied to avoid future introduction of the contaminant (Caporale *et al*., 2001), Traceability serves a number of purposes, though, including protecting animal health and controlling epizootic and enzootic livestock diseases, maintaining public health through safe food supplies, ensuring zoonotic diseases are not transmitted to humans, enforcing animal welfare standards and providing accountability for taxpayer investments through auditing functions (Pettitt, 2001).

The sheer size, scale and complexity of the meat, poultry and seafood industries complicates the implementation of the available technologies, which can be characterized for their availability, use and effectiveness. There are many different technologies available for food product tracing, with none of them being vastly superior to any of the others. The linking of different technologies for identification, logistical location, data entry and record access is hampered by necessary integration factors. This chapter compares and contrasts traceability systems for the different muscle food industries, with emphasis on the differences and similarities in production, processing, logistics and marketing. Identification of products through the supply chain is a key factor in traceability. Radio frequency identification (RFID) is being increasingly used in the food industry, and special attention will be paid to this technology because of its broad applications and current commercialization.

21.1.1 Definitions and scope of traceability

Traceability allows the history and location of a specific activity, process or product to be tracked in detail, step by step. It encompasses all aspects of food and feed chains, with two key functions being tracking information to end users and tracing of information back to the source (Schwägele, 2005). The information may be used at any point in the food chain, from production or early in the supply chain to later in the delivery process at the point of purchase (Regattieri *et al*., 2007). Traceability, in the context of this chapter, follows the ISO (International Standards Organization) 8402:1994 and EU regulation 178/2002 definition as 'the ability to trace and follow a food, feed, food producing animal or ingredients, through all stages of production and distribution' (European Parliament, 2002). This definition is similar to the one in the Codex Alimentarius Commission (CAC) Procedural Manual (CAC, 2011). In the United States, food manufacturers must develop traceability of the food supply to comply with the provisions of the 2011 FDA Food Safety Modernization Act (Nachay, 2011). Traceability systems protect animal health, public health and food safety as a key tool in risk management in both industry and government and are increasingly becoming a requirement for international trade, with traceability and nutritional labeling being international concerns. Contamination of foodstuffs can occur at any stage from food production to preparation, and cooperation and participation along the whole value chain are essential (Tyrchniewicz and Tyrchniewicz, 2010).

For full traceability, tracking and labeling are necessary at each step of the food chain (Miller, 2009) with product identification, data records, product routing and

traceability tools required for an efficient and effective system (Regattieri *et al*., 2007). Traceability tracking and tracing may rely upon the use of identification attached to the animal or product, or on the devices and automation that accompany the animal or product at and to/from each location (Mousavi *et al*., 2002). There are three forms of traceability systems. In the first, each link or step in the supply chain receives relevant tracking information from the previous link in the system; this system reduces transaction costs but depends on a high degree of trust and excellent coordination between the different steps. The second type of system has each step receiving relevant data from all previous links, which increases the speed of tracking and tracing, and allows control of information completeness, but the amount of information to be transferred increases with each successive link. The third type of traceability system uses a separate organization to which each supply link sends relevant information. This type of system resolves issues of trust, allowing rapid tracking and tracing (in principle), and provides for maintenance of the system, but a disadvantage may be the total costs (Meuwissen *et al*., 2003). Backward and forward tracing in a reliable system allows any costs of foodborne illnesses and economies from preventive measures to be attributed to the appropriate food chain segments (Vitiello and Thaler, 2001). Variations on these three types of systems can also be employed. A traceability system could require each step to obtain information from the previous step and transmit relevant information to the next step (one step back, one step forward) or might have two interlinked data repositories, one for raw materials and the other for finished products.

Traceability begins when materials are received, with the operational flow for most food facilities being: raw material receiving, raw material storage, production, finished product storage and shipping (Kelly, 2009). An underlying difficulty after harvest of raw meat is the division of the animal carcass into multiple parts that may be merchandised as multi-component products in many different retail and food service venues, making the sheer volume of data difficult to manage and perhaps necessitating several software applications running on different database systems (Diospatonyi *et al*., 2000). Critical points for systematic loss of information are encountered in transformation of the resources into another form (Donnelly *et al*., 2009). An additional problem is that many food products are composed of multiple ingredients from different commodity categories, so a food safety or quality difficulty early in the supply chain may affect multiple different components later in the supply chain (Miller, 2009). An integrated agricultural and food supply chain traceability system must have product traceability of the physical location at any stage, process traceability to verify the type and sequence of activities that have affected the product, genetic traceability of the product's constitution, inputs traceability to determine the type and origin of all inputs, disease and pest traceability of the epidemiology and biotic hazards, and measurement traceability to relate individual results and records.

The motivation for traceability can vary. It may be to protect the product's reputation, to ensure differentiation of products among suppliers, to guarantee the product's origin when origin is an attribute of interest, to improve supply management, for monitoring and assurance of production and processing methods, or to increase the effectiveness of product recalls in the case of food safety

or product quality difficulties (Pouliot and Sumner, 2008). There are three major areas of risk in meat production traceability: identity retention from animal birth to consumption of the product, food safety to prevent adverse health effects and consumer confidence that the meat consumed is what it is purported to be on the label (Shackell, 2008). Functional traceability attributes of organizational efficiency, chain monitoring and individual responsibility are important to all consumers, while production methods are of interest to only specific market segments (Gellynck and Verbeke, 2001). One study discovered that consumers in Italy were interested in sensory and nutritional attributes but that quality signals such as labels of origin, traceability and quality certifications were considered particularly useful in food choices (Banterle and Stranieri, 2008). There are many certification scheme examples in meat supply chains (Meuwissen *et al*., 2003). Issues to be considered when developing identification and traceability capabilities for the meat industry include: the identification of animals and meat; the required information depth (forward or backward tracking requirements from an individual step in the production chain), breadth (amount of information collected) and precision (degree of assurance of tracing a specific animal or package); and the authenticity of records (Smith *et al*., 2008b). Full traceability is feasible as long as there are benefits at the segment that demands traceability across all ingredients and as long as there is a willingness to pay for the benefits (Souza-Monteiro and Caswell, 2010). Pouliot and Sumner (2008) suggest that consumer willingness to pay for safer products is increased when food is safer with increased traceability.

The core of traceability systems are the hardware and software technologies for implementation of the chosen scheme (Opara, 2003). Product tracing challenges include identifiers of food at each stage from production to consumption, insertion of information captured within current efficient handling and processing steps, and integration of data on bulk ingredients (McEntire, 2010). Transparency of practices and procedures and assurance to validate standards at each level of the marketing chain are important adjuncts to a traceability system (Liddell and Bailey, 2001). Companies must work closely with their suppliers and customers to ensure that information necessary for traceability is apparent and accessible. Activities might include mock recalls, on-site visits and rigorous inspection and testing of raw materials and finished products (Opara, 2003). Examples of supply chains are shown in Fig. 21.1 (for meat and poultry) and Fig. 21.2 (for seafood).

Packages must be identified or labeled in many farm to table traceability schemes. Primary packaging is packaging in direct contact with the product (consumer packaging), while secondary packaging contains several primary packages (outer packaging) and tertiary packaging is the assembly of multiple primary or secondary packages on a pallet or roll container (group packaging). Information on primary packaging is targeted to consumers and information on secondary and tertiary packaging is used inside the wholesale and retail supply chains. Packaging fulfills the logistical needs of handling and distribution, necessarily providing information about the products, conditions and locations so packaging must be considered an integral part of the traceability system as an interface between segments of the supply chain. Plotting the physical flow of packaging from filling

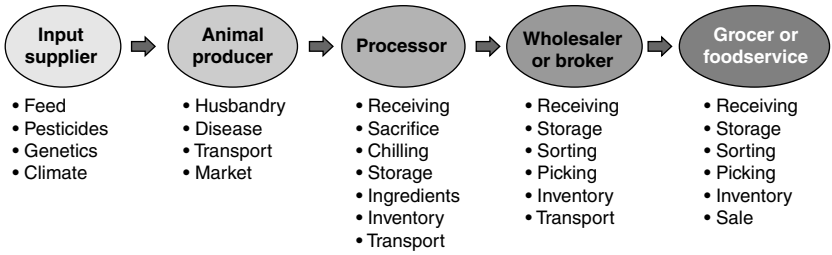

Fig. 21.1 Supply chain for meat and poultry with examples of traceability controls at each segment.

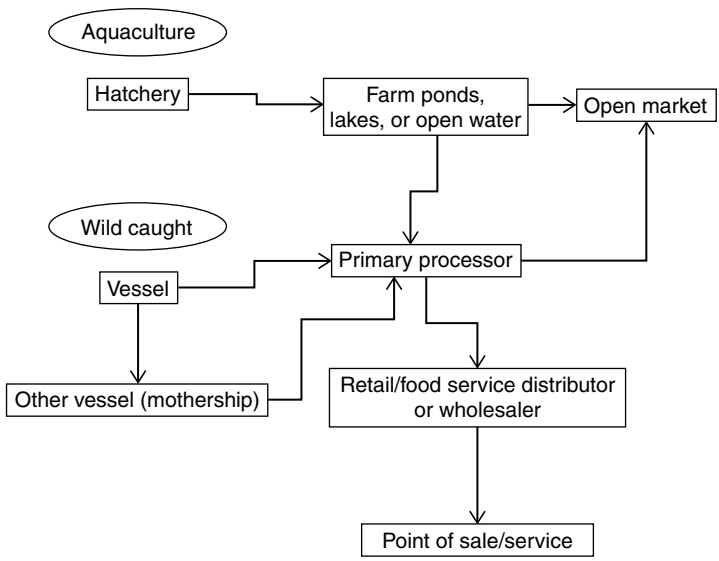

Fig. 21.2 Schematic of the seafood supply chain. (Courtesy of National Fisheries Institute, 2011)

primary containers to outer packaging and group packaging through manufacturer warehousing, carrier transport, distribution center activities (receiving, warehousing, picking, shipping), carrier transport and retail outlets (receiving, replenishing) gives a concept of the intricacies of logistics (Hellström and Saghir, 2007) and thus the complexity that must be considered in traceability systems.

21.2 Current technologies available for muscle food industry tracing systems

The following sections look at the various stages of the identification processes involved with following meat, poultry and seafood products from their origin to the plate, and the associated technologies.

21.2.1 Animal identification methods

Animal traceability is completely dependent upon accurate identification of individual animals, or groups of animals, and the origin and movement records after identification (Smith *et al*., 2005). Individual animal identification has been practiced for many years by visual and non-visual means. Visual identification methods include: hide brands (either hot or freeze) or horn brands; ear, shoulder, or lip tattoos; tags or collars in ears, around necks or tails, in wings, or glued to hide; earnotching or marking; and photographs of color patterns or markings. Ear tags, collars and wing bands may be plastic or metal, and tags may be button or dangle and plain or electronic. Electronic identification methods include bar codes, two-dimensional symbology, RFID and optical character recognition. Transponders may be in dangling neck chains, skin implants or rumen boluses, while biometric identification takes several forms (Smith *et al*., 2005). Biometric means for individual animal identification, including subcutaneous skin implants, ceramic boluses, deoxyribonucleic acid (DNA) genetic profiling, antibody identification (Yordanov and Angelova, 2006) and retina scanning, are relatively expensive and time consuming (Wiemers, 2000). Immunosensors based on antibody antigen recognition are simple, accurate and sensitive, and rely upon the biological sensor antigen or antibody component being detected by a signal transducer. Common biosensors are piezo electric crystals that detect a change in crystal mass due to antigen-antibody binding (Schwägele, 2005). Many identification techniques have advantages over conventional visual tags but are too costly, too slow, or have been insufficiently tested for use in national identification or traceability systems (Stanford *et al*., 2001).

The difficulties with technologies that trace animals, products and by-products through associated rather than direct identification means can be overcome through DNA technology. Once DNA is extracted from the organic source, molecular markers are used to obtain a fingerprint or specific allelic frequencies (Dalvit *et al*., 2007) through real-time polymerase chain reaction (PCR) and other DNA analytical methodologies (Opara, 2003). DNA identification technology is based on the principle that each animal is genetically distinct and that the unique code of the animal can be used to identify it and its products (Yordanov and Angelova, 2006). DNA sampling can be done on live animals to provide positive proof of identity to validate pedigree, origin or fraud (Shackell, 2008). Genetic data, when coupled with information about genetic trait expression, such as growth rate, tenderness or marbling, also allows the determination of the specific sequences of the DNA that are associated with the variation in trait expression or even the specific genes for each trait. Using DNA as an identifier has many advantages, including that the product acts as its own label or identifier, the code is permanent and remains throughout the life history of the animal or product and DNA can be taken at any point in the production chain for matching with the animal history. Tracking can link a product directly to the source, allowing the bypassing of intermediate steps, and samples can be detected in cooked products, raw samples or even sometimes in stomach contents. DNA typing is very accurate and relatively free from human error, allowing it to be

used to audit and verify other traceability identifying and recording systems that are subject to human error (Yordanov and Angelova, 2006). However, problems of high costs for routine tests and attainment of agreement on markers and approaches are impediments to DNA use in most traceability schemes (Dalvit *et al*., 2007).

In some instances, reliable methods of rapid determination of animal species are necessary for regulatory compliance or customer assurance. Electrophoresis (starch, polyacrylamide, agarose gel), isoelectric focusing, immunological and proteomic techniques used to separate enzyme and myoglobin proteins, lipid composition through gas chromatography (with or without mass spectroscopy) and DNA methods have all been used to test for animal species authentication. Methods to determine geographical origin include all the previous methods, together with stable multi-isotopic parameters and near infrared and mid-infrared spectroscopy (Schwägele, 2005). Element contents in mutton samples analyzed by inductively coupled plasma mass spectroscopy with linear discriminate analysis gave a correct classification rate of 94% and a cross-validation rate of 89% for differentiation based on different pastoral and agricultural regions in China (Sun *et al*., 2011). It is also sometimes necessary to detect particular parts of the animal. Stable carbon and nitrogen isotopes have been used to trace the inclusion of poultry offal meal in broiler feeding but the need for additional research has been suggested (Oliveira *et al*., 2010).

21.2.2 Integrated traceability systems

An integrated production chain control system should be able to accurately identify and substantiate all materials and ingredients, production processes, personnel involved and final products (Augsburg, 1990). The benefits of a successful traceability system include improved data collection and reporting, enhanced supply chains, increased inventory accuracy and cost reductions from raw material amounts, higher yields, less labor, reduced cycle times and increased equipment performance that improves cost accounting and process productivity (Gay, 2010). A determination must be made of the physical, mechanical and chemical properties that must be measured and recorded in a traceability system (Opara, 2003). Decisions must be taken about the number of measurements, type of measurements and degree of detail required, data storage requirements, confidentiality and public information and the necessity for constant checks and automatic alarms, among other topics (Regattieri *et al*., 2007). Different company and organizational structures will have different complexities and different methods of implementing traceability (Buhr, 2003).

Record keeping is fundamental in traceability systems (Nachay, 2011) and can vary in complexity from paper records to computer-based information technology methods, with biological technologies included in the most sophisticated systems (Bevilacqua *et al*., 2009). Each system offers mechanisms to trace animals through farm records, record ownership changes and movement through processing and distribution. Paper systems are slow, unwieldy and prone to error, with

immense records generated by even a small company (Miller, 2009). A simple paper register of movements of animals to and from a farm can allow control of epizootic diseases but the record book of each farm must be complete and accurate, and tracing through paper records is laborious and time consuming (Pettitt, 2001). There is potential error when humans must read identification and then enter data rather than using automatic digital transfer from device to device and a traceability system is only as reliable as the reliability of the methods for reading, transcription and tamper resistance of data (Shackell, 2008).

Enterprise resource planning or accounting electronic software will not by themselves provide adequate traceability because of the constraints of lot traceability, production management and labeling. These are more effectively managed by process-oriented systems for warehouse management or production management. The availability of data storage, access and manipulation on web-based applications provides for effective user and product costs (Miller, 2009). Automated systems provide access to more reliable real-time information. Various approaches have been proposed for traceability information design, including graphical models, RFID use, taxonomy classifications, event-driven models, entity-based model and activity-based costing. An event-driven process chain has elements of functions corresponding to an activity task and process step, events before and after execution of a function, and logical connectors for activities and events. Introduction of a complete traceability system requires identification of companies having a critical role in the traceability, identification of a suitable organizational model, creation of a supply chain, identification of technical instruments, lot identification and information management (Bevilacqua *et al*., 2009).

Many current systems for traceability allow for only intermittent tracking and information flow when each step in the system is only required to retain identity and records from the step previous to the successive step (one step backward, one step forward). If there are disjointed or non-continuous communication segments in the traceability chain, this limits the effectiveness of the entire system. A national food traceability system adoption is dependent upon technology that is standards-based and interoperable so any organization can access it in a uniform way while providing a platform for innovation and improvement. Reluctance to share some data with competitors requires security features that protect information but allow access to needed linkages. Software that has a standards-based protocol operates as a referral service while allowing traceability chain participants access to data about a particular product. Discovery services technologies are being developed that can link hybrid identification systems, such as animal tattoos or RFID chips, can aggregate and disaggregate identifiers at any step in the system and be interrelated with other accrued product data such as temperature, quality parameters, or inventory control (Cute, 2009). Efficient traceability relies upon integration of technology, synchronization of data, capture of detailed information, delivery of item information when needed, use of appropriate best practices at each step and use of the traceability processes at every opportunity (Schrader, 2010).

21.2.3 Product coding and data interchange

After each farm or production facility defines its resource units, an appropriate labeling scheme is necessary. A simple system involves the use of a printed label showing a common lot or batch identification number. The information can be printed on the outer case packaging or pallet label in addition to individual retail items. The batch code is originated by the processor and may contain information on production lines, shifts, production time and other desired data as a series of numbers and/or letters. However, more sophisticated methods are usually used, the most common being bar codes and RFID (Bevilacqua *et al*., 2009). These allow traceability information to be scanned into the retail computer network to automate an audit or recall.

Most food manufacturers and retailers around the world use Universal Product Code (UPC) labels, which are recognized by GS1 US and GS1 (formerly known as the US Uniform Code Council and European Article Numbering [EAN] authority, respectively; Thompson *et al*., 2005). Various different types of bar codes have been developed to suit different applications, for example, GS-128 bar codes and two-dimensional (2D) bar codes contain more information than common bar codes, for example. Identification of whole packets, pallets, or containers is usually with Serial Shipping Container Code (SSCC) and a bar code technique to reduce bar code size based on the different UPC and EAN types is reduced space symbology (RSS; Frederiksen *et al*., 2002). Disadvantages of bar codes include that they may be difficult to scan if covered with water droplets or condensed water from melting ice. Bar code labels may also not be securely affixed and fall from the packaging. Electronic tags (e.g., RFID tags – see Section 21.7) are an alternative. These can be built into boxes, but expense makes it necessary for returnable boxes to be used in the fish industry (Frederiksen *et al*., 2002). Labels must comply with readability and traceability requirements with the pallet label serving as a location or destination marker for identification and verification of products in the supply chain (Hellström and Saghir, 2007).

In terms of future trends, RFID and quick response (QR) codes (Neagle, 2011) are enabling technologies that can be profitably adapted by most chain organizations and create possibilities for technological change in the food industries, although not originated for these purposes (Huang and Yang, 2009). Quick response (QR) codes (two-dimensional, black and white square codes) are widely used in Japan and scanned using a mobile telephone camera. QR codes can be printed on any physical object, including advertisements, billboards, labels, packaging, or even on products themselves (Huang and Yang, 2009). QR readers allow customers to obtain additional information, directly from the reading of the QR label or through access to websites. Consumers using QR technologies can receive immediate discounts on purchases, access nutritional information, redeem coupons, view videos about products and services, and make payments. The voluntary mpXML standards (MPXML, 2010) were introduced to address requirements for meat and poultry with bar codes for variable-measure products that identify the manufacturer or brand owner within the GS1-128 bar code (Neagle, 2011). Examples of QR and GS1-128 bar codes showing the amount of

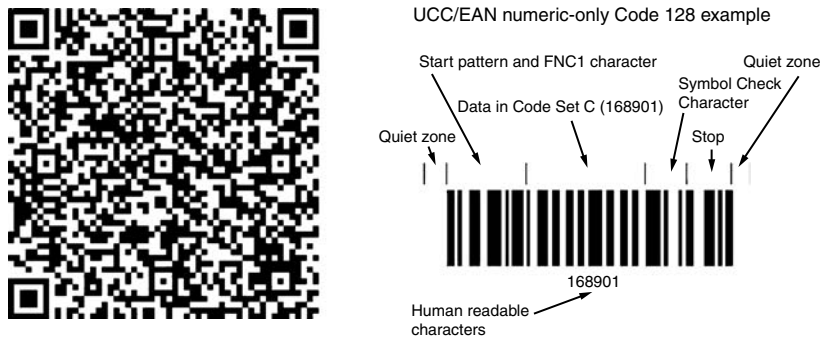

Fig. 21.3 Examples of a quick response (QR) code (left) and GS1-128 bar code (right).

additional information that can be contained compared with electronic data in less complex bar codes and configurations are supplied in Fig. 21.3.

Software packages that integrate financial and production data into one program package with traceability capabilities, as used by large producers and supermarkets, are too expensive for many smaller suppliers, or fishing and seafood trading companies, so it may not be easy for large companies to exchange information with small companies in this way. Various systems of data interchange have been developed. Electronic Data Interchange for Administration, Commerce and Transport (EDIFACT) coding uses pre-defined identifiers for data items within messages and has the ability to extract the meaning of encoded tags, but the messages are not readable by humans and the system lacks the flexibility of choice. Encoding based on eXtensible Markup Language (XML) allows synchronization by exchanging data (and the underlying data structure) for information processing. Active server pages (ASP) for metadata and hyper text transfer protocol (HTTP) are designed to allow generally accepted interfaces and protocols for digitally encrypted data exchange (Frederiksen *et al*., 2002).

21.3 Traceability in livestock production

Animal and animal product traceability requires unique identification of individual animals or groups of animals. Livestock have been identified throughout history to prove ownership or to support breeding programs through ear punch marks or notches, horn brands and tattoos, although these are not usually recognized as official marks. Ear tags are more common but are susceptible to loss, as are collars (Pettitt, 2001). Brands (hot or freeze) or lip tattoos are more permanent, but small brands or tattoos may become illegible or difficult to read unless there is close proximity to (or restraint of) the animal. Ear tags are common for individual animal identification in all livestock species but these can be lost during routine animal movement or handling, which causes temporary or permanent loss of identity. Several breed associations require ear or lip tattooing with a number unique

to each individual animal, but this system is self-limiting for identifying large numbers of animals, and must be done properly to ensure legibility throughout the animal life. A drawback of using tags is that the amount of information that can be visible on them is limited, but this can be overcome by combining electronic identification technologies with tags. Electronic (ear tags, rumen boluses, injectable transponders) and biometric (nose prints, DNA genetic profiling, iris or retinal scanning) methods of animal identification have been developed more recently for the creation and implementation of livestock identification and traceability systems. Iris scanning requires the animal's head to be held stable during the photographic scan, but provides more points of identification than retinal scanning or DNA sampling. Retinal scanning combines digital camera imaging with linkage to an internal global positioning satellite receiver to give automatic and tamper-proof encryption of date, time and location of image capture. There is cost variation among identification types, dependent on reuse or single use of a technology (Johnston, 2009). Most traceability systems have components for individual animal identification from birth to harvest, movement records, animal termination records, as well as a central database (Bowling *et al*., 2008). The reading distance, ease of reading, retention, cost, ease of application, animal restraint requirement for application, tamper resistance and ease and cost of device collection at slaughter were compared for the animal identification devices available at that point in time (Wiemers, 2000).

21.3.1 Livestock identification and traceability systems in different countries

In the United States, RFID tags have been recommended as the technology for individual animals but there has not been consensus among industry segments on the best approach or methodology for cattle identification. To provide full-chain traceability of all domestic products in Canada, a national animal identification system that encompassed all livestock species was proposed. The Canadian Cattle Identification Agency has migrated from dangle tag bar code technologies to RFID technologies, while sheep owners may use dangle bar code or RFID tags (Murphy *et al*., 2008). Mexico has a goal to develop whole-life traceability with numbered tags and corresponding identity cards. Cattle and sheep imported into the United States must have individual blue metal ear tags and valid sanitary certificates (Murphy *et al*., 2008). Bowling *et al*. (2008) provide a summary of various traceability systems around the world, including: details on EU requirements for individual cattle ear tags, electronic databases, passports and premise registries; property identification codes and approved ear devices or rumen boluses for the National Livestock Identification System in Australia; brands for identification of birth location used in Namibia and rumen boluses with imbedded RFID chips used in Botswana; Japanese and South Korean systems for ear tag use; and classifications of group lots and individual animal identification in Brazil and Uruguay.

Countries with sheep identification systems typically have a code to represent the holding, premises or property on which the animals were grown. The system

for ear tags is very specific in the United Kingdom while the ear tags within the rest of the EU are similar in format. Other countries are in varying stages of determining if and how sheep traceability will be conducted (Bass *et al*., 2008). In the United States, a Scrapie Flock Identification number has been recommended for sheep to accompany their Premises Identification Number (PIN), but it was acknowledged that a purely visual identification system would not allow 24-h traceability, and that more reliable and accurate technologies should therefore be pursued (Murphy *et al*., 2008).

21.3.2 Traceability in different types of livestock

Swine

Swine in the United States are identified by group or lot identification, with individual identification needed when animals commingle outside of the production system. A national tattoo number standardization strategy was developed for pigs in Canada to eliminate multiple premises using the same shoulder slap-tattoo numbers (Murphy *et al.*, 2008). The EU, United Kingdom, Denmark and New Zealand have birth-to-harvest traceability programs for pigs. Farm records, producer holding numbers and herd marks are required in the United Kingdom, where several methods of identification, including ear tags, tattoos, slap marks and temporary paint marks are approved. Danish pigs must have an ear tag before leaving the birth herd so this information can be placed in a computerized system. New Zealand swine producers must complete an Animal Status Declaration that gathers details of identification type and specific numbers for the pigs, medicinal record, animal movement and transport which allows tracing through slaughter. Tattooing is not adequate for identifying pigs with dark skin or after soiling. Ear tag losses and failures are affected by fencing type and results with injectable intraperitoneal transponders have been variable. Half-duplex transponders have proved to be more readable than full duplex transponders and longer reading distances are recommended for transceivers. One difficulty with transponders is their low recovery rate at slaughter because they did not adhere to the omentum (Gosálvez *et al*., 2007). Average losses of 3.5–3.7% in slaughter lines were similar for visual tags and half-duplex transponders, but were almost triple (11.5%) for full duplex transponders. Manual recovery of the intraperitoneal transponders from offal trays was 89%, so total identification from farm to slaughter was greater for intraperitoneally injected transponders than for ear tags (Santamarina *et al*., 2007).

It was previously shown (Caja *et al.*, 2005) that transponders in the auricle base of the ear were recovered more by cutting or sight than by palpation at slaughter, and intraperitoneal transponders were mainly recovered loose (86%) in the abdominal cavity. Caja *et al*. (2005) reported that intraperitoneal transponder injection was effective for pig identification and traceability, ensuring the transfer of information from farm to slaughterhouse. Visible readability was similar for all ear tag types before slaughter, but readability of ear tattoos was lowered by

illegibility due to ink fading. Tag loss increased in finishing phases and varied with the housing system used. Identification devices did not affect productivity after application and full duplex electronic ear tags were most efficacious for lifetime identification on the farm (Schembri *et al*., 2007).

Ruminants

Cattle tracing can begin at birth when the calf is ear-tagged and the data entered in a central database and in the farm record. The database issues a passport or certificate that accompanies the beef animal through life; each movement on and off farms and to slaughter is recorded in the appropriate farm register, central database and on the certificate. At slaughter, the movement history can be used for labeling of the meat and the passport or certificate is returned to the central database to record the end of the animal history (Pettitt, 2001). This record keeping in triplicate seems like a lot of extra work but is necessary to maintain the integrity of the tracing system, which is controlled by the physical auditing of a proportion of the animals and documents on a periodic basis. Non-compliance in the United Kingdom can be accompanied by penalties of movement restrictions, loss of value, or loss of premium (Pettitt, 2001). The transition to RFID devices for cattle identification in Canada allows reading of tags in cow/calf operations for improved efficiency in managing production data of breeding choices, veterinary records and herd management histories. RFID panel readers on loading/unloading chutes automatically record the tag numbers of cattle on entrance and exit at auction markets and feedlots. Pilot projects have suggested double identifiers (a visual panel with number and bar code and an electronic ring) for ruminants that technological innovations (IP technology, Bluetooth) should be used for entry and electronic transfer of information, entry and transfer of information manually or electronically by producers and harmonization of identification numbers throughout the industry supply chain to fulfill information exchange agreements with other industry partners (Lavoie and Forest, 2009). Biological markers can be used to trace individual animals since unique patterns in the eye and nose of live animals are recognizable by electronic methods (Shackell, 2008).

Injectable half-duplex passive responders in the armpit of cattle were more suitable on the farm than smaller-sized responders or subcutaneous injection in the ear scutulum or upper lip, but they required more care and a longer time for recovery at the abattoir than for the smaller-sized devices and ear or lip injection (Conill *et al*., 2000). Ghirardi *et al*. (2006a) reported that ceramic or plastic boluses in calf forestomachs varied in retention rate until slaughter. Losses on the farm after subcutaneous injection of half-duplex passive transponders in the armpit or in the retro-auricular ear position for lambs, or after tagging with a small plastic ear tag, were not affected by age at injection, injection position or breed, though ear tag losses were slightly higher than for transponders. Losses in the slaughterhouse were greater for transponders in ears than armpits, but breakage was only 0.3%. Recovery of transponders allowed a harvesting speed of 200 lambs per hour, but use of injectable transponders for fattening lambs was not recommended because of difficulties with recovery at the abattoir (Conill *et al*., 2002).

Ceramic boluses of varying dimensions, volume and specific gravity containing half-duplex glass-encapsulated transponders did not affect disease in or growth of sheep of varying ages. Ear tag losses were 7.5%, while bolus retention varied with bolus features and sheep age. Boluses of 16–45 g, with a volume of 3–22 mL, and specific gravity of 2.0–5.2 were estimated to have a 99.5% retention rate, while boluses of less than 15 mm, specific gravity higher than 3 and weight greater than 20 g were recommended for use in sheep (Ghirardi *et al*., 2006b). Ceramic mini-boluses with these parameters did not cause feed intake or growth differences and had 100% recovery at slaughter (Ghirardi *et al*., 2007). Ceramic boluses with the previous parameters had greater retention than ear tags for goats (Carné *et al*., 2009). Retinal imaging was an accurate technique for auditing the ear tag and electronic mini-bolus identity of lambs from weaning to yearling ages (Rojas-Olivares *et al*., 2011).

21.4 Traceability in poultry production

Poultry production in most countries has evolved from small enterprises to huge sophisticated national or international companies marketing millions of poultry carcasses annually. These companies are generally highly integrated, which facilitates a level of control (Fallon, 2001). Poultry have different traceability requirements than livestock because of the large numbers of birds in many production systems, the vertical coordination or integration of larger operations, high-speed processing lines and the high degree of automation and mechanization. The development and use of automated slaughter and processing has been aided by minimization of carcass size variation and the use of standardized cutting techniques, according to customer specifications (Mousavi *et al*., 2002). Management information systems which exchange relevant information can simplify the complexity of large operations and assist in management decisions. Collection of a standardized data set results in data that is uniform, relevant, accurate and consistent, which simplifies the traceability of product information. Differences in methods of data collection at breeder farms, hatcheries, broiler farms and processing plants cause variability in data quality. This provides suboptimal use of the data in decision making and traceability that requires an exchange of information among different segments in the chain (Yassin *et al*., 2011). In general, though, farm to table tracing is possible in the poultry industry if there is total control of all factors involved in producing the birds and products that extends back to previous breeding stock generations. This allows auditable guarantees to consumers (Fallon, 2001).

Only a few primary breeding companies supply the breeding stock from which almost all commercial poultry meat is derived. This facilitates the tracing of a carcass from primary processing back to the farm of origin and even to one or two previous generations of bird. For most purposes, each flock can be treated as a single homogeneous unit, with each bird having a similar status. Poultry are processed in batches of either an individual flock or in batches of 5000 or more from

a flock on continuous line systems, at speeds of 6000–12 000 or more birds per hour (Fallon, 2001). Batches are kept separate from each other, and flock identity maintained through weighing and grading stages. Each part of the production and processing enterprise has extensive recording of many production parameters to enable performance and profitability to be maximized and output to be optimized. The need to maximize productivity and minimize losses has led to sophisticated traceability all the way from the elite breeding stock to primary processing plant level.

Individual bird identification is unlikely to be applied to commercial poultry because it does not appear to provide advantages or contribute information in addition to the data already collected on flocks. Some techniques have been investigated by researchers. Wing or leg bands are used to identify individual breeding birds and could possibly be adapted for individual chicks (Fallon, 2001). Passive integrated transponders implanted on the neck nape of 3- and 7-day old chicks caused no differences in survival, rate of body mass gain, or susceptibility to pecking by other chicks over 40 days compared with control chicks. This system would provide a viable method for individual identification of juvenile birds but a 5% tag loss and costs might make its implementation prohibitive (Jamison *et al*., 2000). The behavioral and physiological homeostasis of hens was affected by the use of individual identification systems such as leg bands, wing bands, neck tags and livestock markers. Social stress was increased with wing and leg banding, resulting in lower weights (Dennis *et al*., 2008); it is possible that these same negative effects might impact broiler performance and efficiencies, though further research is required.

21.5 Traceability of seafood

The fishing industries exhibit particularly challenging supply chains since fish from marine stocks are caught in the wild, and individual or batch identification are used for different species or types; additionally, trading systems are complex due to quality variations and catch uncertainties. Fishing vessels may be segregated by size, primary target species, catch gear and other differences, but individual vessels may land a variety of species. Pelagic fish are caught in great numbers and stored in tanks on vessels, while trawls are used to catch demersal fish that are stored on ice. Some vessels sort, grade and package seafood onboard, while others bring their catch to processing facilities. Factory ships may prepare frozen products ready for retail sale (Jensen *et al*., 2009).

Traceability requirements for seafood include certification for environmental and sustainable purposes to prevent illegal, unreported, or unregulated fish from entering the supply chain (Donnelly and Karlsen, 2010). Wild fish segments and farmed fish sectors both have great incentives to develop and use traceability systems to protect against allegations regarding the quality of the fish. The importance and complexity of traceability is increased with the typical schemes for seafood marketing, where many demersal fish are sold through auction markets

to accumulators who often mix landings from numerous vessels (Jensen *et al*., 2009). Separation of batches may also be difficult since large fish catches from several trawls or catch areas are mixed (Donnelly and Thakur, 2010). Other difficulties include the renaming and mislabeling of seafood along with chain of custody standards to maintain the identity of species, country of origin and catch or production methods for labeling (Jacquet and Pauly, 2008; Moretti *et al*., 2003). Determination of origin is restricted by different non-related databases, poorly or non-defined fishing grounds or product sites and by lack of information on movement among different industry segments (Sioen *et al*., 2007). International seafood supply chains are also getting longer and increasingly divided into processing steps in different countries (Schröder, 2008). The globalization of trade and lack of international standards has raised concerns from retailers, food service operators and consumers about the safety of seafood supplies; international databases would improve traceability in the international seafood trade. Seafood traceability systems must be compatible with the standardization of data and defined traceable resource units, which must follow each fish or lot through all processing, distribution and retail stages (Thompson *et al*., 2005). Development of voluntary standards for fish chains and data transmission protocols by an EU consortium did not result in a complete system for collecting and transmission of traceability data, including software (Thompson *et al*., 2005). Using bar codes and serial shipping container codes were shown to identify each resource unit and track each delivery in a Danish system (Frederiksen *et al*., 2002). Companies at different stages of fish supply chains have different perceived benefits, but implementing radio frequency time-temperature indicator tags on master boxes will bring benefits only if recall reductions and labor savings are included; processing companies may have less positive cost benefits than trading companies (Mai *et al*., 2010).

Various analytical methods are required for the authentication of seafood products. Species identification is an important tool of traceability in the seafood chain because of the similarity in appearance of many species and the loss of external characteristics during processing (Moretti *et al*., 2003). Reliable analytical methods are needed for authentication when processed products lose their morphological characteristics and low-grade fillets are fraudulently substituted for higher-valued products. Analytical methods are also needed to determine if different production methods, such as organically farmed and wild-caught fish, may contribute more value than conventionally farmed fish. Lastly, the geographical origin of species can be determined by variations in stable isotopes (Schröder, 2008), with the two main analytical methods being nuclear magnetic resonance with mass spectroscopy of isotopic ratio and site-specific natural isotope fractionation by nuclear magnetic resonance (Peres *et al*., 2007).

Species identification is usually based on separation and characterization of specific proteins through isoelectric focusing (IEF), capillary electrophoresis, high-performance liquid chromatography or immunoassays (Moretti *et al*., 2003). Proteome analysis using isoelectric focusing has been applied to identify species and muscle tissues, characterize postmortem changes in arctic and

tropical species, and to determine the effects off during processing of fish muscles (Martinez and Friis, 2004). Thermal treatments cause proteins to lose their biological activity, biochemical structure and chemical properties, so sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) and urea-IEF are suitable unless the products are sterilized. DNA analyses based on polymerase chain reactions are alternatives to protein-based techniques (Moretti *et al*., 2003). Quinteiro *et al*. (1998) were able to identify six canned tuna species using DNA amplification of restriction fragments, even though the DNA had been degraded during the canning process. A 132 bp region from the mitochondrial DNA allowed differentiation of five tuna species (Bottero *et al*., 2007) and fluorescence resonance energy transfer was used to discriminate four tuna species with real-time PCR and melting curve analysis (Dalmasso *et al*., 2007). Trotta *et al*. (2005) used a multiplex PCR method to differentiate grouper fillets from closely related Nile perch (*Lates niloticus*) or the wreck fish (*Polyprion americanus*) species. Amplified fragment-length polymorphism combination markers were used to differentiate and identify 20 different species, and were developed into a database by Maldini *et al*. (2006). DNA-based methods for fish and seafood identification and monitoring of fishery products were reviewed by Rasmussen and Morrissey (2008, 2009). A simple one-step PCR method was described for identification between European and American razor clam species (Fernández-Tajes *et al*., 2010). Use of the amplified and sequenced cytochrome b gene marker allowed species authentication of octopus, cuttlefish, bobtail and bottle squids (Espiñeira *et al*., 2010). DNA bar coding of analyzed shark slices were compared with reference sequences from different databases, revealing much fraudulent identification of commercial samples (Barbuto *et al*., 2010). DNA bar coding had previously been used to identify species of smoked products of fish in ten families and four orders (Smith *et al*., 2008a). Fishery genetics have concentrated on DNA-based species and genetic stock identification, with microsatellites being used extensively, but accumulation of sequence information for genome-wide single nucleotide polymorphism (SNP) analysis will support or displace microsatellites in the future. DNA bar coding uses a fragment of mitochondrial cytochrome oxidase I gene as a standard marker for the identification of animals, with bar coding of all fish species likely to be possible in the future (Kochzius, 2009).

21.6 Traceability of meat, poultry and seafood products

The increase in the proportion of cut-up parts and deboned meat sold has been possible through developments in image analysis, online weighing and tracking, real-time computerized monitoring and tracking and data systems to optimize quality, yield and speed. Systems that monitor carcass parameters early in the slaughter process to optimize steps later in the process require the maintenance of identity or, at a minimum, the same sequence on continuous processing lines (Barbut, 2010). A disadvantage of many animal identification methods is that traceability

ceases or must be transferred at slaughter, even if ear tags, RFID implants, or boluses allow tracking during transport. The complex disassembly of carcasses into parts, muscles, trimmings and by-products raises the difficulty of individual identification and data recording, but batch traceability can be achieved. Several methods for complete traceability in the cutting of carcasses have been proposed. One proposal is that a processor could assign each animal a unique sequential production number corresponding to the animal passport information that would be transferred using RFID and bar code readers from the carcass to a traceability database linked to the plant material handling system (Mousavi *et al*., 2002). In some slaughter plants, tags are scanned after exsanguination and an identification tag printed for the carcass. Some plants have rail trolleys with imbedded RFID microchips to maintain animal identification to the point of fabrication; some plants even maintain identification beyond this point. Carcasses could be cut one carcass at a time, placing all pieces from one carcass in a single identified container for distribution to the retailer where bar-coded tags would be applied to each retail cut. This resolves data transformation difficulties from one step to another step, but is not considered realistic for modern meat slaughter, processing, distribution and retailing operations (Opara, 2003). The product identification method suggested in this study was a tag on the basic raw material with data that is transferred to a bar code on the food product. Another potential method is the transfer of data from live animal to carcass at slaughter, tracking of carcasses with RFID, bar coding primal cut labels and transporting them in returnable totes imbedded with RFID chips and sale of retail cuts with RFID, QR or bar code labeling. Movement of cuts across RFID-imbedded cutting boards sterilized between animals and the handling of more complex cuts by maintaining part sequences correlated with the individual animal would allow all data to be transferred to a general information system for use on packaging and labeling (Bulut and Lawrence, 2007; Murphy *et al*., 2009). Caporale *et al*. (2001) proposed a bovine meat traceability system using animal electronic transponders where all information regarding the animal was recorded and updated to a central database, with the electronic transfer of data from transponders to electronic labels at the processing plant and the reading of electronic labels to supply information to consumers at the retail store. Gledhill (2002) reported that today's meat traceability software systems can enable producers to track meat products from the animal's birth to the supermarket display case. Systems specific to individual meat plants have been described (Golan *et al*., 2004; Smith *et al*., 2008b; Xiong *et al*., 2010).

DNA is increasingly used to trace individual cuts of meat or for auditing conventional meat traceability systems because it is a built-in unique identifier that cannot be manipulated. DNA sampling can be taken on products at any point in the food chain to provide positive proof of identity to validate pedigree, origin or fraud. Effectiveness of a DNA-based system requires an auditable production trail through the processing facility and strict protocols to avoid product contamination (Shackell, 2008). DNA technology using a statistically based strategy can match genotypes of mixtures to correct production batches (Vetharaniam and Shackell, 2005). The use of eight informative markers was able to distinguish the

origin of retail meat independent of genetic population structure (Arana *et al*., 2002). Using only a small number of specific microsatellites allows cost reduction and ease in analyses (Orrú *et al*., 2006). The use of DNA technologies for identifying the animal origin of individual meat cuts appears the easiest DNA traceability system to implement, but its effectiveness is reliant on collection of hair samples from every animal at birth (Dalvit *et al*., 2007). Adulteration or contamination of meat species with other non-labeled species can also be determined by DNA (Calvo *et al*., 2002). It would be advantageous to use DNA from cooked meat to allow traceability in pre-cooked or ready-to-eat products, but DNA quantity has been shown to be reduced in cooked samples (compared to raw samples); smaller amplicons are present in cooked meat, suggesting that a DNA analyses should be designed to use small amplicon sizes for cooked meat samples (Aslan *et al*., 2009).

Some companies carry traceability forward to retail products by using highly organized packaging, portioning and added-value product operations, coupled with advanced computerized labeling, coding and data collection systems (Fallon, 2001). Various automated identification systems have been tested using inkjet and laser technologies, but consumers resist the appearance of marked parts. With either technology, identity is lost with further processing of the carcass (Fallon, 2001). Several companies offer multipurpose labels for products. Examples are labels that give the standard product information of name, weight, unit price, total price and code date, and include safe handling instructions and an irreversible time-temperature indicator with thermochromic ink that changes the color of the printing, or shows temperature abuse or quality changes in the product by other means. Labels with microchips that record temperatures and times are being used in transport of seafood in food-grade plastic bags. These are less bulky, cheaper and do not require mailing back to the supplier for record checking. After activation by bending the corner and affixing to a package, case, or pallet, the labels can be read with inexpensive readers to download the data into a spreadsheet, which is then emailed back to the processor or distributor. The labels are also useful in gauging other distribution and handling aspects, such as the effectiveness of pallet covers, times at different destinations and temperature variations in trailers or distribution centers (Higgins, 2009).

In Australia, processors have primary responsibility for reading and recording cattle transactions in the National Livestock Identification System database by scanning the animal RFID tag and assigning a unique identification number attached to a bar code. The animal numbers and slaughter time and date are linked to live animal information, carcasses, hides and by-products, but do not extend to retail products (Bowling *et al*., 2008). Processors producing animal by-products must have a traceability system in place in the EU (Bass *et al*., 2008). In Denmark, birth herd ear tags on pigs are recorded and the identification number on the slaughterhouse gambrel is automatically read and these are linked to the supplier number for storage in a computerized system. Additional carcass data, lean meat percentages and veterinary observations are also entered into the computer database and form the basis for payment to the producer. Meat cuts and meat products

must be identified by the lot number of the slaughter plant and processing plant, if different and the distributer or packager, must be labeled on retail meat packages (Meisinger *et al*., 2008).

Full traceability demands that a label or identifier relating the meat from a specific animal be attached to each individual piece of meat and remain with it at all times. Meat processors must build traceability into the finished products through tracking and record keeping. Raw material receipt procedures, purchasing invoices, batch records, matching of labels and codes to production records, inventory records and shipping records must be accurate and include lot numbers and other product-identifiable information (Kelly, 2009). Much of the necessary data is already in company records to create powerful decision instruments and build an alert system, but often the data is in different databases that cannot be easily combined. Some existing resource planning systems can be altered or updated, but are generally not suited to provide ingredient-level traceability across transformations and cannot handle event or attribute metadata. Decision support systems must include existing company databases and major operating applications that combine third-party data systems to form consolidated data systems to solve specific operational problems and issue appropriate operational alerts (Cutler, 2009). Flexible software systems that can integrate numerous technologies such as RFID, bar codes and geographical positioning systems allow a product search initiated by a product code that can expand into a virtual map and timetable of locations and events. A few companies have farm-to-retail traceability, with some having mechanisms for consumers to trace meat purchases via the internet back to the farm or animal source (Meisinger *et al*., 2008; Shackell, 2008). Data can also be integrated from enterprise resource planning software and customer manufacturing execution software (Johnston, 2009).

Meat tracing back to a farm or animal can be a complex and costly process, especially for smaller companies. Operations that are larger and more complicated increase costs of traceability to meet a given safety or quality standard because total variable cost of traceability increases with size, though the average fixed cost of traceability implementation decreases with the number of animals processed (Bulut and Lawrence, 2007). The audit trail becomes even more difficult when meat enters distribution centers or brokerages because most distributors or brokers supply multiple retailers or multiple retail stores. Tracking is further complicated because many large retail chains distribute meat within their own organizations. A key to traceability systems is the number of ownership changes or movements that cause each change in custody, which increases the numbers of potential points that might compromise system reliability (Shackell, 2008). Verification of the workability of a traceability system must be made through audits and conducting of mock recalls. Another means of checking traceability of raw materials is to track a sensitive raw material through the process to distribution (Kelly, 2009). Long-term identification requirements will be dictated by retailers and consumers, which might necessitate additional technological developments and implementation (Fallon, 2001).

21.7 Electronic identification (EID)

Methods for EID include bar codes, two-dimensional symbology, RFID and optical character recognition. Except for RFID, most EID methods are hampered by line-of-sight reading and cleanliness requirements (Wiemers, 2000). RFID is more expensive than bar coding, a low-cost system (Bevilacqua *et al*., 2009), but bar codes are less effective than RFID for tracking meat because they can be affected by contamination and are vulnerable to temperatures and chemicals in cleaning and disinfection.

21.7.1 Overview of ratio Frequency identification (RFID) technologies

RFID use has become commonplace in many countries and has proved to be useful and economical where large numbers of animals need to be processed in a relatively short time (Bass *et al*., 2008). RFID uses wireless microchips to create tags that are read through radio waves which use little power and are minimally disrupted by ferromagnetic fields (Regattieri *et al*., 2007). RFID systems based on frequencies between 100 kHz and 30 MHz operate using magnetic induction, while systems using microwave frequencies between 2.45 and 5.8 GHz operate using electromagnetic wave capture (Kumar *et al*., 2009). A basic RFID system consists of three parts: the identifier (a tamper-proof device that is permanently attached or implanted), an activating and/or reading device and software for electronic recording and transfer of data. Both the electronic identifier and reader must have antennae. Use of RFID is characterized by poor directional control (Yordanov and Angelova, 2006) and there may be minor difficulties due to lack of standardized RFID protocols and scanning problems due to electromagnetic interference (Bevilacqua *et al*., 2009). Tags can be chip-based (being ready-only, write once/read many times or suitable for read-write applications) or chip-less (for anti-counterfeiting and anti-theft purposes). Tags can be active, passive or semi-passive: active tags have a battery with a transmitting distance of up to 30 m to a reader, while passive tags are read when passed through the electromagnetic field of the reader and so work well in extreme circumstances and in the cold (−20°C; Huang and Yang, 2009). Semi-passive tags have an internal battery, but the reader transmits the electromagnetic waves.

Some food companies and retail chains have integrated RFID into manufacturing and distribution (Brody *et al*., 2008). Costs of implementing RFID traceability vary with segment of the industry (Mai *et al*., 2010). An RFID system can reduce labor costs, accelerate product flows, provide more efficient control of stock inventory and production monitoring for perishable products, allow improved knowledge of consumer behavior, improve tracking and tracing of quality issues and enhance management of product recalls (Regattieri *et al*., 2007).

RFID technologies must be matched with the specific food application (Kumar *et al*., 2009). Foods with high moisture content can interfere with signals and the read rate (Clarke *et al*., 2006) as water absorbs the RFID signal, especially in the microwave frequency ranges. The read range is 1–4 m, dependent upon

frequency, and the reader cannot communicate accurately with a tag oriented perpendicular to the reader's antenna (Kumar *et al*., 2009). Tags facing outward toward the reader antenna have the highest likelihood of a successful read (Clarke *et al*., 2006). The size of readers is still a limitation for in-store use by consumers, even though RFID antennae can be printed directly on packaging using conductive inks with the RFID chip attached (Moore, 2009). Metal packaging has strong detuning effects on RFID tag antennae, but can be compensated for by increasing bandwidth (Chau *et al*., 2006). A slower forklift truck speed has been shown to improve readability of palletized goods with RFID tags, but RFID readability was dependent on the loading pattern of products on pallets, which varied with food type (Singh *et al*., 2009). Another study reported that power and antennae polarization were more important than sample type and inlay design, but concluded that multi-parameter assessment is required for use of ultra-high frequency RFID (McCarthy *et al*., 2009). Smaller packages of modified atmosphere packaged meat, a minimum sample distance from the antennae, larger antennae and circular polarization were preferred for performance, while increased conveyor speeds decreased average detection rates (McCarthy *et al*., 2010). Energy transfer devices attached to reusable plastic containers for case-ready retail food distribution allowed the passage of radio frequency signals to the interior regions of a unified load to improve the readability of all RFID tags on the plastic containers. Once again, lower speeds of pallet movement improved readability while optimum pallet pattern selection for RFID reading was dependent on the type of product on the pallet (Singh *et al*., 2011).

21.7.2 Applications of RFID in the meat, poultry and seafood industries

RFID transponders injected into animals or imbedded in ear tags are remotely activated receiver-transmitters that use short range and pulsed echoes at about 150 Hz. Livestock transponders are primarily used for identification purposes and transmit information only when requested. Operation should be throughout the animal's lifetime and transponders should therefore be small, lightweight and robust. The electronic identifier must have a protective covering, often made of bioglass (for injectable transponders) or plastic (for ear tags), which allows the penetration of radio waves but is sufficiently strong to withstand being inserted in the animal and able to function until no longer needed. The placement of injected transponders in the body is important to prevent migration. Migration or loss due to improper insertion can lead to problems with recovery of implanted transponders at slaughter. Heads do not remain with the carcasses through the slaughter process, so placement of transponders in the head result in the separation of the transponder from the carcass, requiring a method to automatically link the carcass identity with a gambrel or trolley. Detectors must be present and working at the end of each slaughter product line (whether carcass or edible/inedible by-products) to ensure that all transponders are recovered. EID ear tags are one way to try to avoid transponder appearance in the food chain (Yordanov and Angelova, 2006).

Chen *et al*. (2008) describe a traceability system combining RFID technologies for chicken from the farm, to slaughter and a processing factory, and then to the retailer using a read/write chip to trace and track with personal data access devices and websites. RFID has also been useful in the shrimp industry, where harvested shrimp are placed in containers marked with RFID tags for coding of geographical origin. RFID read/write high-frequency tags allow process information from harvest through to sorting, grading and repacking as long as a centralized database is maintained and there are sufficient mobile or fixed readers at strategic locations. During the retail packaging process, a bar code or QR code is printed on the label, while the RFID tag itself is collected for recycling or reuse. This system is especially adaptable to shrimp production using multilayer cultivation boxes (Huang and Yang, 2009). Elsewhere, RFID smart tags and a commercial reader/writer have been used to facilitate traceability and cold chain monitoring of fresh fish in an intercontinental logistic chain (Abad *et al*., 2009).

21.8 Future trends

Consumers will likely continue to be concerned about the products they purchase and eat, making accurate sourcing and labeling important in muscle food choices. Regulations are not sufficient to prevent food fraud, so robust analytical tests are still required to verify authenticity (Ballin, 2010). The tracking of information to end users and the tracing of products back to source will require more rapid and accurate methods of animal and product identification, efficient communications and information transfer among industry segments and comprehensive systems to link traceability components. In addition, the continuing changes in laws and regulations regarding animal disease prevention, foodborne illness prevention and surveillance and increased globalization of food supplies are likely to dictate an increased use of traceability systems capable of handling complex data formats. The Food Modernization Act in the United States expands the Food and Drug Administration's authority to establish a product-tracing system for domestic and imported foods. Many grocery chains around the world are involved in GS1 standards to improve the efficiencies of supply chains and electronic commerce through standard product identification and electronic data interchange (Mermelstein, 2011).

One challenge is in the linking of existing systems with bar code data capture, with no standard software available to electronically share information across the supply chain. Another difficulty is in meshing traceability systems with the many auditing components that are being increasingly mandated by many distributors and grocery chains. The Global Food Safety Initiative is just one example of collaborative efforts among retail, processor, food service and provider companies to enhance food safety, ensure consumer protection and strengthen consumer confidence. Each scheme to provide auditing of practices among different industry segments is highly reliant upon interconnected identity and information

exchange, and on the standardization of expectations and procedures. EAN-13 and GS1-128 applications and even more sophisticated two-dimensional point-of-sale identification labels such as QR will continue to replace the limited UPC codes (Neagle, 2011). There are an increasing number of internet QR generation sites and QR code-reading devices. These will help to improve the access and reliability of information in assurance programs that are complementary to traceability needs. Assurance schemes can cover health risks and animal welfare, as well as other areas such as organic, non-genetically modified and environmentally friendly products that are considered valuable or important by consumers (Pettitt, 2001). The use of critical tracking events (CTE) in determining food traceability requirements has been gaining broad acceptance. Analysis to identify CTE, linkages of specific key data elements, secure access to centralized data reservoirs and improved data collection technologies (such as machine vision cameras and laser marking) will continue to move traceability systems closer to real-time applications (Welt and McEntire, 2011).

21.9 Sources of further information and advice

An important aspect of animal traceability is to provide security and monitoring of animal disease transmission from country to country, and from animals to humans. Strategies to track and trace animal disease, developed collaboratively by the National Institute for Animal Agriculture and the United States Animal Health Association, were published in 2010 (NIAA and USAHA, 2010). A comprehensive report and implementation plan on animal disease traceability was developed by the USDA Animal and Plant Health Inspection Service in 2010 and amended the next year (USDA Animal and Plant Health Inspection Service, 2011).

The Institute of Food Technologists examined traceability in food systems for the US Food and Drug Administration with collection of information related to product tracing and assembly of reports on technical aspects and recommendations (McEntire *et al*., 2010) and addressing of cost considerations and implications (Meija *et al*., 2010). The Food Standards Agency (UK) made a preliminary study of traceability in the food chain (2002), and a report from the Commission of the European Communities (2004) provided details on establishing a system for the identification and registration of bovine animals, and on the labeling of beef and beef products. The US implementation guides for traceability of meat and poultry (mpXML, 2010) and for the traceability of seafood (National Fisheries Institute, 2011) explain the GS1 standards and how they can be implemented by these industries. Specifications for the information to be recorded at chicken slaughter/processing establishments and other links in chicken distribution chains have been developed (Donnelly *et al*., 2008). A summary of the influence of the International Standards Organization (ISO) standards on the interactions of RFID and bar code applications is in Oehlmann (2008).

21.10 References

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22

Labelling of meat, poultry, seafood and their products in the EU

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Abstract: Meat, poultry, seafood and their products form an important part of national diets and international trade. Consumers want to know that the food they eat is safe, and they are also seeking more information as to the quality, authenticity, origin and method of production of the products they purchase. Therefore, it is not surprising that, in addition to the standard requirements of labelling legislation, there is a raft of specific legislation and standards that provides consumers with this extra information. This chapter details the existing rules for labelling meat, poultry, seafood and their products on an international, European Union and UK basis. The labelling rules, consumer protection measures, assurance schemes, protection of origin and specific rules on production (e.g. organic) that apply to all foods are examined. Also the specific rules, which apply to the labelling or claims on the particular commodities such as beef, poultry, fish, shellfish and their products, are also discussed.

Key words: EU labelling rules, EU beef and fish labelling rules, EU poultry marketing regulation, Codex standards for labelling and canned fish, protected denomination of origin, protected geographic indications.

22.1 Introduction

Meat, poultry, seafood and their products had an estimated total (retail and catering) UK market value exceeding £27 billion in 2008.[1,2] A breakdown of retail sales of meat, poultry, fish and shellfish with an estimated value of £8.2 billion in 2008[3–5] is shown in Table 22.1. Given this very large market size, and their importance as one of the main sources of protein in the diet of consumers, it is not surprising that there have been specific labelling requirements for meat products in UK national rules for over half a century, as well as general labelling requirements. More recent changes in the labelling of meat, poultry and seafood and their

Table 22.1 Retail market value of meat, poultry and seafood

	2007 UK	2008 (estimated) UK	2009 EU[1]
Red meat[2] (beef)	£3.06bn(£1.63bn)	£3.21bn	—
Poultry[3] (chicken)	£2.53bn(£2.11bn)	£2.69bn	—
Meat products including poultry	—	—	€33bn (estimated)
Seafood (fish and shellfish)	£2.21bn[4]	£2.30bn[4]	€48bn (France €10.3bn Spain €10.5bn Italy €8.6bn Germany €4.9bn)

Notes: [1] Leatherhead Food Research Foodlineweb;[2] total of beef, lamb, pork and offal sales; [3] includes whole birds, portions and further processed poultry; [4] includes canned fish sales (£0.4 billion).

products has been to effect harmonisation in the European Union (EU) either as general rules for all foods or specific rules affecting the marketing of poultry, beef and seafood. The details required by the EU general labelling rules are very similar to the general standard for labelling in Codex Alimentarius, a WHO/FAO organisation encouraging standardisation in world trade.

22.2 General (horizontal) food labelling requirements

Protection of consumers from misleading or inaccurate description and labelling of foods has been in place for over 100 years in the UK. More recent legislation has been developed to harmonise this protection for all citizens living within the EU, and also to facilitate trade within the internal market. There are general laws – Food Safety Act 1990,[6] EU General Food Law Regulation (EC) 178/2002[7] and the Consumer Protection from Unfair Trading Regulations 2008,[8] which make it an offence to mislead consumers when describing foods, whereas the Food Labelling Directive 2000/13/EC[9] details the specific information required in the description and labelling of food. These rules apply across all foods (horizontal rules), although there are specific provisions for individual foods within them. In addition, there is vertical legislation, which applies to specific foods or groups of foods, and requires special labelling in addition to the general rules.

22.2.1 Food Safety Act 1990

This Act and its predecessors have been the basis of food legislation in the UK for over 130 years, and it still remains the main vehicle by which to implement secondary food legislation.[6] Two sections in particular deal with consumer protection as regards description and labelling. Section 14 makes it an offence for anyone to sell, to the purchasers prejudice, any food which is not of the nature, substance or quality demanded. Section 15 of the Act makes it an offence to give or display

a label with any food sold or publish an advertisement, which falsely describes a food or is likely to mislead as to the nature substance and quality of the food, as well as to sell any food which is misleadingly presented. These two sections have provided a great deal of established case law on misleading and inaccurate labels, and because the maximum amount of fines that can be imposed is greater than for secondary legislation – for example, the Food Labelling Regulations 1996,[10] they have been the preferred route in many prosecution cases.

22.2.2 The Consumer Protection from Unfair Trading Regulations 2008

These Regulations implement the Unfair Commercial Practices Directive 2005/29/EC.[7,11] They effectively replace the main provision of the UK's Trade Descriptions Act 1968,[12] which made it an offence to offer or supply goods to which a false description applied, although some minor provisions of the Act still apply. The Regulations apply to all goods for sale to consumers, not just food. Its main provisions prohibit traders from making misleading actions covering the marketing or advertising of goods, which contain false information or cause the average consumer to take a transactional decision he (or she) would not have taken. The Trade Description Act 1968 has been used to prosecute misleading labelling as higher penalties could be imposed than under labelling rules; however, there is as yet little case law for the 2008 Regulations.

22.2.3 General Food Law Regulation 178/2002/EC

The objective of Council Regulation 178/2002[7] is to ensure that all Member States of the EU have adequate national provisions in place to provide public health protection and protection of other consumer interests in relation to food. It has some similarities to the UK's Food Standards Act 1999,[13] for example, in establishing the European Food Safety Authority to carry out risk assessments. Although it was largely compatible with existing provisions of the Food Safety Act 1990, the Food Safety (Amendment) Regulation 2004[14] and General Food Regulations 2004[15] fully align UK national legislation with Council Regulation 178/2002/EC as regards requirements and enforcement provisions.

This EU Regulation introduces compulsory traceability requirements for all food businesses, as well as making Member States responsible for preventing food fraud, food adulteration and practices which mislead consumers. Article 16 of the 178/2002 requires that labelling, advertising and presentation of food shall not mislead consumers and hence reinforces Sections 14 and 15 of the Food Safety Act 1990.

22.2.4 Council Directive 2000/13/EC and the Food Labelling Regulations 1996

Council Directive 2000/13/EC[9] is, in fact, a consolidation of earlier Directives (including the first Labelling Directive 79/113/EEC) controlling the description and labelling of foods for the final consumer when purchased in retail or in a catering

outlet.[10] The mandatory information required on pre-packaged food in particular has been in place since the 1980s. Any other information can be given voluntarily, but must not be misleading; there are also many 'vertical' rules which require mandatory specific information on certain foods. There have been more subsequent amendments since 2000, and these, along with the main Directive, have been implemented nationally under the Food Labelling Regulations 1996 (as amended). The Directive requires that any labelling, advertisement or presentation of the food should not be misleading to the purchaser to a material degree, particularly:

- as to the characteristics of the food, and, in particular, as to its nature, identity, properties, composition, quantity, durability, origin or provenance, method of manufacture or production process
- by attributing to the food effects or properties that it does not possess
- by suggesting that the food possesses special characteristics when, in fact, all similar foods possess such characteristics.

Pre-packed foods

The Directive details the mandatory information required for pre-packed food which includes:

- *the name of the food*: This can be a legal name (i.e., a name prescribed by EU or UK law, such as cod, haddock, beef), a customary name (e.g., shepherds pie), or a name precise enough to indicate the true nature of the food and distinguish it from other foods (e.g., beef meat balls in gravy). The name must be accompanied by any physical condition or treatment (e.g., frozen, dried, concentrated) – if omission of such mention is misleading to consumers. If the food or any ingredient has been irradiated then this must be mentioned, and also if any GMO ingredient is used.
- *a list of ingredients*, provided the food is not a single-ingredient food. The name given to an ingredient can be a legal name, a customary name or the name used if the ingredient was sold on its own. The ingredients are given in descending order of ingoing weight into the product. There are only a few generic ingredient descriptions detailed in the Directive (e.g., cheese, vegetable oil and fats, sugar). One of relevance is the ingredient description 'fish', which is any species of fish or mixture of species. Ingredients which have a technical function in the food – that is, additives – have to give the functional category of the additive (e.g., stabiliser, colour, flavouring etc.), as well as the name or E number of the additive. Where the ingredient is itself a compound food (e.g., sausage in 'sausage and mashed potatoes ready meal') then all the component ingredients have to be listed in brackets.
- *the quantity of certain ingredients or category of ingredients*: When an ingredient is mentioned in the name of the food or is associated closely with the food (e.g., steak pie), then the amount of beef steak going into the pie as a percentage of the final pie weight has to be declared either in the name of the food or in the ingredients list. This is known as quantitative ingredient declaration (QUID).

- *the net weight of the food* (i.e., the weight of the food minus the weight of any packaging): Drained weights of canned foods need to be given in addition to the net weight, unless the covering medium is a sauce and consumed with the contents of the can. Frozen products with a protective ice glaze such as fish fillets or prawns have to declare the weight net of glaze, as well as the net weight.
- *an indication of the durability of the food*: Durability dates are the responsibility of the manufacturer or retailer and give an indication to consumers when the food should be consumed. Perishable foods that are susceptible to pathogenic bacterial growth are given a 'use-by' date, and it is illegal to sell a food after its 'use-by' date. Less perishable foods are labelled with a 'best before' date, which indicates a loss of quality of the food after the durability date. As storage and handling of foods is not standardised, manufacturers usually build in a margin of safety into their durability dates.
- *any special storage conditions or conditions of use*: Many products will mention whether they are suitable for home freezing or, once opened, how quickly they need to be consumed.
- *the name and address of the manufacturer or packer, or EU seller*: There must be an address which consumers can contact for advice, more information or complaints.
- *lot identification*: The requirement to mark each batch or lot with a visible, legible and indelible identification, prefixed by the letter L, is to establish a common lot identification system. The details of lot identification are given in Food (Lot Marking) Regulations 1996.[16]
- *place of origin, if omission would mislead to a material degree with regard to its true origin of provenance*: Origin labelling is still open to interpretation, and can be difficult where a food has many ingredients from different sources and these are changed according to market conditions. Under WTO rules, the origin of a food is the last location where a transformation or processing of the food took place. However, Food Standards Agency advice has recommended that where consumers have a special interest in the origin of a food for welfare or ethical reasons, then fuller information should be given – for example, bacon produced in Britain from Danish pigs. This advice has been reinforced by the results of a study, which indicate that consumers do not recognise the WTO definition of origin but want to know the origin of the ingredients.[17] There are more specific rules for certain foods – for example, beef and fish – where origin information is mandatory.
- *instructions for use if appropriate use of the food could not be made of the product without those instructions*: It is usual to give cooking or serving instructions on the label.

Non pre-packed foods

Foods sold loose or just overwrapped, even in a tray, do not have to give all the mandatory information required for pre-packed foods. Member States still

have the option of deciding how much information should be given with non pre-packed foods, given the practical difficulties; in the UK only minimal information is required. Only the name of the food needs to be given and the category of the class of additive, if used in the food. If the food is described by a legal name, then it still has to conform to the requirements associated with that name.

Nutritional information

The requirements for nutritional labelling are detailed in Directive 90/549/EEC.[18] Unless a nutritional claim is stated – for example, low fat – then, at present, nutritional information is given on a voluntary basis. If nutritional information is given, then it must follow a set format based on 100 g portion of the food. The usual information given is either the 'big 4' or 'the big 4 and little 4' as shown in Table 22.2.

Loose or non pre-packed food also gives nutritional information only on a voluntary basis, and may give just one of the stated nutrients. If a claim is made such as 'low fat pâté', then the fat content per 100 g has to be stated.

Allergen labelling

The Food Labelling Directive 2000/13/EC was amended by Directive 2003/89/EC[19] to require ingredients known to cause allergies or intolerances to be labelled. Subsequent amendments have been made to the Food Labelling Regulations 1996 in 2004, 2005, 2007 and 2008. There are 14 categories of ingredients that need to be declared, as well as a list of exemptions of ingredients no longer considered to be allergenic. The three of interest to this chapter are fish, molluscs and crustaceans. Other provisions were to remove some of the generic descriptions of ingredients so that every ingredient is listed, and a previous exemption that allowed compound ingredients to be listed if less than 25% of the food.

22.2.5 Permitted additives and processing aids

The use of enzymes and additives in food are controlled by Regulations 1332/2008[20] and 1333/2008[21] respectively, which replace the previous additives legislation Directive 95/2/EC.[22] These two Regulations set up processes for approving enzymes and additives in order to try and simplify the existing provisions. Enzymes are principally considered as processing aids – substances which aid processing but do not have a technological function in the final product, and are not usually limited to specific foods. Generally, additives are not permitted in unprocessed foods

Table 22.2 Format of nutritional labelling for the main nutrients per 100 g of food

Energy kJ or kcal	Energy kJ or kcal
Protein g	Protein g
Carbohydrate g	Carbohydrate g of which: sugars g
Fat g	Fat g of which: saturates g
	Fibre g
	Sodium g

unless specifically allowed. For example, vacuum packing and modified atmosphere gases such as nitrogen can be used for 'fresh meat and poultry' and all other foods. As tenderising enzymes are regarded as processing aids, they can be used in fresh meat. Also the use of polyphosphates and triphosphates (E451, E452) in frozen/quick-frozen fillets of fish and shellfish to a level of 5 g/kg of product is permitted to reduce thaw and cooking loss. Water-retention agents such as polyphosphates, soya or milk proteins or hydrolysed collagen proteins if added to fresh meat (including poultry) then transform the product into a meat preparation, and the product must be described accordingly to indicate the presence of these ingredients and water. Nitrites and nitrates are the most common and traditional preservatives in meats. The permitted levels have been reduced to keep nitrosamine levels to a minimum while maintaining microbiological safety. Cured products are based on added levels rather than residual ones, and vary depending on whether the cured meats are raw or heated treated. Traditional products, for example, which have been immersion or dry cured, are still based on residual amounts.

22.2.6 Codex general standard for the labelling of pre-packaged foods

The objective of Codex Standards is to facilitate international trade, but they are not mandatory for countries to adopt.[23] However, under the WTO-SPS (World Trade Organization Sanitary and Phytosanitary) Agreement,[24] countries cannot impose health or safety requirements on imports that are stricter than Codex Standards or Guidance, and similarly under the WTO-TBT (Technical Barriers to Trade)[24] Agreement, higher requirements cannot be imposed on imports than exist in international standards (Codex being one of the main sources). This has given Codex Standards a much higher profile than they enjoyed previously.

The Standard is, in essence, similar to the EU Labelling Directive 2000/13/EC in detailing the basic information required on the labels of pre-packed foods: the name of the food, list of ingredients, allergen labelling (only eight groups), QUID, durability dates (only best before), net contents and drained weights, address of manufacturer or packer, origin (if misleading to omit), lot identification and instructions for use.

22.3 Origin, assurance and 'eco-labelling' schemes

Consumers are increasingly concerned about how their food is produced and where it comes from. Purchasing decisions are also made on the basis of animal welfare considerations and environmental and social impact. Manufacturers, suppliers and retailers can demonstrate to consumers that their products are produced in a sustainable way, or have protected origin by putting logos linked to assurance schemes on the labels of foods. Some of these schemes are regulatory based, such as organic and protected origin logos; others, such as farm assurance schemes (e.g., 'red tractor'), 'fairtrade', 'freedom' food, are private initiatives, which still have inspection and certification requirements.

22.3.1 Protected designation of origin (PDO), protected geographical indication (PGI) and traditional speciality guaranteed (TSG)

In 1993, EU legislation came into force which provided for a system for the protection of food names on a geographical or a traditional recipe basis. The idea was to have a system similar to 'appellation controlleé' for wines, but applied to other foods and beverages. The scheme is open to regional and traditional foods that can guarantee their authenticity and origin. Once registered, the name of the food or beverage is given legal protection against imitation throughout the EU. Council Regulations 509/2006[25] and 510/2006[26] give the basis of the schemes for TSGs, PDOs and PGIs respectively. The procedures to apply to one of these protected schemes are detailed in Commission Regulation 1898/2006[27] for PDOs/PGIs and Commission Regulation 1216/2007[28] for TSGs. Producers normally apply through their national organisations (in the case of the UK this is ADAS), and the European Commission publishes a full list of foods that have been registered.

PDO

Open to products which are produced, processed and prepared within a particular geographical area, and with features and characteristics which must be due to the geographical area

PGI

Open to agricultural products and foodstuffs closely linked to the geographical area. At least one of the stages of production, processing or preparation takes place in the area.

TSG

Open to products which are traditional or have customary names and have a set of features which distinguish them from other similar products. These features must not be due to the geographical area where the product is produced, but highlight traditional character, either in the composition or means of production.

22.3.2 Organic foods

The description 'organic' can only be used if the food has been grown, reared or prepared according to the strict criteria laid down in EU law. It can also be used for processed products if 95% of the ingredients are produced organically. The Regulation governing organic production has recently been reviewed and revised, and Council Regulation (EC) No 834/2007[29] covers organic production and labelling, whereas Council Regulation (EC) 889/2008[30] gives more detail rules on the implementation and certification of organic production. Defra is the government body responsible for the regulation in the UK, and it approves certification bodies to inspect and certify organic producers. Table 22.3 gives a list of the UK certifying bodies and their codes. The certification code has to use the acronym of the country as set out in ISO (International Standards Organization) Standard 3166, hence the change from UK to GB.

The Regulation covers all aspects of production from the breeding stocks and strains of animal, location of holding, the stocking density, feed, veterinary treatments and management practices to support for animal health and welfare. Certification of organic holdings is carried out by audit and there is also an annual inspection.

Organic foods have to label the certification number of the certifying and inspecting body and, optionally, each of the certification bodies can add their own logo. After July 1, 2010, all organic foods produced in the EU have to carry a European logo (see Fig. 22.1a) and mention of the place where the agricultural

Table 22.3 Approved UK organic certification bodies (previous certification number)

Organic Farmers and Growers Ltd	GB Organic [Certification] 2 (UK 2)
Scottish Organic Producers Association	GB Organic [Certification 3] (UK 3)
Organic Food Federation	GB Organic [Certification] 4 (UK 4)
Soil Association Certification Ltd	GB Organic [Certification] 5 (UK 5)
Biodynamic Agricultural Association	GB Organic [Certification] 6 (UK 6)
Irish Organic Farmers and Growers Association	GB Organic [Certification] 7 (UK 7)
Organic Trust Ltd	GB Organic [Certification] 9 (UK 9)
Quality Welsh Food Certification Ltd	GB Organic [Certification] 13 (UK 13)
Asisco Ltd	GB Organic [Certification] 15 (UK 15)

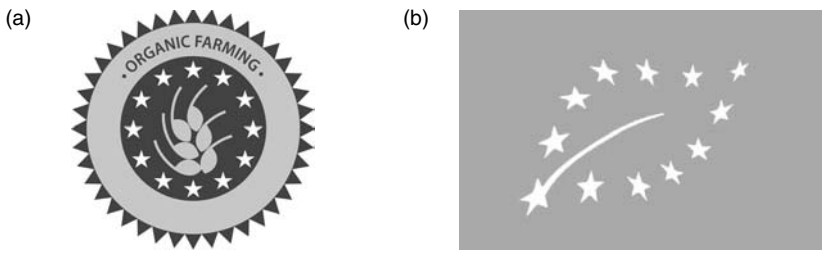


Fig. 22.1 Designs of European organic food logos.

products were farmed. The new logo, which was decided by an open voting system on the European Commission website,[31] is shown in Fig. 22.1b), and has replaced the previous logo that was different in each of the Member States' languages. Organic foods from outside the EU can use the logo, but it is not compulsory. Where a food contains less than 95% organic ingredients, the logo cannot be used and the food has to label which of the ingredients are organic and their proportion in the list of ingredients. Packaging that conformed to the previous organic regulation can be used until January 1, 2012.

22.3.3 Private assurance schemes and eco-labels

There are now a number of voluntary schemes that either set quality standards or aim to reduce the environmental effects or improve animal, welfare which require audits or inspections. Once accredited, the food producers can use the logos to inform consumers that their product achieves a certain standard. Most of the schemes are self-financing. The International Standards Organization has developed a series of guides for different types of schemes, the most important guide (ISO 14024:1999[32]) being for schemes which have independent auditing or inspection. Table 22.4 gives details of a number of these schemes.

22.4 Specific (vertical) requirements for raw meat and minced meat labelling

Although origin labelling is only given where omission would mislead consumers, meat and meat products are one of the main food areas where consumers have indicated that they would like to see origin information. According to recent research by the Food Standards Agency,[17] in 2009, 78% of meat and meat products now carry a country of origin statement compared to 69% in 2005. This includes, however, the compulsory origin labelling of beef, which has been in place since 1997.

22.4.1 Protected origin descriptions

The following UK meats have obtained protected origin either as a PDO or PGI; in addition two other meats have applied for TSG status. Some French and German examples of protected origin of meat are also given in Table 22.5. The European Commission holds a list of all protected origin foods in the EU (http://ec.europa.eu/agriculture/quality/door/list.html).

22.4.2 Beef labelling

The concerns about health risks in the transmission of bovine spongiform encephalopathy (BSE) from cattle to humans and other animals prompted the compulsory origin information that enabled beef to be traced back to where it came from. Council Regulation (EC) 1760/2000[33] replaced earlier regulations and established

Table 22.4 Examples of assurance, eco-label and welfare schemes

Name and website	Objective of scheme	Logo
Red Tractor Scheme www.redtractor.org.uk	Unifying different sector standards under a single assured food standards umbrella along food chain. Checks food safety, animal health, welfare and protection of the environment.All farm standards can be found on the red tractor website and include standards for beef and lamb, poultry and pigs.	
LEAF- Linking Environment and Farming www.leafmarque.com	Promotes environmentally responsible farming to produce good food with care and to high environmental standards. Provides an environmental audit to give improvements and cost benefits.	
Freedom Food Ltd. www.rspca.org.uk/*freedomfood*	RSPCA's farm assurance and food labelling scheme. The scheme focuses on improving the welfare of farm animals reared for food including fish. Covers all stages from rearing to slaughter. Audits by RSPCA trained assessors	
Marine Stewardship Council. (MSC) www.msc.org	An initiative founded by WWF and Unilever which works with FAO to tackle overfishing and difficulties of future supplies of fish. Has developed standards for sustainable fisheries with traceability system along the food chain.	
Earth Island Institute (EII) www.earthisland.org	Standards developed by EII and Heinz to monitor tuna fishing and processing companies to ensure that tuna is not caught by methods that harm dolphins. There many national logos, but EII works with 90% of world tuna companies.	

Table 22.5 Examples of meats with protected origin in UK, France and Germany

PDO	PGI	TSG
UK		
Isle of Man Manx Loaghtan Lamb	Scotch Beef	Red Poll Beef (in progress)
Orkney Lamb	Scotch Lamb	Gloucester Old Spot Pork
Shetland Lamb	Welsh Beef	
Orkney Beef	Welsh Lamb	
France		
(Beef) Taureau de Camargue	(Lamb)Agneau de Lozère	
(Lamb) Prés-salés de la baie de Somme	(Veal)Veau d'Aveyron et du Ségala	
	(Lamb)Agneau de lait des Pyrénées	
Germany		
(Lamb) Lüneburger Heidschnucke	(Beef)Bayerisches Rindfleisch (Pork)	
(Lamb)Diepholzer Moorschnucke	Schwäbisch-Hällisches Qualitätsschweinefleisch	

a system for beef labelling and Commission Directive (EC) 1825/2000[34] gives the details rules on the labelling of beef. A recent amendment (Commission Regulation (EC) 275/2007[35]) gives more flexibility on the labelling of trimmings and mince from batches prepared from a mixed origin of beef cuts. The rules apply to all sales of raw beef, whether chilled or frozen, beef mince, including uncooked beef burgers (without any added ingredients), and require information on where the animal was born, reared, slaughtered and cut up. This information is required to be printed on pre-packed beef labels, but can be displayed on a notice for beef sold loose in butchers, for example. Figure 22.2 gives two examples of beef labels and the compulsory origin information that has to be provided. Each beef product is given a reference number of code which serves as a batch code and permits the product to be linked back to the source animal, group of animals or batches of beef used in the trimmings for example for minced beef.

22.4.3 United Nations economic commission for Europe (UNECE) standards for meat

The UNECE is one of the five UN regional commissions set up to promote pan-European economic integration. Its members cover all European states and North America. Its activities include developing internationally harmonised standards that facilitate fair international trade and prevent technical barriers to trade; defining a common trading language for sellers and buyers; promoting a high-quality, sustainable production; and creating market transparency for buyers and consumers. Development of the standards includes not just European and North American participation but also other exporting countries such as Australia, Argentina, Brazil, New Zealand and Japan, therefore gaining international status. There are

British Beef **Rump Steak** 06/03/10/324578/4 – (reference no. or code) Weight: **500g** Price: **£5.25** Unit Price: £10.50/kg **Born in:** *Orkney* **– UK** **Reared in:** *Orkney and Aberdeenshire* **– UK** **Slaughtered in:** *Aberdeenshire* **– UK (1245)EC** **Cutting in:** *Aberdeenshire* **– UK (1789)EC** (plus all the information required by FLR 1996 – use by date, supplier address, storage and cooking instructions)	**Irish Organic Lean Minced Beef** 04/04/11/146239/5 – (reference no.) Weight: **800g** Price**: £3.40** Unit Price: £4.25/kg **Minced in: UK** **Slaughtered in: Ireland** UK 9876 EC **Origin: Ireland** **Cutting in: Ireland** **GB Organic Certification 7** (plus all the information required by FLR 1996 – use by date, nutritional information especially fat content per 100g, supplier address, storage and cooking instructions)

Fig. 22.2 Examples of beef labelling for meat and mince.

standards for beef,[36] pork,[37] goat,[38] sheepmeat[39] and meat co-products.[40] The standards deal with quality and principally give a specification of cuts and carcase quality classification. The labelling sections are without prejudice to national labelling legislation, but detail recommendations for mandatory information and additional information (see Table 22.6) that should be given to consumers.

22.4.4 Fat content of minced meat

In the UK, minced beef accounts for around 47% of beef sales by volume,[3] and hence represents an important component of household cooking. There are some legal limits of fat content in certain types of minced meat prescribed in hygiene legislation. Commission Regulation 2076/2005[41] amended the general animal products hygiene Regulation 853/2004[42] to reintroduce, on a transitional basis, earlier compositional requirements for certain descriptions of minced meat, shown in Table 22.7.

These requirement are not applied to minced meat described differently – for example, minced beef – and hence there no prescribed fat limits for these products, although there is established case law based on previous prosecutions that foods described as beef mince should not contain more than 25% fat unless described accordingly. Claims are frequently used for mince such as 'lean' or 'extra/super lean' which are not considered as nutritional claims, but are used to convey lower fat contents than standard mince. The UK Association of Public Analysts has issued guidance[43] on the fat content of mince so that 'lean' should not have more than

Table 22.6 UNECE meat standards - mandatory and additional labelling information

Labelling information	Unpackaged carcases, quarters or cuts	Pre-packaged meat
1. Mandatory		
Health stamp	✓	✓
Slaughter number or batch number	✓	✓
Slaughter date (not for goat, meat co-products)	✓ (for pork in additional information)	
Packaging date		✓
Name of the product		✓
Use-by information as required by each country		✓
Storage methods: chilled, frozen, deep frozen		✓ (not for pork)
Storage conditions		✓
Details of packer or retailer		✓ (or in documentation)
Quantity (number of pieces)		✓ (or in documentation)
Net weight		✓ (or in documentation)
2. Additional (verifiable through traceability)		**Pre-packaged only**
Country of birth		
Country(ies) where reared		
Country where slaughtered		
Country(ies) where processed or cut		
Country of origin – where birth, rearing, slaughter, cutting and packaging have taken place in same country		
Production and processing systems		
Characteristics of the livestock, production and feeding systems		
Slaughtering procedures		
Processing/packaging date		
Quality/grade/classification		
pH, lean and fat colour		
Quantity (number of pieces) if given in documentation for goat, sheepmeat and meat co-products		

Table 22.7 Compositional and labelling requirements for certain descriptions of minced meat

	Fat content	Connective tissue: meat protein ratio
Lean minced meat	≤ 7%	≤ 12%
Minced pure beef	≤ 20%	≤ 15%
Minced meat containing pig meat	≤ 30%	≤ 18%
Minced meat of other species	≤ 25%	≤ 15%

16% fat, and 'extra/super lean' should not be more than 9% fat. A Food Standards Agency survey[44] found that there extensive overlap of fat contents of the three types of mince (standard/lean/extra or super lean). A qualitative consumer survey[45] carried out on behalf of the Food Standards Agency indicated that most consumers expect a fat content of between 10% and 15% fat for minced beef described as lean. Discussions are still in hand as to whether the fat content of descriptions of minced meat such as lean and extra lean should be prescribed by European law.

22.5 Specific (vertical) requirements for poultry meat labelling

The following sections look at various regulations affecting the ways in which poultry meat can be described, covering special designations and marketing.

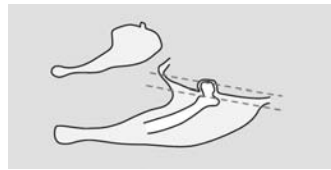
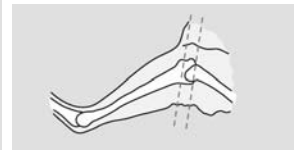
22.5.1 Protected names

There is only one UK poultry product, which is registered as a traditional speciality guaranteed (TSG); that is *Traditional Farm Fresh Turkey.* On the other hand, France has 32 PGIs for poultry from different regions, and also (turkey) *Dinde de Bresse* and (guinea fowl) *Pintadeau de la Drôme*.

22.5.2 The EU poultry meat marketing regulation

A standard for marketing raw poultry meat (whole, parts and offal of chicken, turkey, duck, goose and guinea fowl) has been in place since 1975. The standard has now been incorporated into a consolidated marketing regulation for all agricultural products – Council Regulation (EC) No 1234/2007.[46] The detailed rules for the poultry meat standard are given in Commission Regulation (EC) No 543/2008.[47] The standard covers the quality classifications, description of cuts of poultry meat, temperature ranges of the storage of chilled, frozen and quick-frozen poultry meat, as well as the limits of unavoidable water uptake as a result of preparation and cooling. In addition, claims such as 'corn-fed', or 'free range' are specified. A recent amendment to the Marketing Standard (Council Regulation (EC) No 1047/2009[48]) widens the scope of the Standard to include salted chicken and poultry preparations. The change affects the raw materials that can be used to make poultry preparations, and only permits fresh (not previously frozen) poultry meat to be used in the production of poultry preparations sold fresh (chilled). It does not affect poultry products that are cooked. Salted chicken can enter the EU on a lower import tariff, and salt is the only ingredient that can be added up to 1.2% and the food can still be described as 'poultry meat'. Added water (except extraneous water within limits) or water-retention agents such as polyphosphates can be added to poultry meat or other 'meat', but then the food becomes a meat/poultry preparation, and labelling rules apply. Some of the definitions that are

Table 22.8 Examples of marketing terms defined in the poultry meat marketing standard

Marketing term	Summary definition
Class A and Class B	Defined in terms of appearance and blemishes on whole birds and cuts
Poultry cuts – breast, leg, drumstick, thigh etc. For example: see opposite	Leg- consists of femur, tibia and fibula: two cuts made at joints  Drumstick – tibia and fibula together: two cuts made at joints 
Fresh poultry meat	Poultry meat not stiffened by the cooling process (i.e., freezing), and kept between −2°C and 4°C – i.e., cannot market previously frozen poultry as 'fresh'
'Corn-fed'	Poultry fed with at least 50% maize during rearing
'Free range'	Stocking rate defined and birds must have had access to open air runs during at least half lifetime to an area with vegetation, and fed with at least 70% cereals during rearing
Extraneous water content (technically unavoidable water content)	Limits on the unavoidable water uptake during cooling of frozen whole birds and cuts, especially immersion chilling prior to freezing. Checks on uptake carried out in processing plant.* If the limits are exceeded the product can only be marketed if a declaration 'Water content exceeds EC limits' is on the label. Also food labelling rules on added water may apply
Poultry preparations are included in the Standard	Frozen salted (usually max. 1.2% salt) chicken can only be used for cooked poultry products, whether chilled or frozen, but not fresh poultry preparations

Notes: *The extraneous water limits depend on the method of cooling and the method of analysis. For frozen whole chickens the limits determined by analysis are as follows:

Cooling method	Drip/thaw loss analysis	Chemical analysis
Air cooling	1.5%	2.0%
Air-spray cooling	3.3%	4.5%
Immersion cooling	5.1%	7.0%

given in the Standard and used in the labelling and description of poultry meat are outlined in Table 22.8, and an example of a poultry meat label in Fig. 22.3.

22.5.3 UNECE standards on poultry

UNECE have published quality standards for chicken,[49] duck[50] and turkey.[51] These standards are similar to the meat standards, and give specifications for whole birds and cuts in an international language, as well as quality assessments. Like the meat standards, these standards lay down mandatory and additional information (see Table 22.9) that should be given along with any national requirements.

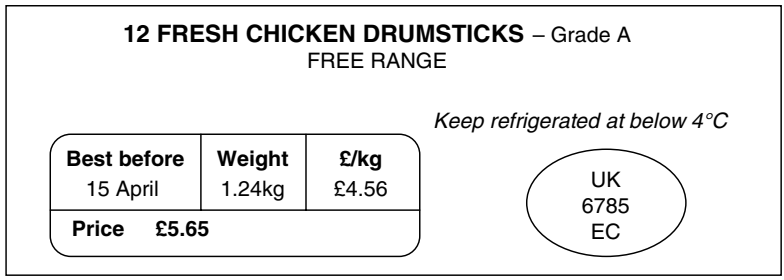


Fig. 22.3 An illustration of a label with poultry meat marketing terms.

Table 22.9 UNECE standards on poultry – mandatory and additional information on pre-packaged poultry carcases and parts

Mandatory Information
Name of the product
Health stamp/inspection stamp
Sell-by/use-by date as required by each country
Storage conditions: e.g., 'Store at or below XX °C'
Appropriate identification of packer, distributor or dispatcher
Net weight in kg (and optionally lb)
Percentage of additional water, i.e., unavoidable water gained during processing and depending on cooling treatment (This information is 'additional' or 'other claims' in the Duck Meat Standard)
Additional or Other Claims as Required by National Legislation or on Purchasers Request or Voluntarily
Country of birth — Country(ies) of raising
Country of slaughter — Country(ies) of processing/cutting
Country(ies) of packing
Country of origin: where 'country of origin' is reserved to indicate that birth, raising, slaughter, processing/cutting and packing have taken place in the same country
Production and feeding systems — Processing/packaging date
Quality/grade/classification — Slaughtering procedures
Chilling system — (Breed only for Duck Meat Standard)

22.6 Specific (vertical) labelling of meat and poultry products

The following sections look at various regulations affecting the ways in which meat and poultry products can be described, including considerations of factors such as meat content and method of recovery.

22.6.1 Protected names

There is only one UK protected geographical indication (PGI) product which is *Melton Mowbray Pork Pie*. France and Germany have many more PGI meat

products and some examples are: (ham) *Jambon de l'Ardèche* (F), (sausage) *Saucisson sec de l'Ardèche* (F), (ham) *Jambon de Bayonne* (F), (beef sausage) *Hofer Rindfleischwurst* (G), (liver sausage) *Thüringer Leberwurst* (G), (salami) *Greußener Salami* (G).

22.6.2 Meat and poultry content of meat products

In 1984,[52] the UK introduced a requirement to declare the minimum meat content for meat and poultry products (except for products with added water, where the added water content had to be declared). In order to achieve a practical solution for this requirement, a simple but wide-ranging definition of meat was used which limited the fat content of the meat or poultry ingredient. Meat and poultry products have to be labelled, as any other compound foods, according to food labelling rules. However, when quantitative ingredient declaration was made mandatory for most food products, this replaced the UK system of minimum meat content with a more problematic requirement. Named ingredients have to be quantified; therefore, in place of giving a minimum total meat content for a pork and beef sausage, both pork and beef have to be quantified separately. In addition, it was necessary to define meat at a European level, so that the labelling of meat products circulating within the single market is all on the same basis. Commission Directive 2001/101/EC[53] amended the Food Labelling Directive 2000/13/EC (and subsequent amendment to the Food Labelling Regulations 1996), giving a definition of meat from all species for the purposes of labelling and calculating the meat and poultry content (QUID) of meat products. The definition of meat is summarised in Table 22.10.

Although the definition benefits the consumer in ensuring that declarations reflect the amount of meat as the consumer would understand the term, it causes more difficulties for manufacturers when calculating meat contents. Quantitative ingredient declarations are based on ingoing ingredients as a percentage of the final product. However, in this case, limits on fat and connective tissue are placed on the ingoing ingredients, and any excess of either fat and/or connective tissue

Table 22.10 European definition of meat for labelling

Meat (plus species name) or in English – beef, lamb, pork, chicken etc. Defined as: skeletal muscles of mammalian and bird species with naturally included or adhering tissue, where the fat and connective tissue content does not exceed the values given below, and where the meat is an ingredient of another foodstuff. Mechanically separated meat (MSM/MRM) is excluded.

Species	Fat%	Connective tissue* %
Mammals (other than rabbits or porcines) and mixtures of species with mammals predominating	25	25
Porcines	30	25
Birds and rabbits	15	10

Note: *Ratio of collagen and meat protein content.

has to be declared separately in the list of ingredients. The excess fat can be declared as chicken fat or pork fat, for example. Excess connective tissue depends on the type of tissue, and can be declared as chicken skin, pork rind or beef connective tissue. Hence, a manufacturer has to know the fat and connective tissue content of the meat ingredients that are being used. Two methods are available in Food Standards Agency guidance[54] to undertake the calculation of meat contents, which are both recipe based.

The CLITRAVI (liaison centre for the meat processing industry in the EU) method

This method is named after the European Trade Association that proposed it, and is the method recommended by the European Commission. It requires that the meat ingredients are analysed to determine whether the fat and connective tissue contents are greater than the limits in the definition of 'meat'. If all the meat ingredients of one species are considered to be skeletal muscle (assembled or disassembled), then a composite analysis of these ingredients in the proportion that they occur in the recipe can be carried out. The marker used for connective tissue is hydroxyproline, which is an amino acid occurring only in collagen and not muscle, and the connective tissue percentage equals hydroxyproline g/100 g multiplied by 37. Fat is determined by solvent extraction following an acid hydrolysis, and the protein also has to be determined using nitrogen as the marker.

The FSA method

The FSA method was devised as a practical rather than analytical method for calculating the meat content. The calculation relies on the manufacturer's estimation of the visual lean (VL) content of each meat ingredient by eye. The fat and connective tissue contents of the meat ingredients are taken from reference tables in the FSA's guidance,[54] based on species, VL and type of meat cuts. These values are used to calculate the total amount of fat and connective tissue for each meat species ingredient, based on the ingoing weight in the recipe, and these are compared to the limits allowed in the definition. If the fat and/or connective tissue is/are in excess, the meat ingredient content is adjusted down by allowing the fat and/or connective tissue only up to the permitted limits. A spreadsheet calculator[55] is available on the Agency's website to simplify the calculation.

Provided the visual lean is estimated accurately, then a survey[56] carried out by the Food Standards Agency in 2007 showed that there is good agreement in the meat content declarations between the two methods. However, the survey found that in nearly 60% of samples examined, manufacturers (which in the majority of cases were small businesses and butchers) underestimated the visual lean by more than 10% when compared with chemical fat determination.

22.6.3 Mechanically separated/recovered meat (MSM/MRM)

Because MSM/MRM is outside the definition of meat for labelling purposes, if it is used in a meat product, it cannot count towards the meat content declaration

(QUID) and has to be labelled separately as 'mechanically separated chicken', for example. MSM is defined in Regulation (EC) 853/2004[42] as 'the product obtained by removing meat from flesh-bearing bones after boning or from poultry carcases, using mechanical means resulting in the loss or modification of the muscle fibre structure'. Conventional machines recover the product from flesh-bearing bones using very high pressures (around 200 bar), which results in the total loss of muscle structure. However, newer and modified machines operate at lower pressures (less than 100 bar) and remove 'meat' from bones under a different process, which results in a product (usually described as 'desinewed meat'), where the muscle structure is substantially intact. A relatively simple microscopy method[57] has been developed which examines the muscle structure of the raw material and enables a decision to be made whether it is MSM/MRM or 'meat'.

22.6.4 Reserved descriptions of certain meat products

The UK has protected the names of certain meat products such as sausage, meat pie and sausage roll since 1967, and an expanded list in 1984.[52] The names are not mandatory, but, if used, then the product has to conform with the definition, and equal or exceed a minimum meat content. Because certain meat products are important in both retail and catering, consumer protection was maintained when the regulations controlling these names were reviewed and amended in 2003[58] (and same requirements in Scotland, Wales and North Ireland). The compositional requirements were also maintained but converted to meat contents based on the European definition of meat. These are UK and third-country requirements only and products from other EU countries do not necessarily have to abide by them. Fortunately, most major retailers follow the requirements in their own label specifications, in whichever country the products are manufactured. Some examples of the reserved descriptions are given in Table 22.11. Many other European countries have mandatory standards for traditional meat products.

22.6.5 Added water

Many meat and poultry products have water added to them as an ingredient. Cured meats are tumbled, injected or immersed in curing brines in order to distribute the brine containing the curing agents, sodium nitrite and nitrate, throughout the meat. When meat or poultry are cooked they lose water and soluble proteins as a result of protein denaturation, and it is common practice in order to improve palatability, to add water and water-retaining agents before cooking to compensate for this loss. Therefore, although some products, whether raw or cooked, look as though they are a piece, cut or joint of meat, they contain added water, and usually other water-retaining agents. General food labelling rules require that the name of the food should distinguish between the two cases – that is, meat with or without added water – to enable consumers to distinguish between the two types of product. There has been a more detailed rule in the UK as to how products with added water should be labelled since 1984.[52] These rules have changed in the detail required

Table 22.11 Examples of reserved descriptions in UK meat product regulations

Reserved description	Pork	Meat from poultry, rabbits or both	Other mammalian meat (e.g., beef, lamb) or mixtures
Burger with any	67%	55%	62%
other name except hamburgerHamburger	67%	N/A	62%
Meat pie – when ingredients uncookedMelton Mowbray pie– when ingredients cooked, name qualified by type of meat only and pie weighs	12.5%	12.5%	12.5%
	12.5%	—	—
≤200g and ≥100g or	11%	11%	11%
when pie weighs <100g	10%	10%	10%
Sausage	32%	26%	30%
Pork sausage	42%	N/A	N/A

Table 22.12 Summary of Regulation 5 of the UK meat products regulations 2003

Added water and other ingredients have to be declared in the name of the food or meat products with the appearance of a cut, joint, slice, portion or carcase of meat or of cured meat (whether cooked or uncooked).

- The declaration is necessary for meat products, raw or cooked or cooked cured meat, when the added water is greater than 5% of the weight of the product.
- The declaration is necessary for uncooked cured meat products when the added water is greater than 10% of the weight of the product.The added ingredients that must be named include added animal ingredients from different meat species, but exclude:
- Any additive, curing salt, garnish or decorative coating ingredient used as solely as flavouring, including sugar.

in the name of the food over the years, and in response to problems found with chicken breast injected and/or tumbled with added water, but also with undeclared added hydrolysed collagen proteins from beef and pork.[59,60] Table 22.12 summarises the main requirements of Regulation 5 of the Meat Products Regulations 2003[58] as amended for meat products which look like a cut, slice, portion or joint of meat, and further details can be found in FSA guidance.[61]

22.6.6 Meat products sold loose

Although meat products sold loose are not required to give a full list of ingredients, there is still a requirement for such products to give consumers information about how much meat they contain, and whether they contain added water and certain ingredients including any GMOs or irradiated ingredients. Only the meat content has to be declared in a product – for example, 'contains 50% pork'. The name would also still have to mention added water, similarly to pre-packed products.

22.7 Specific (vertical) labelling of fish and shellfish

The following sections look at various regulations affecting the ways in which fish and shellfish can be described, focusing on origin, production methods and so on.

22.7.1 Controls on labelling of fish and shellfish

As well as being subject to the general labelling rules, fish and shellfish have very specific labelling and description requirements, which derive from European marketing rules. Council Regulation (EC) 104/2000,[62] which controls the marketing of fishery and aquaculture products, makes the following requirements for describing fish and shellfish:

- the commercial designation of the fish/shellfish (i.e., an agreed commercial name for that species)
- the production method (i.e., whether it is farmed or wild, and, if wild, whether caught at sea or inland waters)
- the catch area (i.e., an area of the ocean in the case of sea-caught fish, or country of production in the case of farmed fish or fish caught in inland waters).

These requirements only cover fish and shellfish products in Chapter 3 of EU Customs Code Combined Nomenclature (CN codes). Whether a product has to follow these rules sometimes appears inconsistent because it depends on its presentation, and whether it falls within the Chapter 3 groups of CN codes. Table 22.13 illustrates this by giving details of what is or is not included in Chapter 3.

More detailed rules on implementing these requirements for the above products are given in Commission Regulation (EC) 2065/2001,[63] and the enforcement of these rules have been enabled in national legislation in England[64] (as well as Scotland, Wales and North Ireland).

22.7.2 Commercial designations

Each Member State is required to draw up and publish a list of commercial designations for fish species accepted within their territory. The commercial designation is the common name for a species of fish described by its international, scientific (Latin) name. For example, only fish of the species *Melanogrammus aeglefinus* (L.) can be described as 'haddock' in the UK; no other fish species can use this name. The European Commission has lists of commercial designations from each Member State. The Food Standards Agency (and now Defra) is responsible for drawing up and amending the list of commercial designations for the UK. Lists are updated by amending the Fish Labelling Regulations 2003, and updated lists are also published on its website.[65] The UK also has to accept commercial designations agreed in the Republic of Ireland in English and vice versa. In some cases, a specific commercial designation covers a family of fish species – for example, the designation 'hake' covers all species of the family *Merluccius*. Commercial designations are legal names; hence the link between the common name and scientific name covers all fish products, not just those in Chapter 3 of the CN codes, as well as food sold in

Table 22.13 Fish and shellfish subject to labelling and description rules

Fish and shellfish products included (Chapter 3 products)	Fish and shellfish products excluded (Non-Chapter 3 products)
Live fish	
Fresh, chilled or frozen fish (whole, gutted, headed, fillets, steaks, minced fish) with no other ingredients except salt	Products with added ingredients or which have been further processed, preserved, treated or cooked: poached salmon, poached salmon fillets/slices
Fish blocks (fillets or mince) without other ingredients except brine	Composite products where the fish is an intrinsic component of the end-product: coated, battered, breaded fish products – e.g., fish fingers, coated scampi
	Ready meals, fish pies etc. where fish or seafood is an ingredient
Smoked fish (cold or hot smoked by natural processes) with only salt – e.g., - smoked salmon- smoked herring (e.g., Buckling), - kippers, - smoked haddock	All smoked fish with colours, flavours etc. e.g., - smoked salmon with honey and sugar - smoked mackerel with colourings and other ingredients (e.g., peppered smoked mackerel)
Surimi (i.e., processed fish protein)	Surimi-based preparations and/or products such as crabsticks, fishsticks and similar products
Fish with butter and/or sauce packaged separately	Fish where butter and/or sauce is added directly onto the fish, considered a further process
Dried fishSalted or brined fish – e.g., salted cod, anchovies	Dried or salted fish with no other ingredient except salt and water
Crustaceans, whether in-shell or not – e.g., prawns, crabs, lobsters Cooked in-shell crustaceansCooked, unpeeled crustaceans Peeled, uncooked crustaceans	Crustaceans which are both cooked and Peeled – e.g., cooked and peeled prawns
Raw molluscs, whether in-shell or not – e.g., mussels, scallops, oysters	Cooked molluscs – e.g., cockle meat out of shell, winkle meat with or without shell

catering. Thus, any food sold as 'cod in batter' has to be made from *Gadus morhua*, *Gadus macrocephalus* or *Gadus ogac.* A product can still be described using only the generic ingredient name 'fish' rather than a commercial name – for example, 'fish pie'; however, the commercial name of each species of fish used will need to be given in the list of ingredients. The scientific names for fish are only required on documentation for traceability purposes, especially in international trade.

22.7.3 Production method

The method of production has to be given with the commercial designation and describes how the fish or seafood was 'harvested' – that is, whether it was produced by

aquaculture or caught 'wild' in the ocean or inland waters. The details in Regulation 2065/2001 define how the production method should be declared to the consumer:

- for fish/shellfish products of aquaculture, the terms 'farmed' or 'cultivated' must be used to indicate that they have been farmed – for example, farmed seabass. In the UK, the term 'cultivated' is used only occasionally for certain molluscs – for example, 'cultivated oysters'
- for products caught at sea or in freshwater, the terms 'caught' or 'caught in freshwater' must be used. However, these terms can be omitted if it is clear from the name that the fish is wild – for example, North-East Atlantic haddock.

22.7.4 Geographical origin

The Regulation requires that for fish or shellfish produced by aquaculture, a declaration as to the country of production, where the final development took place before harvesting, has to be given. In the EU, the named country has to be a Member State. Hence a food described as 'farmed Scottish trout' would also need to add the description (somewhere on the label) 'produced' or 'farmed' in the UK, because Scotland is not a Member State.

Regulation 2065/2001 also details how the catch area for fish and shellfish caught at sea should be described. The Regulation divides the oceans into 12 catch areas, based on FAO statistical classifications, as shown in Table 22.14 and Fig. 22.4. While this is an accurate division of the world's catch areas, many of catch areas detailed in Table 22.14 are not so well known among average consumers. The designated catch areas have to be declared even if more local areas are used in the description. For example, fish described as 'North Sea mackerel' would still need to mention the designated catch area 'North-East Atlantic'. The designated catch areas can be abbreviated – for example, NE Atlantic.

Table 22.14 Designation of catch areas of world's oceans

Catch area	FAO identification of the area
North-West Atlantic	FAO area 21
North-East Atlantic (excluding Baltic Sea)	FAO area 27
Baltic Sea	FAO area 27.IIId
Central-Western Atlantic	FAO area 31
Central-Eastern Atlantic	FAO area 34
South-West Atlantic	FAO area 41
South-East Atlantic	FAO area 47
Mediterranean Sea	FAO areas 37.1, 37.2 and 37.3
Black Sea	FAO area 37.4
Indian Ocean	FAO areas 51 and 57
Pacific Ocean	FAO areas 61, 67, 71, 77, 81 and 87
Antarctic	FAO areas 48, 58 and 88

Source: FAO yearbook, Fishery Statistics. Catches. Vol.86/1. 2000.

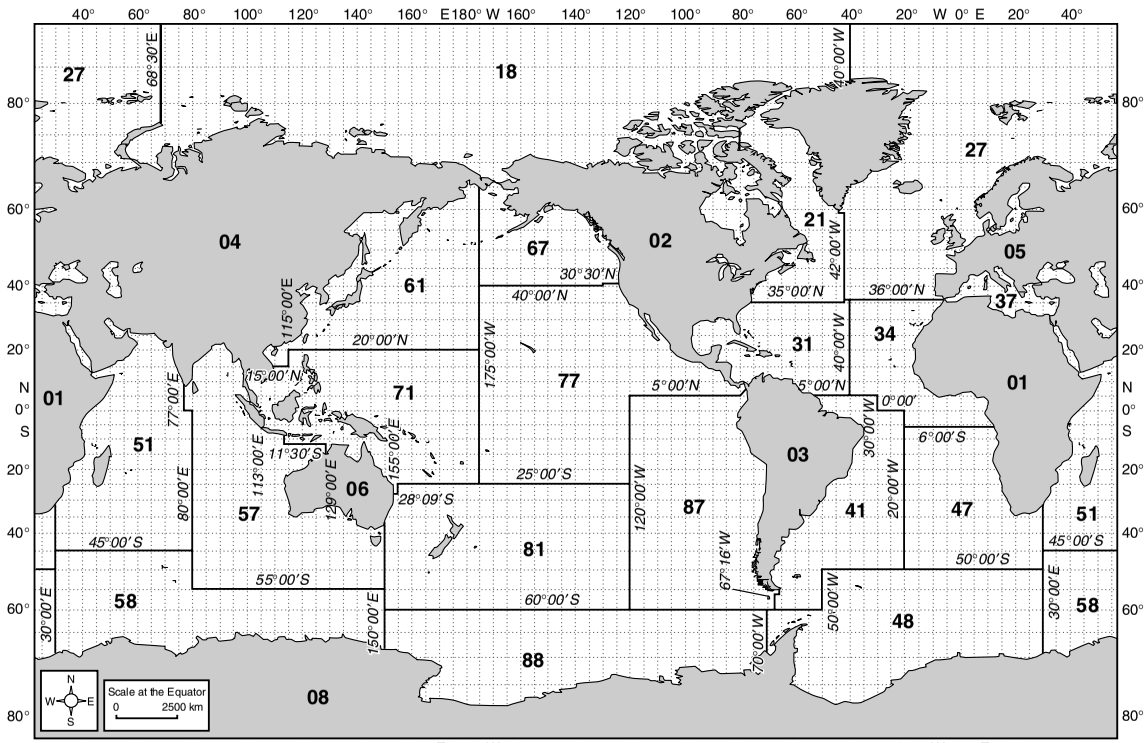


Fig. 22.4 FAO map of major fishing areas of the world.

In the case where a more precise local name for farmed, or 'wild' (caught at sea or in fresh water) fish or shellfish is used, there is some flexibility where the mention of the country of origin or designated catch area is placed. It does not necessarily have to be next to the name, but in the case of a label can be in another position or on the back label, for example. For fish and shellfish sold loose, it is possible to have a 'readily discernible' declaration on a label or ticket or on the wall of the retail outlet as a poster or notice in full view of customers – for example, 'Fish caught in the NE Atlantic'.

For mixtures of fish of the same species coming from a variety of production methods, the Regulations require that the labelling must state each production method – for example, 'a mix of farmed Scottish cod and cod caught in the NE Atlantic', in the order in which origin predominates. For mixtures of fish of the same species coming from different catch areas or fish-farming countries, the origin that is most representative of the batch in terms of quantity must be stated. Hence, a batch of 'farmed salmon steaks' may originate predominantly in Scotland, but also in Norway or Chile, and could be described as 'farmed salmon steaks originating from Scotland, Norway and Chile'.

An amendment to the fish labelling regulation, Council Regulation 1224/2009,[66] has not only emphasised the need to ensure that all the information required by Commission Regulation 2065/2001 is given to the consumer at retail sale, but also whether the fish/seafood has been previously frozen.

Protected names

In the UK there are some registered products with protected geographical indication (PGI):

Arbroath Smokies, *Whitstable Oysters*, *Cornish Sardines*, *Scottish farmed salmon*, *Traditional Grimsby Smoked Fish*.

The PGI 'Scottish farmed salmon' is an interesting case since it takes precedence over the fish labelling rules, but any seafood within the scope of the Fish Labelling Regulations has to comply with them as regards giving the required information to consumers. The PGI covers salmon from the species *Salmo salar* and, among other criteria, has to be farmed in the defined geographical area of the western coast of mainland Scotland, Western Isles, Orkney and Shetland Isles. Salmon farmed in Scotland not conforming with the PGI cannot use the name 'Scottish farmed salmon' or any variation of it such as 'farmed Scottish salmon' or 'salmon farmed in Scotland'. Such products can use the description 'farmed in the UK' or even 'produce of Scotland' with additional reference to the UK somewhere on the label. Products covered by the PGI include not only whole or gutted salmon but also salmon fillet, steak portions, smoked salmon, salmon pâté and ready meals where 'Scottish farmed salmon' is the main ingredient.

Examples of protected names in France and Germany for fish and shellfish include: (mussels) *Moules de Bouchot de la baie du Mont-Saint-Michel* (PDO), (anchovies) *Anchois de Collioure* (PGI), (oysters) *Huîtres Marennes Oléron* (PGI), (carp) *Holsteiner Karpfen* (PGI), (trout) *Schwarzwaldforelle* (PGI).

22.8 Specific (vertical) labelling of fish and shellfish products

The following sections look at various regulations affecting the ways in which fish and shellfish products can be described, considering, in particular, fish content and methods of preservation.

22.8.1 Fish content and description of fish ingredients

General labelling rules require that, in most cases, fish and shellfish products (not subject to the fish labelling regulations) have to give a fish content declaration (QUID), and if more than one commercial designation is used in the name of the food – for example, 'cod and salmon pie' – the amount of cod and salmon used as a percentage of the final weight has to be given. Although this requirement is the same for many foods, like meat, there are some special problems associated with fish and shellfish processing in regard to defining fish and shellfish as ingredients. Many of the stages in fish and shellfish storage and processing involve water, and indeed the use of clean water is a requirement under good hygienic practice. The most important of these stages are icing of the freshly harvested fish, washing at several stages of preparation, freezing followed by ice-glaze application and thawing by immersing in or spraying with water. It is likely that at each of these stages there are changes in weight and composition of the fish. Unlike poultry processing where limits of unavoidable uptake of water during processing are laid down in Commission Regulation (EC) 543/2008,[47] fish and shellfish processing, which has a greater potential for water uptake, has no such legal limits. However, in the UK there is an industry/enforcement authority Code of Practice,[67] which defines fish ingredients produced under good hygienic practice in terms of limiting the uptake of water during preparation. In addition, the names of fish ingredients and products using different forms of fish blocks (i.e., fillet or mince) are defined, along with wholetail and reformed scampi (*Nephrops norvegicus*), as shown in Table 22.15.

Fish blocks are the normal presentation for raw material fish in world trade, and serve as the ingredient for most coated fish products. The description 'wholetail' (whole) scampi can only be used if one or more wholetails are used, and the product does not contain any minced scampi. Controls on the labelling the products, especially the amount of ingredient, can be checked in the case of a product made from one species of fish by analysis of the product. The fish content is calculated from the total fish nitrogen and using nitrogen factors. A list of interim nitrogen factors are detailed in the Code of Practice and take into account loss of soluble nitrogen and uptake of water during good hygienic practice in preparing a fish ingredient. This chemical analysis approach to measuring fish content of products manufactured from fish blocks has been adopted in the Codex Standard for Quick Frozen Fish Sticks (Fish Fingers), Fish Portions and Fish Fillets – Breaded or in Batter.[68]

22.8.2 Labelling of canned or preserved tuna and sardines

Canned sardines and tuna are two fish products that have their own marketing standard in the EU.

Table 22.15 Recommended names of ingredients and products from different fish blocks

Description of block	Name in list of ingredients	Name of product
100% fish fillets	Fish or fish fillet (x%)	P fillet or steak (made with 100% fish fillet)
90% fish fillet with 10% polyphosphate and/or salt solution	Fish or fish fillet (x%)	P fillet or steak
80% fish fillet with 10% V-cut mince and also 10% salt and/or polyphosphate solution	Fish or fish fillet, including up to 10% minced fillet (x%)	Fish P or fish fillet P (indicate the presence of up to 10% minced fillet)
100% fish portions (made from fish cuts from fillets after prime cuts removed)	Fish pieces (x%), pieces of fish (x%)	P from pieces of (more than one) fish
100% minced fish	Minced fish (x%)	P made from minced fish

Note: Where P = product name, and x% is quantity of fish ingredient in product, 'fish' can be replaced by commercial designation of fish.

Table 22.16 Commercial and scientific names of fish that can be called tuna or bonito

Tuna	Bonito
Albacore or longfinned tuna (*Thunnus alalunga*)	Atlantic bonito (*Sarda sarda*)
	Pacific bonito (*Sarda chiliensis*)
Yellowfin tuna (*Thunnus albacores*)	Oriental bonito (*Sarda orientalis*)
Bluefin tuna (*Thunnus thynnus*)	Other species of genus *Sarda*
Bigeye tuna (*Thunnus obesus*)	
Other species of genus *Thunnus*	
Skipjack or stripe bellied tuna (*Euthynnus (Katsuwonus) pelamis*)	Atlantic little tuna (*Euthynnus alleteratus*)
	Eastern little tuna (*Euthynnus affinis*)
	Black skipjack (*Euthynnus lineatus*)
	Other species of the genus *Euthynnus*
	Frigate mackerel (*Auxis thazard*)
	Auxis rochei

Canned tuna and bonito

Council Regulation 1536/92[69] is a marketing standard for the canned product that defines which species of fish can be called 'tuna' and which 'bonito'. It also defines descriptions which can be used in the labelling of canned tuna, such as 'solid', 'chunks', 'flakes' and 'shredded' or 'grated', as well as descriptions of the covering media – 'natural' (own juice or brine), 'olive oil' and 'vegetable oil'. Table 22.16 lists the species that can be called 'tuna' and those that have to use the name 'bonito'. In the case of tuna, the description can also use the commercial name where appropriate.

Canned sardines

The humble canned sardine has been the subject of heated world dispute over which species of fish can use the description 'sardine'. An earlier European marketing

Table 22.17 Species that can be marketed as canned sardines

Sardina pilchardus *Sardinops melanostictus*, *S. neopilchardus*, *S. ocellatus*, *S. sagax*, *S. caeruleus*, *Sardinella aurita*, *S. brasiliensis*, *S. maderensis*, *S. longiceps*, *S. gibbosa*, *Clupea harengus*, *Clupea (Strangomeri) bentincki, Sprattus sprattus*, *Hyperlophus vittatus*, *Nematalosa vlaminghi*, *Etrumeus teres*, *Ethmidium maculatum*, *Engraulis anchoita*, *E. mordax*, *E. Ringens, Opisthonema oglinum*

standard[70] restricted the species using this name to *Sardina pilchardus*. However, as this favoured the Atlantic and Mediterranean processors, it was challenged by Peru in the WTO. Because the Commission Regulation was more restrictive than the Codex Standard on Canned Sardines (STAN94),[71] Peru won the case, and the European Commission had to amend its Regulation. Commission Regulation 1181/2003[72] and an amending Commission Regulation 1345/2008[73] give a revised marketing standard, which allows other species of fish to be labelled as canned sardines, provided this name is joined with the scientific name of the fish and the geographical location of where it was caught using the same catch areas outlined in Table 22.14. Canned sardines made from *Sardina pilchardus* do not have to be qualified with the scientific name or catch area. The Regulation gives a list of species (Table 22.17) that are included in the marketing standard.

22.9 Future trends

In January 2008, the European Commission published a proposal for an EU Food Information Regulation that would replace the Food Labelling Directive 2000/13/EC and the Nutritional Labelling Directive 90/496/EEC.[18] After four years of discussion, the European Council and Parliament agreed a revised Regulation, which was published in November 2011.[74] Businesses will have three years to implement the labelling changes and five years to implement the changes to nutritional labelling. The Regulation consolidates all the amendments and changes that have previously occurred, and removes any differences in national provisions on labelling in different Member States of the EU, which were allowed in the Food Labelling Directive. Other main changes in the Regulation are:

- strengthening the provisions concerning the clarity of information to consumers, and setting a minimum font size for the obligatory information to consumers
- introduction of a requirement for allergenic labelling for foods sold loose and in catering, and allergens will need to be highlighted in the ingredients list
- origin labelling will still remain on the basis of giving the origin of a food or ingredient if omission is misleading. In the case of meat (other than beef and third-country poultry, which are compulsory anyway), country of origin will

be mandatory for meats such as lamb, pork and goat. The Commission has two years to decide on the details of what origin information has to be given

- where the origin of a food is given but it is not the same as the origin of the primary ingredients (making up more than 50% of the food), then the origins of the ingredients have also to be given. Also the Commission has three years to examine extending origin labelling to meat, and milk ingredients that represent more than 50% of a food, unprocessed and single-ingredient foods
- provisions on labelling minced meat and setting fat limits for descriptions such as lean and extra lean have been transferred from hygiene legislation to labelling legislation
- the compulsory declaration of energy, fat (including saturates), carbohydrates (including sugars) and salt (expressed as amounts per 100 g) or per portion in the principal field of vision – that is, front of pack. Voluntary national schemes (such as Guideline Daily Amounts (GDAs) or the UK's traffic light scheme) will still be allowed
- adoption of the UK specific rules on added water and hydrolysed proteins in the Regulation. Hence, where hydrolysed proteins are used in foods with meat or fish ingredients, but are derived from a different species, then the presence and species of these proteins have to be declared in the name of the food. Meat, poultry and seafood which contain more than 5% added water and which look like a cut or portion have to declare the amount of added water in the name of the food, and this includes the addition of water to cooked and uncooked cured meat."

22.10 Acknowledgements

I would like to thank my former colleagues at the Food Standards Agency for their help and information in writing this chapter, especially Pendi Najran.

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23

Food packaging laws and regulation with particular emphasis on meat, poultry and fish

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Abstract: The chapter gives an overview of the regulatory regime for food contact materials in the EU (European Union) and in the United States, contrasting the differing approaches taken in each jurisdiction. The impact of these different approaches is examined in terms of globalization of supply and the impact upon product development. Possible future developments in legislation and regulation of food contact materials are discussed.

Key words: food contact materials, food contact substances, EU law, USFDA, globalization, food packaging law.

23.1 Introduction to food contact material legislation

It has been estimated by the European Union Directorate General for Enterprise that in the EU the food industry is the third most regulated industry after automobiles and chemicals. As packaging materials straddle both food and chemicals, it is to be expected that it is subject to a very high degree of regulation. Any brief review of packaging regulations globally will confirm this. Despite this regulatory regime, we can still see numerous incidents of product recalls due to heavy metals in ceramics, formaldehyde or primary aromatic amines in kitchenware or migration of photo-initiators from printing inks. The regulation of food can be seen as different to other classes of goods. Food, being a fundamental human right, demands protection for consumers and political sensitivity will ensure that regulation will address any threat to its integrity.

Among the public there exists a general perception that packaging materials are inert (Colwell, 2010). While this may be desirable, it is, of course, not the case. Under certain conditions, practically all materials will release some of their constituents. This can be as a result of the chemical migration of molecules from the packaging material and their absorption by the food or even their physical transfer through abrasion with the food material. Hence, the correct questions are not so much whether there is migration or not, but how much migration there is and whether it is in quantities which are safe for the consumer. Thus, fundamentally, all regulatory regimes stipulate, first of all, methodologies for estimating the degree of migration from the packaging materials and, second, specify that the level of migration is within tolerable safety limits. There are, of course, philosophical differences regarding the way these objectives are achieved and this will be explored later while examining the differing regulatory systems in use.

The regulation of packaging has a particular relevance to muscle foods (meat, fish and poultry). They are particularly vulnerable to spoilage from both pests and microbes, hence packaging plays a major role in their protection. However, the water and fat content of muscle foods can itself facilitate the migration of chemicals from the packaging material. Thus, the correct selection of a packaging material is imperative in order to ensure that it is fit for purpose.

Packaging is regulated as part of the control of all food contact materials. While packaging materials comprise the largest volume of these materials, food contact materials also include items as diverse as gloves, knives, chopping boards, mixing bowls – even the gaskets in those bowls, table tops, conveyer belts, piping, free toys in children's fast food meals, fish boxes and, arguably, even fishing nets; thus, anything which has the potential to transfer some of its constituents to food is potentially regulatable as a food contact material.

The regulation of food contact materials can be difficult and complex for a number of reasons. There is an enormous diversity of food contact materials in use, from paper to plastics (in all its varieties), metals to melamine, cellulose to ceramics. In addition, there are multi-layer combinations of these materials. Furthermore, there are also inks, adhesives, seals and waxes which can be applied to the package, all of which need to be regulated. The food to be packaged is equally diverse in terms of its constituents, its moisture, fat or alcohol content, its pH, whether it is fresh, processed or ready to eat. The conditions in which the food contact materials are expected to perform vary widely: temperatures from freezing to roasting, vacuum to high pressures, a range of atmospheric gas compositions and storage durations varying from moments to months. In addition, the development of 'smart' packs, edible films and the environmental demands for the use of potentially contaminated recycled materials further complicate any regulatory regime.

While the primary purpose of the regulation of food contact materials is the protection of the public, it also has a role in encouraging free trade – the transparency and harmonization of regulations facilitating the free movement of goods. Regulation, and its enforcement, protects the honest producer against the unfair

advantage taken by their unscrupulous competitor. This chapter will examine regulations as they apply to food contact materials only; however, it must be added that other regulatory measures also apply, such as contract law and civil liability or tort law but these are outside the scope of this chapter. There are also various private standards which encompass food contact materials, such as the British Retail Consortium, Global Standard for Packaging, but again they are outside the scope of this work.

23.2 The regulation of food contact materials in the European Union (EU)

The EU was founded in 1957 as the European Economic Community (EEC) by six countries with the objective of improving trade and political cooperation. It has now grown to 27 member states sharing a single market and strong economic, political and social cooperation (Madelin, 2008). Given that the EU is currently the world's largest producer of food and drink products (O'Rourke, 2005), the regulations in place for the control of food contact materials within its borders are of global significance.

23.2.1 Food contact materials regulation within the context of European food law

It is interesting to note that neither food nor consumer protection were mentioned in the Treaty of Rome, the treaty that founded what is now the EU. Food was considered an issue to be regulated by the member states themselves. What regulation there was focused mainly upon commodities, was piecemeal and in place primarily to ensure free trade between the member states. However, a series of dioxin and BSE crises in the 1990s radically changed the way in which food and food contact materials were to be regulated. Consumer protection is now the overriding objective of all food-related regulation within the EU.

The general food law, Regulation 178/2002, states that there should be a high level of human health protection, that decisions in this regard are to be based upon risk analysis, that there is to be traceability of all food from farm to fork and that food business operators have the primary responsibility in ensuring the wholesomeness of the food which they sell. It also established the European Food Safety Authority (EFSA) as an independent risk assessment and communications agency and upgraded the Rapid Alert System for Food and Feed (RASFF) within the Community as a monitoring tool for food safety emergencies.

23.2.2 The regulatory enactment process in the European Union

All regulations, or changes to regulations, are proposed by the European Commission, the administrative arm of the EU. These proposals are debated by both the European Parliament, representing the citizens of the EU, and the

European Council, which represents the governments of each of the member states. In most areas, including food contact materials legislation, both bodies must agree in order for the regulation to become law, a process formerly known as 'co-decision', now referred to as the 'normal process' given that it is predominant. Community legislation may be introduced as a directive, a regulation or a decision. 'Directives' specify the objectives to be achieved by the legislation and it is up to each member state to transpose these into their national laws. There is a limited time allowed for the completion of this process after which time the legislation comes into force. In contrast, legislation in the form of a regulation does not require transposition and comes into force on the date specified in the regulation. However, national legislation might still be required where a regulation demands penalties to be imposed as the EU has no jurisdiction in criminal matters. Decisions are normally administrative in nature and apply to specific individuals or situations. It is perhaps worth noting the nomenclature – directives (and decisions) will always have the year followed by its number, while a regulation will have the number first, followed by the year.

While the directive allows for adaptation to suit local conditions (in keeping with the subsidiary principle), the regulation has the advantage of uniformity of guidance and control. In the case of food contact material legislation, the regulation has become the favoured implementation method (Schäfer, 2010). Minor amendments to the framework legislation, such as altering thresholds or adding or removing specific materials from approved lists, does not require the co-decision process. They can be approved by agreement of a committee comprising representatives of each member state of the EU council through the process of comitology (van der Meulen and van der Velde, 2008).

23.2.3 The regulatory enforcement process in the European Union

It is the duty of each member state to have in place adequate official controls to enforce EU legislation within their jurisdiction. Official controls in the EU are regulated according to Regulation (EC) No 882/2004 of the European Parliament and of the Council of 29 April 2004 on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules. The EU Food and Veterinary Office, a unit of the DG Sanco, the Health and Consumers Directorate General of the EU Commission, audits the official controls in each member state. These controls include the implementation of food contact material (FCM) regulations for all food exported from or sold within the EU. Failure by a member state to implement regulations will result in action by the Commission, ultimately in the European Courts of Justice (ECJ).

23.2.4 Mutual recognition and the national food contact material (FCM) legislation of other member states

Food contact materials are regulated both by harmonized EU legislation and, to the confusion of those outside of the EU, non-harmonized national legislation.

Any additional national measures or exceptions to the harmonized EU regulations are justifiable only on the grounds of the protection of human health. This is illustrated by the recent controversial Danish ban on the use of Bisphenol A (BPA) in babies' bottles on the grounds of a potential but unproven risk. This was later extended EU wide in Regulation (EU) 321/2011, invoking the precautionary principle. However, the stakes have been raised somewhat with the French National Assembly (October, 2011) approval of a ban on the use of BPA in all food packaging materials from 2014 and warning labels on packaging containing BPA for pregnant women and children under three from 2013.

Member states are free to introduce national legislation where materials are not included in specific EU legislation, but it cannot be done with the intention of creating a technical barrier to trade. The free movement of goods between member states is a cornerstone of the EU treaty. Thus the principle of mutual recognition as established in the case commonly referred to as *Cassis de Dijon* also applies to food contact materials. Hence, any FCM lawfully produced and/or marketed in one member state should be free to move throughout the EU.

As outlined in *Cassis de Dijon*, any exceptions must be justified on the basis of overriding reasons of public interest. This has been an evolving concept which numerous court judgments, Council Resolution of 28 October 1999 (European Council, 2000) and Regulation (EC) No. 764/2008, have sought to clarify. Hence, what constitutes 'justifiable public interests' has not always been clear. Regulation (EC) No. 764/2008 allows for prior authorization of food contact materials by a member state, but it must be proportionate to the risk and be non-discriminatory. Also, economic operators who might be effected by any proposed national regulations must be informed and consulted in advance of their introduction.

Given that 21 of the 27 member states have national provisions in place for food contact materials (EU Commission, 2011), it is imperative that, in seeking guidance on FCM regulation, both EU and national provisions are consulted in tandem. A current list of all national provisions is maintained on the EU website (listed in Section 23.10 below).

23.2.5 Framework Regulation (EC) No 1935/2004

Regulation of food contact materials in the EU began 1976 with the adoption of a framework directive (76/893/EEC) establishing the general principles for the sale and use of all FCMs in the EU. This was replaced by Directive 89/109/EEC, which in turn has been replaced by the current Regulation (EC) No. 1935/2004. An outline of the Community legislation on Food Contact Materials is presented in Fig. 23.1.

The framework regulation sets the general requirements and rules for FCM. It defines food contact materials as 'any materials and articles which are or may be in contact with food and or transfer their components to food through normal and foreseeable use'. It excludes antiques, fixed water supplies and coverings or coatings which are consumed with the food (e.g., cheese rinds) (Evans, 2011a).

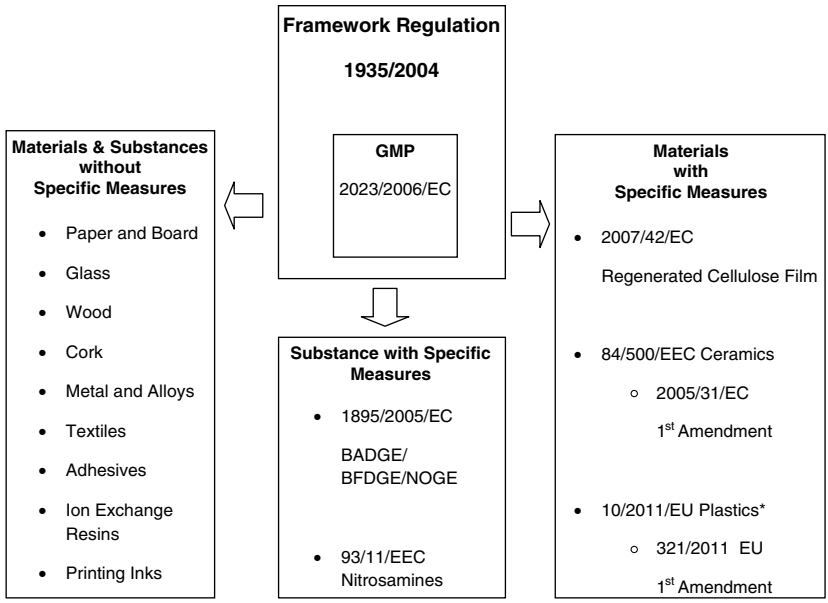

Fig. 23.1 Overview of EU food contact material legislation (last update November 2011). Note: This regulation replaces a number of older directives and regulations; however, some of the older measures remain in place for a transitional period. These are not shown in the figure but are referred to in the text.

The regulation ensures that food is safe and the consumer protected by setting five basic requirements:

1. The FCM should not endanger human health.
2. It should not unacceptably change the composition of the food.
3. It should not change the organoleptic characteristics of the food.
4. The FCM must be manufactured according to good manufacturing practice (as specified in a separate regulation).
5. The consumer must not be misled – by advertising or labelling, for example.

The regulation allowed an exemption for active and intelligent materials and articles (Schäfer, 2010).

The Commission is empowered to adopt specific measures for groups of materials, listing 17 such materials in the annex to the regulation. To date, specific measures have been adopted for ceramics, plastics, recycled materials and active and intelligent materials, along with three other substances.

An authorization procedure (positive list) applies where there is a specified measure; however, currently only regenerated cellulose and plastics are so regulated. The application is made through the member state to the EFSA with an accompanying dossier. The EFSA will conduct a risk assessment and the commission, who are responsible for risk management, will decide based

Fig. 23.2 Article 15 of the Framework Regulation (EC) No 1935/2004 on materials and articles intended to come into contact with food specifies the labelling requirements. It says that food contact materials shall be accompanied by the words 'for food contact' or this symbol, unless it is obvious that the article is for food contact.

upon the safety evaluation from the EFSA and other factors. All authorizations are general in that they apply to everyone wishing to use the material (Schäfer, 2010).

All FCMs must be labelled to indicate their suitability for food use (unless obviously for food use – for example, a fork). This can be the words 'for food contact' or the use of the approved symbol (see Fig. 23.2). Any special instructions for the safe use of the material must accompany the FCM, along with the name and address of the manufacturer, processor or seller to facilitate traceability.

While the general food law, Regulation 178/2002, demands full traceability of food to ensure efficient recall in the case of an emergency, it does not apply to the package; however, Article 17 of Regulation 1935/2004 extends traceability to the packaging component. It applies to all stages of production and distribution. Records must be maintained of customers and suppliers, and these must be available for inspection to the competent authorities on demand.

When a specific measure is adopted the materials or articles must be accompanied by a declaration of compliance. This must be available to the competent authorities on demand. Detailed declarations of compliance are required for plastics, recycled plastics, ceramics and active and intelligent materials. The declaration must include the name and address of the EU manufacturer or importer of the material or substance, facilitating traceability. The material used should be identified and it should be confirmed that it complies with the regulations. The Declaration of Compliance should include the safe use of the material in terms of suitable foods, time/ temperature, and surface to volume ratios in addition to any specific restrictions under the regulations.This information is essential in allowing customers to know that their use of the material will, in turn, comply – thus facilitating the safe use of the food contact material (Hegarty, 2010).

23.2.6 Good manufacturing practice: Commission Regulation (EC) No 2023/2006

Framework Regulation 1935/2004 specifies that the manufacture of FCMs should comply with detailed rules on good manufacturing practice (GMP). To ensure uniformity among member states, Commission Regulation (EC) No 2023/2006 on GMP for materials and articles intended to come into contact with food was enacted. Article 2 states that GMP should apply to all stages of food contact material production, from the manufacturers of the FCM to the converter to the food industry, but excluding the basic raw materials suppliers. There should be an effective quality management system detailing the quality assurance steps in place to control the process and ensure that degradation products are not included in the final package.

Recent cases of ink causing taint in cereals and concerns about mineral oils from inks contaminating recycled cardboard are addressed in the annex to the regulation. Specific rules are included with regard to the avoidance of offset whereby ink from the printed side contaminates the non-printed side of a package, potentially contaminating the food. The annex to the regulation was amended by Regulation (EC) No. 282/2008 on recycling to add rules on recycled materials. It states that GMP should be applied to all stages of the recycling process.

23.3 EU legislation on specific materials

Specific measures apply to only four materials, with vinyl chloride having been the first area addressed in 1978. This was followed with extensive legislation on plastics and, at the start of 2011, a comprehensive overhaul of plastics regulation was enacted as Commission Regulation (EU) No 10/2011.

23.3.1 Plastics: Commission Regulation (EU) No 10/2011 on plastics materials and articles intended to come into contact with food

This regulation, which came into force in May 2011, is better known as the Plastics Implementing Measure (PIM), on which work commenced in 2004 (Langhorn, 2011). While the aim of the PIM was to clarify and consolidate previous directives and their amendments and delete obsolescent regulations, it has gone far beyond those goals. Figure 23.3 summarizes the changes in the PIM. The regulation recognizes that technological advances, together with industry demands for better, cheaper and less polluting materials, often result in packaging comprised of up to 15 layers, which may also include paper or metal. In addition, the layers may include adhesives, coatings and inks. It addresses new technological advances such as nanotechnology, fermentation-derived materials and the concept of functional barriers. The PIM also included major changes to the migration testing regime, with specific consideration for particular packs and consumers, such as children.

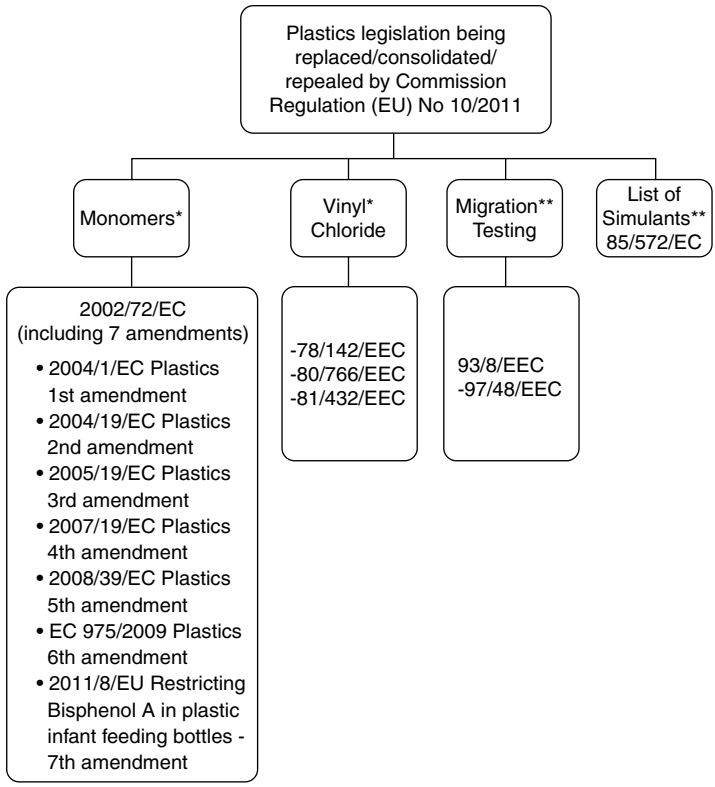

Fig. 23.3 Legislation replaced/consolidated or repealed by the new plastics regulation, Commission Regulation (EU) 10/2011. Notes: *Commission Regulation (EU) 10/2011 replaced these from the 1 May 2011; **Commission Regulation (EU) 10/2011 replaces these from the 1 January 2013.

23.3.2 The EU toxicological approach

The plastics regulation recognizes that polymers, due their large molecular size, are unlikely to migrate, hence control must focus upon the safety of the monomers used in their manufacture together with any impurities or by-products of the polymerization process. Thus, all monomers and additives to be used must have been risk assessed by the EFSA based upon the worst foreseeable conditions of use. They must appear in a positive list in order to be approved for use. Impurities are defined as 'non-intentionally added substances' (NIAS) and, while not requiring authorization *per se*, their use should be assessed in accordance with the internationally recognized scientific principles of risk assessment. The same rule applies to degradation and reaction products and the catalysts used in the manufacture of polymers. Neither colorants nor solvents present in the polymer are subject to authorization as yet. Materials in nanoform require separate assessment from the material in its parent form, given their potentially altered properties.

23.3.3 Compliance testing

In order to ensure the safety of packaging materials it is necessary to ensure that the migration of substances into food is below allowable limits. There are two measurements of migration: one is of specific substances, the specific migration limits (SML); the other is of the total level of migration of all substances, known as the overall migration limit (OML).

The OML sets a generic limit for inertness of 10 mg/dm^2 of packaging material. This limit is derived from the general assumption of a maximum daily intake of up to 1 mg/kg of body weight. Thus for a 60 kg person the limit would be 60 mg. As a 60 kg person consumes 1 kg of food per day, the food, if packaged in the form of a cube, would have a surface area of 6 dm^2 available for migration. Thus the limit for total migration is 60 mg/6 dm^2 or 10 mg/dm^2 (Evans, 2011b).This is a worst-case scenario as it assumes that migration occurs with 100% efficiency which, in reality, overestimates exposure, thus enhancing the margin of safety.

It is important to note that this OML derived from 1mg/Kg body weight is based not upon toxicological concerns per se, but from two other factors (Schuup, 2012). The first is from the general observation that above this limit the quality of the food is effected (Schuup, 2012). The second is that the testing regime for food contact materials takes a tiered approach. Thus the higher the migration rate, the more stringent the testing requirement (Barlow, 1994). The testing requirement for levels in excess of 1mg/ Kg Body weight (or 10mg/dm^2) would be excessive and likely incur high failure rates hence 10mg/dm^2 is a practical cut off point.

The SML is the maximum permitted amount of a given substance allowed to migrate into a food or food simulant, and is specified in Annex I of the regulation. The SML is expressed in mg of substance per kg of food (mg/kg). It is established through a specific authorization procedure conducted by the EFSA based upon a favourable toxicological assessment (Schäfer, 2010). The SML is set to be equivalent to the tolerable daily intake (TDI), the safe consumption limit for the substance. Thus, again assuming that a 60 kg individual consumes 1 kg of food per day and that the food is wrapped in the plastic material measuring 6 dm^2 , the SML is set as the amount of migration from that 6 dm^2 of packaging material which is equal to the TDI. For substances without a specific migration limit (so not in Annex 1) a generic specific limit migration limit of 60 mg/kg is applied.

23.3.4 Food simulants

Migration can be measured either in the food itself or in a suitable food simulant. A food simulant is a test medium imitating food. For example, substances will migrate into a 10% ethanol solution at approximately the same rate as they would into fresh meat. The use of simulants standardizes the testing and can simplify the testing methodology. Annex III specifies six simulants and assigns foods to each.

23.3.5 Migration testing

The methods of testing migration rates are laid out in Annex V of the regulation. It states how the foods are to be exposed to the contact material in terms of time and

temperature conditions. The testing is now split into two separate testing regimes, one for the SML and a second for the OML. In testing for specific migration, the testing conditions are to be equivalent to the worst possible exposure conditions the food might face, so, for example, if meat was to be stored at between 5°C and 20°C, the testing has to expose the food to packaging at 20°C for whatever the maximum foreseeable storage or cooking duration might be. To replicate long-term storage conditions, the testing is done for 10 days at between 40°C and 60°C, depending upon whether the food is intended to be frozen, at room temperature, or stored for more or less than 6 months.

OML testing conditions are set out in Table 3 of Chapter 3 of the Annex. There are seven standard testing conditions listed, OM1 to OM7, which stipulate different time/temperatures, depending upon the intended food contact conditions. These may be different from the SML conditions, so the OML testing may not be sufficient for establishing SML compliance (Langhorn, 2011). However as OML testing is more frequently employed that SML testing, the simplification of the new regime will result in reduced laboratory costs and better comparability (Schuup, 2012).

The regulation recognizes that migration modelling is a much cheaper method than chemical testing and allows for its use provided it is scientifically verified and is likely to overestimate the migration rate. As lipophilic substances readily migrate into fat, and the diet normally comprises less than 20% fat, migration is overestimated. Hence, the fat consumption reduction factor (FRF) is applied to correct for this overestimate.

Methods of analysis are set out in Regulation (EC) No 882/2004. Testing is complex so technical guides are to be produced. Given the significant changes involved in Regulation (EC) No 10/2011, particularly in testing, industry has been given time to achieve compliance. Implementation of the regulation is staggered with full implementation in 2016, the older directives remaining in place for the interregnum.

23.3.6 Special considerations regarding migration limits

As food consumers, infants and children differ from the average 60 kg adult from whom the OML of 10 mg/dm^2 is derived. This is because children's diets are less diversified than adults and, given that they are growing, their consumption of food per kg body weight is greater than that of adults. Hence Article 12 stipulates that for food aimed at them the limits are based upon what is actually in the food consumed (mg/kg food), rather than the migration from the food contact material.

A functional barrier is a layer within food contact materials designed to prevent the migration of substances from behind that barrier into the food. It is, so to speak, a bag within a bag, and is used for items such as promotional toys in cereal boxes or McDonald's happy meals. Such barriers may also be used for the widgets in active and intelligent packaging systems. Behind a functional barrier non-authorized substances or recycled material may be used, provided that they

are within a maximum migration level of 0.01 mg/kg (10 ppb) in food. However, substances that are mutagenic, carcinogenic or toxic to reproduction are excluded, unless they have previous authorization. Also excluded are nanoparticles, unless a full risk assessment is positively concluded.

The calculation of OML is based upon 1 kg of food being covered in 6 dm^2 of contact material. However, with low-volume packages or individually packed sliced meats, for example, the surface area to volume ratio is much greater. In these cases, the OML should be related to its content in the food rather than the migration rate from the packaging.

23.3.7 Declaration of compliance and supporting documentation

The traceability requirements of Article 16 of Regulation (EC) No 1935/2004 have been extended in the new PIM regulation. Each food business operator (FBO) in the chain, from the plastic raw material to the retailer, must provide a declaration of compliance to the FBO to whom it sells. The declaration of compliance should also be available to the national competent authority upon request. The declaration, apart from the traceability details, must contain sufficient information necessary to ensure that their customer is, in turn, compliant. Thus, it requires adequate information on any substances subject to restrictions in food. It must also specify the use of the material or article in terms of the appropriate type of food, the time and temperature treatment and storage intended, and the ratio of surface area to volume intended. This information is essential to allow an end user to ensure that the proposed material is safe for the intended use.

The written declaration must be reviewed if there are changes in the migration level due to changes in production or composition or when new scientific data becomes available. Article 16 states that all supporting documentation – for example, on testing, calculations or analysis – must be available to the competent national authority upon request.

23.3.8 Recycled plastics: Commission Regulation (EC) No 282/2008

The recovery and reuse of packaging materials is part of EU policy as stated in Directive 94/62/EC. The difficulty is that such material may contain residues from previous use, contaminants from misuse or non-authorized substances. Commission Regulation (EC) No 282/2008 of 27 March 2008 on recycled plastic materials and articles intended to come into contact with foods and amending Regulation (EC) No 2023/200 sought to harmonize the rules within the EU. There are two processes used in recycling plastics. The chemical process breaks down the substance to its constituent monomers and oligomers, which are subject to the PIM, Regulation (EU) 10/2011 *supra*.

The safety of the plastic coming from the mechanical process depends upon a number of factors: the input material sorting efficiency; the effectiveness of the process to reduce contamination; and the definition of the use for the recycled material. The combination of these processes is specific to the site and material

used. Hence, authorization must be on an individual basis. There must be an effective quality assurance system in place which ensures that, for example, the cleaning process is validated and the sorting process efficiency is appropriate for the material being sorted (polyolefins require 100% efficiency while PET requires less).

Authorization of the process is through the submission of a dossier to the EFSA. Guidelines for such submissions are available from the EFSA website (a link to which is available below) (EFSA, 2008). This applies for third countries as well as EU member states. Plastic recyclers must appear on a Community register and are subject to inspection by the national authority of the member states according to Regulation (EC) No 882/2004 on official controls.

23.3.9 Active and intelligent materials: Commission Regulation (EC) No 1935/2004 and Commission Regulation (EC) No 450/2009

Active and intelligent food packaging is based upon a deliberate interaction of the packaging with the food or with its environment. This challenges the principle of inertness, which underpins all EU legislation on food contact materials and articles. To allow for their use, a new Framework Regulation 1935/2004 was enacted (Schäfer, 2010). The purpose of active packaging is the extension of the shelf life of the food and the maintenance or even improvement of its quality. The purpose of intelligent packaging is to indicate or monitor food freshness (Dainelli *et al.*, 2008). While this technology has great potential benefits for the consumer, there are also risks associated with their use. Any released materials, whether intentionally or unintentionally, must be safe, hence the regulation states that they must be authorized. If the released material has a function that is separately regulated, it will be subject to that specific legislation. For example, if it is an additive, it will be subject to Regulation (EC) No 1333/2008 on additives. The material should be effective and not be just a marketing gimmick (Irvine, 2009). It should not mislead the consumer, for example, by masking spoilage.

Interpretation of the safety aspects of Framework Regulation (EC) 1935/2004 proved inconsistent throughout the EU, so a specific measure on active and intelligent materials, Commission Regulation (EC) No 450/2009, was enacted (Irvine, 2009). This lays down the specific rules to be applied, in addition to the general requirements of Regulation (EC) 1935/2004. All substances to be used in the manufacture of an active or intelligent component must be authorized and appear on the Community list. This is a positive list and the EFSA must have performed a risk assessment and issued an opinion in which the identity, function and any restrictions are stated.

Materials released and intended to have an effect do not have to be authorized but must be in full compliance with all relevant Community and national provisions applicable to food (such as the additive regulations). This is also the case for materials incorporated by techniques such as grafting or immobilization intended to have a technological function in the food (EFSA, 2009). However, even if the substance has been approved under the relevant legislation, its stability under the intended

Fig. 23.4 Do not eat symbol. Note: Article 11 of Regulation (EC) No 450/2009 on active and intelligent materials and articles intended to come into contact with food contains additional rules on labelling. One of these rules is the following: To allow identification by the consumer of non-edible parts, active and intelligent materials and articles or parts thereof shall be labelled, whenever they are perceived as edible: (a) with the words 'DO NOT EAT' and (b) always where technically possible, with the symbol reproduced in Annex I.

packaging, manufacturing and processing conditions must still be verified by the packaging manufacturer if chemical reaction, degradation or decomposition is likely to occur.

The passive parts, such as the widgets or housing material for the active and intelligent material, must be compliant with relevant specific legislation, so, for example, the plastic parts must comply with plastics regulation etc.

Non-edible parts which might be confused with food, such as sachets, must be labelled as such to prevent confusion with spice packets, for example. Figure 23.4 illustrates the labelling required.

As with all specific measures, a written declaration of compliance is required as per Annex II. In addition to the traceability information, it should include adequate information on its suitability and use, together with any restriction applicable. This is to allow end user compliance. It must also comply with Directive 2000/13/EC on labelling where any release is involved.

23.4 Other specific measures of importance

The following sections look at certain particular materials commonly in contact with food – and the regulations pertaining to these.

23.4.1 Ceramics: Council Directive 84/500/EEC

Apart from kitchen crockery, ceramics are used to package meat pies and pates. The danger arises from the heavy metals used in the glazes and colours. This was one of the first food contact materials to be regulated because of the particular poisoning

hazard, mainly from lead and cadmium. The directive and its amendment (Directive 2005/31/EC) set limits upon the leaching of lead and cadmium and proscribe the migration tests and analytical methods required for compliance. The framework directive applies to other heavy metals. As with all specific measures, a declaration of compliance must accompany the product, giving traceability information and stating that the article is within the limits for lead and cadmium (Schäfer, 2010).

23.4.2 Regenerated cellulose (cellophane): Commission Directive 2007/42/EC

All substances, with the exception of dyes, pigments and adhesives used in the manufacture of cellophane and plastic-coated cellophane, must be authorized and appear on a positive list. Synthetic casings such as those used for sausages are excluded, however. This is because the casing is intended to be consumed with the food, and is thus regulated as a foodstuff rather than a contact material. Because of the absorption of water by cellophane films, it is not feasible to measure migration rates so restrictions are expressed as residual content in the film (Schäfer, 2010).

23.4.3 BADGE, BFDGE and NOGE: Commission Regulation (EC) No 1895/2005

Commission Regulation (EC) No 1895/2005 of 18 November 2005 on the restriction of use of certain epoxy derivatives in materials and articles intended to come into contact with food banned the use of BFDGE and NOGE on the grounds of potential health concerns, but the use of BADGE was permitted by the EFSA as concerns about its carcinogenicity proved unfounded (EFSA, 2004). Its migration limit was actually increased to 9 mg/kg in food.

BADGE (Bisphenol A diglycidyl ether) links the monomer Bisphenol A to epoxy groups to produce the epoxy resins used widely in can linings. It is also used as a heavy-duty liner for tanks and water pipes, but these are excluded from the regulation due to the high volume to surface area involved in that particular use which make migration concerns trivial.

While the migration from can linings has become a concern, particularly with tinned baby food, it is the use of BPA in polycarbonate materials for children which has sparked most controversy. This shatterproof plastic is used to make babies' bottles and tableware. The concern is that the estrogenic effect of BPA could affect brain development and behaviour of foetuses and infants. This has resulted in bans or calls for bans of BPA in babies' bottles in Denmark, Sweden and France and has resulted in the first amendment to the plastics regulation, Commission Implementing Regulation (EU) No 321/2011 of 1 April 2011 amending Regulation (EU) No 10/2011 as regards the restriction of use of BPA in plastic infant-feeding bottles. This was despite an EFSA report, which reviewed the safety of BPA and recommended no change in the limits (EFSA, 2010).

However, the controversy continues with the call in Sweden for the complete phasing out of BPA in can linings (since limited to packaging for food aimed at

children under three) and the passing of legislation in the French parliament banning its use for products aimed at children under 3 years old from 2013, followed by a total ban in 2014. This is based upon two reports from the French Agency for Food health Safety (ANSES). In response to the French scientific findings, which sparked the ban, the EFSA have continue to maintain their position on limits but have said that low-dose studies currently in progress in the United States and due to report in 2012 will be monitored closely (EFSA, 2011). In April 2012, the EFSA announced a new comprehensive risk assessment of BPA together with a colloquium devoted to the issue. These will focus upon low dose effects, impacts upon vulnerable groups and the contribution of non-dietary sources to overall exposure.

23.5 The regulation of food contact materials in the United States

The following sections investigate the differences in approach and practice regarding regulation of food contact materials in the United States, as compared to the EU.

23.5.1 Introduction and background

The regulation of food packaging materials in the United States has a longer history than that in the EU. It goes back to 1913 with the passing of the Gould Amendment to the Pure Food and Drink Act of 1906, which aimed to prevent deceptive packaging practices such as the half-filling of large packs (Baughan and Attwood, 2010). It requires that contents be plainly marked on the outside of the food package (Meadows, 2006). However, the key piece of legislation which regulates food packaging materials is the 1958 Food Additives Amendment to the Federal Food, Drug and Cosmetics Act (FDCA) of 1938. To understand how the system works in practice it is useful to examine briefly how it evolved.

The FDCA of 1938 set up the US Food and Drugs Administration (FDA), which regulated, among other things, food additives. It established protocols for their preclearance. In the 1950s in the United States there was considerable concern among consumers about serious diseases such as cancer and there was a suspicion that chemical food additives were the cause. In response to this, the Delaney Congressional Commission was established to enquire upon the veracity of these claims. While the commission found that there was no truth to these allegations, they did find that there could be migration from packaging material into food. The commission classified such materials as indirect food additives and, despite stiff industry opposition, ruled that they should be regulated in the same manner as direct food additives, using the food additive petition process.

Prior to the 1958 Act, packaging producers, primarily at the behest of their customers, would submit to the FDA information on their material and its proposed use and the FDA would issue a letter of sanction. It was envisaged that this system would remain as the main preclearance method and that the food additive petition process would only be used for food contact substances in complex or

controversial cases. However, it was found that these prior sanction letters were being used as a marketing tool and the facility was withdrawn, leaving only the slower, more complex petition route for preclearance (Heckman, 2005).

A combination of industry lobbying and the administrative burden resulted in the passing of the Food and Drug Administration Modernization Act of 1997. This established the Food Contact Notification programme, fundamentally a system similar to the prior sanction letters, which is now the system of choice for the approval of food contact materials.

23.5.2 The US exposure approach to FCM legislation

When discussing the control of FCM in the United States in the context of global FCM control systems, it is very important to recognize the difference in approach from that prevailing in the EU. While superficially, in terms of toxicology assessment and analysis, there are similarities, the two systems have fundamental differences and represent almost polar opposites in terms of regulatory systems.

The EU takes a subjective toxicological approach assessing each substance and placing it on a positive list. The United States takes an exposure approach, assessing the use of the material and its contribution to the diet, basing its decisions on the degree of toxicology testing which is required, if any. It is essentially based upon the Paracelsusian principle 'the dose makes the poison'. Therefore, where exposure levels are anticipated to be low or minimal, a series of exemptions to the regulations are either legislated for or are followed through custom and practice.

The other fundamental difference is the respective responsibilities of the packaging manufacturer and the regulatory authority. In the United States it is up to the manufacturer to interpret the regulations and decide whether approval is needed or not. The FDA considers it unnecessary, and even unadvisable, that it should be consulted, seeing this as the role of the manufacturer (Hutt, 1969).

23.5.3 The regulatory enforcement process in the United States

The FDCA established the US FDA as the body responsible for the regulation of food contact materials. This work is done at the Center for Food Safety and Applied Nutrition (CFSAN) by the Office for Food Additive Safety (OFAS). Food additive status is a crucial part of the evaluation and enforcement process at the Department of Agriculture's Food Safety and Inspection Service (FSIS), which supervises meat and poultry plants and products. The Department of the Treasury's Bureau of Alcohol, Tobacco and Firearms reviews additive status in the clearance of packaging materials (Heckman, 2005).

23.5.4 A practical approach to the US food contact materials regulatory regime

Because of the cost of toxicological testing and regulatory compliance, it is important that manufacturers adopt a structured approach to their decisions regarding

the preclearance of food contact materials (Baughan and Attwood, 2010). The first step is to check if the substance and use are already precleared under a published FAP (Food Additive Petition) regulation, a FCN (Food Contact Notification) or are subject to a pre-1958 prior sanction. The next step is to establish that migration can potentially occur as, by definition, if there is no migration, it is not a food additive and hence not subject to regulation.

Exemptions play a large part in the US system. Thus it must be established if the substance is one that is generally regarded as safe (GRAS), is below the threshold of regulation (TOR), is a houseware or if it falls under the basis polymer doctrine, all of which may exempt it. The OFAS will assist in establishing this if requested.

Once a manufacturer determines that preclearance is necessary for the marketing of their material, there are two protocols available. In the vast majority of cases, they will assemble the required data, submit this to the FDA via the Food Contact Notification system and, assuming that there are no objections from the FDA, the notifier will be allowed to market to their material 120 days later. The notification is posted to the FDA FCN website and applies to the notifier only for the use/s as stipulated. Alternatively (and infrequently since the introduction of FCN process), a Food Additive Petition may be applied for. This includes similar data to the FCN; however, the difference is that, once approved, the petition is published in title 21 of the Code of Federal Regulations (21 CFR) as a regulation which gives approval to all manufacturers. This process from application to publication can take from 2 to 4 years. Table 23.1 summarizes the federal regulations as they apply to food contact materials.

The following sections will discuss each of these steps in turn.

23.5.5 Good manufacturing practice (GMP), the Delaney clause and the constituents policy

As in the EU, the US Code of Federal Regulations includes GMP requirements for the manufacture, packing and holding of foods. Title 21 Section 110.80 of the Code of Federal Regulations (21CFR §110.80) demands that food be suitable for human

Table 23.1 US Code of Federal Regulation: food additive regulations pertaining to food contact materials

Title 21 Code of Federal Regulations	
Part 174	General Provisions
Part 175	Adhesives and Coatings
Part 176	Paper and Paperboard
Part 177	Polymers
Part 178	Adjuvants, Production Aids, Sanitizers
Part 179	Use of Irradiation
Part 180	Interim Use of Substances
Part 181	Prior Sanctioned Substances
Part 182	GRAS Substances for Direct Addition
Part 184	Affirmed GRAS Substances for Direct Addition
Part 186	Affirmed GRAS Substances for Food Contact

consumption and that food containers are safe and suitable for their intended use. Thus, food contact materials are subject to the food additive regulations to ensure their safety. The Delaney clause (see the evolution of US regulations *supra*) specifically excludes any material which is carcinogenic. However, virtually all chemical substances contain impurities of some sort arising from their manufacturing process. Some of these may be carcinogenic but may be present in very small quantities. In the 1950s, when the Delaney clause was adopted, technology could detect, at best, in parts per million (ppm) whereas now detection of fractions of parts per billion (ppb) is technically possible; thus carcinogens at very low levels would prevent the approval of what are 'virtually safe' additives. In response to this problem, the FDA formulated the 'constituents policy'. Impurities are termed 'non-functional constituents' and provided that the additive as a whole is safe it can be approved. The carcinogenic impurities are subject to risk assessment and a 'safe' level being established.

23.6 Exemptions to the regulations

The following sections consider the many and various ways in which aspects of FCMs are exempted from the regulations that would otherwise apply.

23.6.1 What constitutes 'no migration'?

Obviously, where there is no migration from a food contact substance, the material is not a food additive and is not subject to food additive regulations. The question arises as to what constitutes 'no migration'. As discussed *supra*, the advances in detection technology, together with the application of extreme extraction conditions, may result in detectable, if insignificant, levels of migration.

In 1969, Dr Lessel Ramsey, then Assistant Director of Regulatory Programmes at the FDA's Bureau of Science (now known as CFSAN), in what has become known as the 'Ramsey Proposal' suggested that a level of 50 ppb was appropriate unless there were special toxicological concerns, such as with heavy metals. While the proposal was never adopted due to apparent political concerns, the agency deemed the standard scientifically acceptable (Heckman, 2005).

Further guidance can be derived from the judgment in the 1969 case of *Monsanto vs Kennedy*. This concerned the demand from the FDA that acrylonitrile copolymer in unbreakable containers be classified as an unsafe food additive because of the possibility of migration. The FDA lost the case in the US Court of Appeals, the judgment ruling that a substance must migrate into food in more than insignificant amounts to be considered an additive (Heckman, 2005).

The matter was further clarified by a 1974 statement from the FDA's Office of General Counsel. This stated that a material should only to be classified as an additive if, under normal conditions of use, it becomes a component of the food, and in amounts not generally regarded as safe. The threshold should be reduced, however, for high-toxicity substances, for those in widespread use or for those use for sensitive classes such as infants and children (Baughan and Attwood, 2010).

23.6.2 Functional barriers

Natick Paperboard vs Weinberger in 1975 examined the migration of polychlorinated biphenyl (PCB) from paperboard to food. The FDA claimed to have authority to seize such PCB-containing products, as they would adulterate food. The appellant paperboard company claimed that a functional barrier prevented migration to the food. The judgment upheld the view of the appellant on the basis that contamination could not reasonably be expected to occur through the functional barrier, hence the paperboard would not be a food additive.

23.6.3 Sanctions issued prior to the 1958 amendment

Prior to the 1958 FDCA the practice was that manufacturers, normally at the behest of their customers, frequently enquired of the FDA as regards the suitability of their products. In response, the FDA would issue a *de facto* sanction by letter. The FDCA specifically exempted substances which had been granted these letters. While these sanctions have proven robust, Heckman (2005) stating that none has been challenged, they are based upon 1950s science. Hence, the FDA has sought to limit the scope of such exemptions and can still prohibit or set conditions where there exists proof that adulteration is occurring (Baughan and Attwood, 2010).

23.6.4 Generally regarded as safe (GRAS)

As with the aforementioned sanction letters, GRAS substances are also specifically excluded from the definition of food additives under the FDCA. A GRAS determination must be made by trained experts using scientific procedures, published data and to the standard of a food additive regulation. Pre-1958 GRAS determinations are based upon common experience. The determination is the responsibility of the manufacturer. It is notable that the GRAS determination is based upon concentration and use; thus, as was found in the *Monsanto* case *supra*, a substance can be consider GRAS at low concentrations but not at a higher concentration (Heckman, 2005). Should the FDA wish to challenge the determination, it is incumbent upon them to prove that it is erroneous.

In the past, manufacturers wishing to confirm their GRAS determination could file a GRAS Affirmation Petition; however, this policy has changed (though neither policy has a legislative basis). Now the FDA will accept a notification and, provided they do not object upon the grounds of insufficiency of process or information, they are effective after 90 days and appear on the website of GRAS notification (a link to which is provided in the section *Sources of Further Information and Advice* and is titled 'US FDA -Determining the Regulatory Status of Components of a Food Contact Material'). It must also be noted that, because not all determinations are subject to affirmation or notification, this list is incomplete.

23.6.5 Threshold of regulation (TOR)

The TOR exemption was adopted in 1995 and recognized that materials from which migration was *de minimus* (but not carcinogenic) should be exempt from preclearance

regulation as a food additive. TOR is set at levels where the dietary concentration of the substance does not exceed 0.5 ppb or, for substances that have been cleared as food additives, the use does not exceed 1% of the acceptable daily intake (ADI).

While the TOR clearance programme has been largely superseded by the FCN system, it still remains in place. Now, its use lies primarily as a support for a GRAS determination (Baughan and Attwood, 2010).

23.6.6 Housewares

While housewares are not subject to a specific exemption in the FDCA, it is apparent from the Act that Congress intended such an exemption. Housewares would include paper cups and plastic tableware and cooking utensils. Such containers are without food and are intended for use in food service or by the consumer. It does not apply to food manufacturers or where they are packaging food. The rationale behind the housewares exemption is that the contact duration is so brief as not to present a migration hazard. However, they are still subject to FDCA adulteration regulations and the FDA can and does take action should they present a health hazard.

23.6.7 Basic polymer/resin doctrine

The doctrine states that basic polymers or resins are considered to include all of the materials essential to their manufacture (such as catalysts), but to exclude any additional materials such as adjuvant-like plasticizers. Once the polymer is pre-sanctioned, its ingredients do not require preclearance.

The rationale underling the exemption is that these ingredients remain at only insignificant levels in the final polymer, either forming part of the polymer itself or being washed out during its manufacture. The ingredients are, however, subject to GMP provisions.

23.7 The food contact notification system

In 1997 the FDMA introduced the Food Contact Notification system (FCN) in order to simplify the administration of the regulation of food contact materials and to accelerate the approval process. It is the process encouraged by the FDA and is now used for the vast majority of preclearance applications. However, in particular circumstances a Food Additive Petition (FAP) may still be required.

23.7.1 The food contact notification process

Information as listed in the aforementioned system is submitted to the FDA to prove the safety of a substance, offering sufficient detail to make its case. Within approximately 30 days the FDA will issue one of the following responses:

- if the substance has been shown to be safe, an acknowledgement letter, with or without limitations and the date of receipt

- reasoned objections to the application, with a request for further information
- a requirement that a Food Additive Petition be submitted.

If approved with limitations, the notifier can ask for clarifications. The notifier can market the substance 120 days after receipt of the acknowledgement letter, and it will be listed on the FDA website.

23.7.2 Prenotification consultation

While it is not a requirement, it is certainly recommended that submitters consult the FDA prior to submitting either an FCN or an FAP. The FDA will advise on whether the FCN or FAP is the most appropriate route and clarify uncertainties regarding the interpretation of safety data.

23.7.3 The food contact notification requirements

Notification must contain sufficient information to allow a determination of safety to be made. A link to the FDA guidelines for submissions is provided in the section *Sources of Further Information and Advice* and is titled 'US FDA – Food Ingredients and Packaging Guidance for Industry'. It should contain information on the chemical identity, the manufacturing process, the conditions of use, in terms of temperature contact duration, and any proposed reuse. GMP requires that data on the manufacturing specification and the intended technical effect be included. The cumulative estimated dietary intake (CEDI) which will result from use is required to facilitate a decision on the most appropriate toxicological testing. Thus, if the CEDI is below 0.5 ppb no toxicological testing is needed; if between 0.5 and 50 ppb two tests are recommended; if between 50 ppb and 1 ppm five tests are recommended; and if greater than 1 ppm the submission of a food additive petition is generally recommended.

Under the National Environmental Policy Act (NEPA) all federal agencies are required to consider the environmental impact of their decisions. For FCN and FAP submissions an environmental assessment is required; however, there are various categorical exclusions, such as with repeated-use items. A link is provided to the NEPA submission guidelines in the section *Sources of Further Information and Advice* and is titled 'US FDA – Food Ingredients and Packaging Guidance for Industry'.

23.7.4 Food additive petitions (FAP)

The FAP submission is similar to that for a FCN; however, one major difference is the time taken for the clearance to be issued. It takes from 2 to 4 years, and a regulation is published in the federal register. This gives a general approval, which is available to all manufacturers. With the introduction of the more efficient FCN process, it is generally retained for the following only:

- where the CEDI is greater than 1 ppm, or, if a biocide, where the CEDI is greater than 200 ppb or
- where the carcinogen tests are not clearly negative.

In these cases the FDA recommend an FAP, but a presubmission consultation is advised. A link to guidance documents for FAP submissions is provided in the section *Sources of Further Information and Advice* and is titled 'US FDA – Food Ingredients and Packaging Guidance for Industry'.

23.8 Implications of regulations for packaging and product development

The current decade is a very exciting time in terms of the development of packaging. Never has there been a greater confluence of science, technology and consumer demand, leading to rapid innovation in the variety and uses of food packaging. This decade has also seen the rise of the concerned consumer who is more informed of the dangers with regard to the food we consume and is fearful for their own safety and that of their family. Coupled with this is the increased role of the media, ever more anxious to inform the consumer of such dangers.

All innovation carries risks and consumers rightly demand that risks be at least minimized and preferably eliminated, often by way of regulation. However, zero risk is inappropriate as it would lead to a paralysis of technological development and innovation. Regulatory complexity or overregulation add to the cost of development, potentially stifling innovation and limiting the development of packaging for niche uses. It is important that a regulatory regime balances the potential utility of a packaging innovation to the consumer with the protection of their safety. The then Secretary General of DG Sanco, Robert Madelin, discussing food regulations, said that regulation should foster rather that hamper innovation (Madelin, 2008). Recent figures from PIRA International project an annualized growth of 7.5% in the use of active and modified atmosphere packaging to 2014, so it could be reasonably argued that a correct balance has been struck.

It has often be stated as a truism that 'regulation follows innovation and to reverse this would be to stifle the latter'. However, it can be argued that this is not always true. Regulations, or the threat of regulations, can often be a spur to innovation. The waste reduction policies in the EU have caused an increase in recycling activities in Europe, together with down-gauging (the development of stronger films using less material). Thus, waste going to landfill has been reduced by 43% since 1998 (EUROPEN [The European Organization for Packaging and the Environment], 2011). It will be interesting to follow the success of innovation in response to the proposed limitation/banning of BPA in packaging. In the EU, the Lisbon agenda proposed that regulations be simplified so that competition is encouraged. This should lead to the reduction in cost of development, hence an increase in innovation.

Globalization is happening not just in terms of food supply but also in terms of packaging supply and development. The various regulatory regimes increase the costs of compliance so any convergence in global regulations can be expected to impact favourably upon packaging development. An increased market will reduce the overall cost of regulation and development, hence accelerating innovation and facilitating the development of applications suitable for niche markets.

23.9 Future trends in legislation

Legislation can never remain static. It must change to address new circumstances and new challenges. Consumers are constantly changing in terms of their lifestyles. This effects the requirements they place upon the food packaging that they use. Trends such as a demand for greater freshness and reduced environmental impact require innovation in materials and uses. The food industry is also changing in terms of globalized supply. Longer transport times, greater shelf-life requirements and storage needs call for different packaging. Technology is improving. Thresholds of detection are falling, while our knowledge of the chemistry of reaction and degradation products is improving.

In the EU the process of harmonization of regulations between member states will continue in pursuit of the Lisbon agenda. This will probably result in new specific measures along the lines of the plastics implementation measure. The adoption of measures for paper and paperboard are being discussed. The Council of Europe (not an EU body but including all member states of the EU) has already developed voluntary codes of practice in the area. Again, as with the PIM, the legislation is most likely to be adopted as a regulation rather than a directive.

While the US Food Contact Notification system is generally working well, concerns have arisen about the older Food Additive Petition process. Older FAP regulations have proven inflexible, particularly in the recent BPA case. The general approvals so granted have allowed many uses to develop and these are not easy to track. The use does not have to be notified, thus the extent of use is difficult to gauge. In addition, it is a difficult and slow process to withdraw the approval. The FDA is working on a voluntary basis with users to try to overcome the problem. Should results of the impending low-dose BPA studies prove significant, these problems will be brought to the fore.

Recent years have established the consumer as a key driver of legislative change. Consumer concerns and fears are swiftly adopted by pressure groups who lobby politicians. This can be intensified by the media, where issues can gain global news coverage very rapidly. The reticence of business or public bodies to react quickly can allow a story to grow and gain credibility even in the absence of firm evidence. The 'substance of the month' phenomenon builds upon the suspicion of the public of science and scientists. This suspicion did not develop without foundation. The BSE and Dioxin crises dented the faith the public have in the ability of public bodies to protect them. This is exacerbated by our personal, notoriously poor, ability in terms of risk categorization. Risk perception rarely correlates to scientific risk assessment. Ironically, while we worry increasing about the safety of our food, in truth, it has never been safer. Frequently modern media are blamed for being alarmist; however, we just have to look to the evolution of the Delaney clause in the United States to see that it is not so new. (Indeed, the first attempt at the introduction of potatoes to England was considered a plot to poison the populace and met with riots!) Fear of the unknown is not new. Consumers need to perceive a benefit before they will take a risk.

In response to such fears regulators internationally have developed a policy of transparency and openness. The EFSA is a model of transparency, with board

meetings and submissions available on-line. The FDA has demonstrated a similar approach, well illustrated by its response to BPA regulation. It has been recognized that consumers are not ignorant but intelligent and responsible; they can be allowed to make sensible decisions once sufficiently advised (Madelin, 2008).

The other trend, akin to transparency, which is apparent in the making of regulations, is that the process is now a cooperative one. This reflects the shared responsibilities of all of the parties. From producers to consumers, everyone makes a contribution through the consultation process. It is in the interest of producers to ensure that materials are properly regulated. They are subject to due diligence and it is demanded by their customers. Even prior to the USFDA FAP process in 1958, manufacturers were requesting letters of sanction from the FDA to assuage the fears of their customers. In Japan, industry bodies produce voluntary codes of practice which can later be converted to regulations. The Council of Europe has a wide range of industry-inspired codes of safe practice. A problem for one manufacturer inevitably ends up as problem for all, so it is in everyone's interest to prevent such problems.

Globalization of food and packaging supply has many advantages but carries with it difficulties also. While we talk of a global chain, it is in reality a global web (Colwell, 2010). As a result, local legislation has global impact. The transparency and openness required by each jurisdiction can jeopardize the confidentiality of technical data.

The advantages of harmonization are so obvious that it seems inevitable that progress will be made in the future, even though there is little evidence of this from the current situation. There are a number of ways in which harmonization of regulations could occur. Joint regulations would be simplest but unlikely, while mutual recognition could also overcome the problem; the fear is that it might be viewed as a lowest common denominator approach. Japan already has mutual recognition of some US and EU regulations. MERCOSUR has adopted regulations from both jurisdictions. There is already a lot of cooperation in technical organizations such as JECFA, the WHO, the FAO and standardization bodies. Rationalization or mutual recognition of methodologies and tests would be a good starting point (Kopper and Ariosti, 2010). It is interesting to note the use of the US low-dose study by the EFSA in their opinion on BPA. The recent regulation of recycling and active and intelligent materials in the EU is on a propriety basis similar to the US FCN system, so may show a path forward. The precautionary principle which forms part of EU food law is an obstacle. The United States tends to view it as 'one report short of a decision', while the EU consider it essential for consumer protection. Given what we know about consumer behaviour and risk perception, perhaps the consumer needs that time to perceive the benefit and hence accept risk.

It may be instructive to examine the evolution of food contact materials legislation in the EU. When the process started out there were many different approaches among the member states. Only over time and with mutual respect and a lot of negotiation has it gelled into a corpus of law (Heckman, 2005). Even now, the process is far from complete.

The more technically advanced we become, the more difficult it is to regulate food contact materials. The regulation of risk is not simple because the problem

is not a simple one. The best a regulator can hope for is to strike the right balance between scientific certainty, the legitimate expectations of business operators, the feasibility of legislation and the protection of the consumer (Madelin, 2008).

23.10 Sources of further information and advice

European Union Reference Laboratory for Food Contact Materials – Sampling methods and guidance documents http://ihcp.jrc.ec.europa.eu/our_labs/eurl_food_c_m

EU DG Health & Consumers – food contact materials legislation http://ec.europa.eu/food/food/chemicalsafety/foodcontact/index_en.htm

EU food contact materials database https://webgate.ec.europa.eu/sanco_foods/main/?event=display

European Commission list of national legislation http://ec.europa.eu/food/food/chemicalsafety/foodcontact/sum_nat_legis_en.pdf accessed

US FDA Food Ingredients and Packaging Guidance for Industry
http://www.fda.gov/Food/GuidanceComplianceRegulatoryInformation/GuidanceDocuments/FoodIngredientsandPackaging/default.htm

US FDA Determining the Regulatory Status of Components of a Food Contact Material http://www.fda.gov/Food/FoodIngredientsPackaging/FoodContactSubstancesFCS/ucm120771.htm

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Commission Directive 81/432/EEC of 29 April 1981 laying down the Community method of analysis for the official control of vinyl chloride released by materials and articles into foodstuffs.

Commission Directive 93/8/EEC of 15 March 1993 amending Council Directive 82/711/EEC laying down the basic rules necessary for testing migration of constituents of plastic materials and articles intended to come into contact with foodstuffs.

Commission Directive 97/48/EC of 29 July 1997 amending for the second time Council Directive 82/711/EEC laying down the basic rules necessary for testing migration of the constituents of plastic materials and articles intended to come into contact with foodstuffs.

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Commission Directive 2004/14/EC of 29 January 2004 amending Directive 93/10/EEC relating to materials and articles made of regenerated cellulose film intended to come into contact with foodstuffs.

Commission Directive 2005/31/EC of 29 April 2005 amending Council Directive 84/500/EEC as regards a declaration of compliance and performance criteria of the analytical method for ceramic articles intended to come into contact with foodstuffs.

Commission Directive 2007/42/EC of 29 June 2007 relating to materials and articles made of regenerated cellulose film intended to come into contact with foodstuffs.

Commission Implementing Regulation (EU) No 321/2011 of 1 April 2011 amending Regulation (EU) No 10/2011 as regards the restriction of use of Bisphenol A in plastic infant-feeding bottles.

Commission Regulation (EC) No 1895/2005 of 18 November 2005 on the restriction of use of certain epoxy derivatives in materials and articles intended to come into contact with food.

Commission Regulation (EC) No 2023/2006 of 22 December 2006 on good manufacturing practice for materials and articles intended to come into contact with food.

Commission Regulation (EC) No 282/2008 of 27 March 2008 on recycled plastic materials and articles intended to come into contact with foods and amending Regulation (EC) No 2023/200.

Commission Regulation (EC) No 450/2009 of 29 May 2009 on active and intelligent materials and articles intended to come into contact with food.

Commission Regulation (EU) No 10/2011 of 14 January 2011 on plastic materials and articles intended to come into contact with food.

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Council Directive 82/711/EEC of 18 October 1982 laying down the basic rules necessary for testing migration of the constituents of plastic materials and articles intended to come into contact with foodstuffs.

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