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Review

Bioactive packaging technologies for extended shelf life of meat-based products

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Abstract

To prevent the development and spread of spoilage and pathogenic microorganisms via meat foodstuffs, antimicrobial packaging materials could be a potential alternative solution. Instead of mixing antimicrobial compounds directly with food, incorporating them in films allows the functional effect at the food surface – where the microbial growth is mostly found – to be localized. Antimicrobial packagings include systems such as adding a sachet into the package, dispersing bioactive agents in the packaging, coating bioactive agents on the surface of the packaging material, or utilizing antimicrobial macromolecules with film forming properties or edible matrices. The potential of these technologies are evaluated for the preservation of meat and meat products.

Keywords: Antimicrobial packaging; Meat; Active matrices

Contents

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0. Introduction

Due to recent outbreaks of contaminations associated with meat products, as well as growing concerns regarding the safety of intermediate moisture foods, active packaging has been greatly developed in recent years. As mentioned in Quintavalla and Vicini (2002) and Anonymous (2001), a packaging material is defined as "a type of packaging that changes the condition of the packaging to extend shelf-life or improve safety or sensory properties while maintaining the quality of the food". Principal active packaging systems involve oxygen scavenging, moisture absorption, carbon dioxide or ethanol generation, and finally antimicrobial systems. These concepts, which are successfully utilized in the US and Japan, have seen only limited development in Europe. This could be due to legal restrictions, to a lack of knowledge about both the acceptability of these systems to consumers and their effectiveness in packaging, or to their economic and environmental impact.

Although little developed in Europe, in the past decade active packaging has become one of the major areas of research in food packaging. Of these active packaging systems, the antimicrobial version is of great importance. As reported by Oussalah, Caillet, Salmieri, Saucier and Lacroix (2004), carved beef has a short shelf life that varies between 3 and 5 days when kept at +4 °C. *Pseudomonas*, *Enterobacteriacea* and lactic acid bacteria are responsible for meat deterioration. Meat and meat products may also be contaminated by *Listeria monocytogenes*, *Salmonella typhimurium*, *Salmonella enteritidis, Escherichia coli* 0157:H7 and *Yersinia enterolitica*, responsible for food-borne illnesses and deaths. Thus, additional measures should be used to ensure the safety of such products. In addition to the development of pathogens, microbial growth commonly induces undesirable organoleptic changes during the storage of meat. If bacterial growth at the meat surface-packaging film could be delayed or halted, large gains in product shelf-life would be possible (Ouattara, Simard, Holley, Piette & Begin, 1997). Depending on the region, some of the traditional methods of preserving foods from the effect of microbial growth (thermal processing, drying, freezing, irradiation, etc.) cannot be applied to some food products, such as fresh meats and ready-to-eat products. Indeed, ready-to-eat products such as cooked ham, are completely processed prior to final packaging and are consumed without further cooking, thus increasing the possibility of the occurrence of food-borne illnesses if further contamination by pathogens occurs (Marcos, Aymerich, Monfort, and Garriga, 2007a).

Moreover, as discussed by Skandamis and Nychas (2002), despite the extended shelf life of refrigerated products stored under vacuum pack or modified atmosphere packaging (MAP) conditions, there is an increasing concern about the growth and survival of microaerophilic and/or psychotrophic pathogens. Indeed, psychrophiles can grow within a temperature range of −14 °C-21 °C, with a high rate at 0 °C and an optimum growth at 15 °C or less. Most psychrophilic strains are spoilage microorganisms but some are pathogenics, such as *L. monocytogenes*, *Aeromonas hydrophila*, *Yersinia enterolitica*, etc. (Fabrizio and Cutter, 2004; Kwiatek, 2004; Tsigarida, skandamis, and Nychas, 2000). The latter are therefore particularly dangerous, since they can grow to high concentrations at room and refrigerated temperatures. Recently, *L. monocytogenes* and *E. coli* 0157:H7 outbreaks have attracted worldwide attention (Masniyom, Benjakul, and Visessanguan, 2006). *L. monocytogenes*, in addition to its psychrotrophic characteristics, is quite tolerant to high levels of sodium chloride. *E. coli* is a member of the Enterobacteriaceae family that commonly develops in fresh and frozen meats. Moreover, bacteria also differ in their requirements for oxygen. Microaerophilic bacteria, such as the pathogen *Campylobacter jejuni*, and non-pathogen *Lactobacillus*, need a low oxygen level for their development, between 2% and 10% and can tolerate the modified atmosphere more than, for example, spoilage strains such as *Pseudomonas* (Hussain, Mahmood, Aktar, and Khan, 2007; Okolocha and Ellerbroek, 2005). The aerobic spoilage organisms, which usually warn consumers of spoilage, are inhibited while the growth of pathogens may be allowed or even stimulated (Farber, 2001).

For all these reasons, the red meat industry has increased the use of preservative packaging. To reduce the growth and spread of spoilage and pathogenic microorganisms in meat foodstuffs, antimicrobial packaging materials could be developed and used because they can inhibit or kill the microorganisms and thus extend the shelf life of perishable products and enhance the safety of packaged products (Han, 2005). For example, packaging films that release organic acids offer potential for reducing the effect of the growth of slime-forming bacteria on fresh meat (Rooney and Han, 2005). Films that release lactic acid are particularly attractive, as this acid is normally present in the meat and can be effective when applied at the cut surface. Indeed, combining biocide agents directly into a packaging material could provide several advantages. First of all, this system could be more efficient by maintaining high concentrations on food surfaces with a low migration of active substances. Only the necessary amount of biocide would be used (Fig. 1). Secondly, in most cases, the agent would not be a direct additive to the food product. Thirdly, the direct incorporation of bactericidal agents or growth inhibitors into meat formulations may result in partial inactivation of the active substances by the food constituents and is therefore expected to have only a limited effect on the surface flora.

According to Cooksey (2001), there are three basic categories of antimicrobial films:

(1) Incorporation of antimicrobial substances into a sachet connected to the package from which the volatile bioactive substance is released during further storage. Common packaging materials can be utilized without the use of alternative packaging materials.

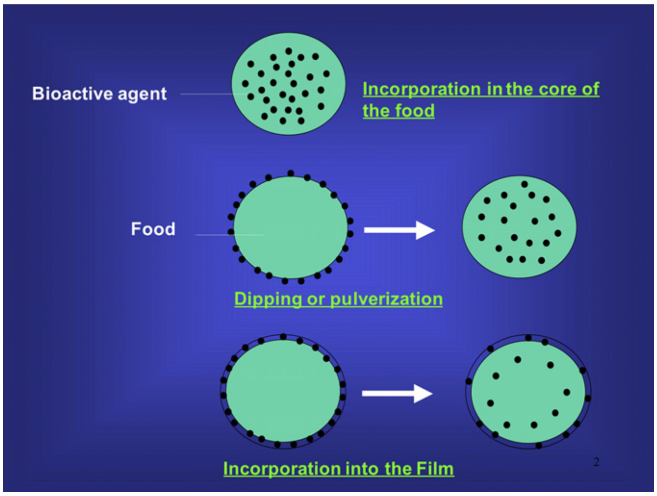

Fig. 1. Different incorporation modes of additives in food products (incorporation into the foodstuff, dipping or pulverization, and finally incorporation into a film) and consequences. The black points correspond to an antimicrobial compound.

(2) Direct incorporation of the antimicrobial agent into the packaging film.
(3) Coating of the packaging with a matrix that acts as a carrier for the antimicrobial agent. These categories of materials can release the antimicrobial agents onto the surface of the food (Appendini and Hotchkiss, 2002; Buonocore, Del Nobile, Panizza, A., Corbo, & Nicolais, 2003; Halek & Garg, 1989; Sebti, Pichavant & Coma, 2002; Wen, Chen & Chen, 1999; Weng & Hotchkiss, 1992). The antimicrobial agents may either be released through evaporation in the headspace (volatile substances) or migrate into the food (non-volatile additives) through diffusion (Fig. 2). The system is more efficient than a direct application of the antimicrobial agent onto meat surfaces, because it slows the migration of the agents away from the surface, and thus helps to maintain high concentrations where they are needed. Moreover, antimicrobial packaging can take two additional forms (Appendini & Hotchkiss, 2002):
(4) Utilization of inherently antimicrobial polymers exhibiting film-forming properties, such as cationic amino-polysaccharides (Begin & Van Calsteren, 1999; Coma et al., 2002; Ouattara, Simard, Piette, Bégin & Holley, 2000; Pen & Jiang, 2003), or polymers which are chemically modified to produce bioactive properties. Packaging materials with bioactive agents chemically bounded to the polymer can be included in this category and the immobilization of the biocide on the packaging polymer could be used for non-food-grade molecules. For this system, the release of the biocide agent is not required, or is prohibited in the case of non-food-grade biocides. Therefore, legal issues and standards concerning the rate of migration of substances in packaging into food products do not limit the development of such bioactive materials. However, the limitation of such a system is the direct contact between the packaging and the food.
(5) Utilization of bioactive edible coatings directly applied onto the foods. The limitation is that the bioactive agent should be approved as a food additive.

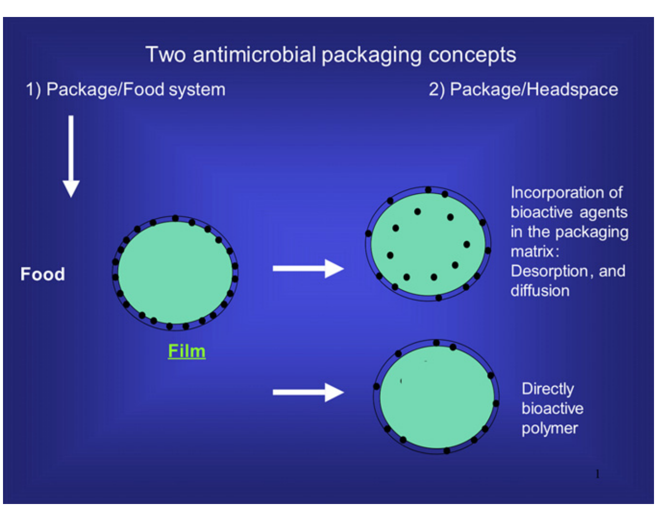

Fig. 2. Both antimicrobial packaging concepts: package-food and package-headspace systems.

This paper, after a brief discussion of developments in antimicrobial packaging materials producing an atmosphere modification by adding a sachet into the package, surveys the other antimicrobial packaging systems based on the dispersion or on the coating of bioactive agents in/on the packaging. It also surveys the utilization of inherently antimicrobial polymers or edible matrices. The potential of these technologies are evaluated to extend the shelf-life and assure the innocuousness and preservation of meat and meat products.

1. Antimicrobial packaging produced by adding a sachet into the package

Besides the use of antimicrobial packaging materials or antimicrobial inserts in the package headspace, gaseous agents have been used to inhibit the growth of micro-organisms. Common gases are carbon dioxide, ethanol vapor and sulfur dioxide (Han, 2005). Oxygen-scavenging packagings also possess indirect antimicrobial properties against aerobic micro-organisms. Among all the literature developed on the subject, only the most relevant studies related to meat product preservation will be presented here. Nadarajah, Han, and Holley (2005a) tested the antimicrobial potential of allyl isothiocyanate, a volatile and aliphatic sulfur-containing compound, potentially found in black and brown mustard associated with a filter paper disk which was packaged with a ground beef patty. The patty and the filter paper were placed together in a bag made of a multilayer system. Due to the release of the allyl isothiocyanate into the headspace of packaged beef, these authors showed that this bioactive agent could substantially reduce the *E.coli* 0157:H7 in fresh ground beef during refrigerated or frozen storage. However, only a few research studies deal with new antimicrobial systems incorporated into sachets and most of the developed systems are commercially available. Commercial generators of antimicrobial substances may be based on CO_2, ethanol of chlorine dioxide generators. An Ethicap sachet (Freund, Japan) could be used as a source of ethanol vapor (Table 1). However, ethanol-generators are more developed in bakery packaging (MAP) due to their antifungal activity. Other systems such as Silver zeolite are also proposed.

1.1. O_2-scavenging technology

High levels of oxygen present in food packages may facilitate microbial growth and although oxygen sensitive foods can be packaged under MAP or vacuum conditions, such techniques do not always facilitate the complete removal of oxygen (Kerry, O'Grady, and Hogan, 2006). O_2 absorbers applied to meat product packaging can prevent the growth of moulds, aerobic bacteria such as *Pseudomonas* and oxidative damage of muscle pigments and flavors to avoid discoloration (Vermeiren, Devlieghere, Van Beest, De Kruijf, and Debevere, 1999).

Table 1
Some commercial active packaging materials for food applications

Concept	Trade-name	Company	Forms
CO_2-emitting/ O_2-scaventing	Ageless G	Mitsubishi Gas Chemical (Japan)	Sachets
CO_2-scaventing/ O_2-scaventing	Ageless G	Mitsubishi Gas Chemical (Japan)	Sachets
CO_2-emitting	Verifrais	SARL Codimer (France)	Sachets
CO_2-emitting/ O_2-scaventing	Freshpax M	Multisorb technologies (USA)	Sachets
CO_2-scavenging	Freshlock	Multisorb technologies (USA)	Sachets
CO_2-emitting		Standa (France)	Gel into sachets in contact with the food
Absorption of liquid water		Standa (France)	
Antimicrobial substances			
Ethanol vapor emitting	Ethicap	Freund (Japan)	Sachets
	Negamold		
	Oiteck	Nippon Kayalan (Japan)	Sachets
Silver Zeolite	Aglon™	Agion Technologies	Paper, plastic packaging
Triclosan	Microban	Microban prod. (UK)	Plastic packaging
Ally lisothiocy anate	WasaOuro	Lintec Corp. (Japan)	Sheets
Glucose oxydase (H_2O_2)	Bioka	Bioka ltd (Finland)	Sachets
Chlorine dioxide	Micro sphére	Bernard Technologies (USA)	Sachets, film, wraps, plastics

As specified in Anonymous (2001), among the packaging technologies developed by and for meat and meat products, MAP has led to the evolution of fresh and minimally processed food preservation. Residual oxygen can, however, be responsible for various degradation phenomena. O_2-scavenging technology may be used appropriately to remove residual O_2 after MAP or vacuum packaging. Moreover, this system can absorb the O_2 that permeates through the packaging film. O_2 absorbers can also be a complement of vacuum packaging to avoid photo-oxidation phenomena, in particular for sliced delicatessen products. Indeed, presentations in small packages with a transparent cover to show the food product are more and more appreciated. Unfortunately, if oxygen traces are still present when the package is put on the shelf, the photo-oxidation phenomena start to take effect, leading to a rapid discoloration of the meat.

The majority of the currently commercially available O_2-scavengers (Table 1) are based on iron oxidation and are incorporated into a sachet (Ageless®-Mitsubishi Gas Chemical Co., Japan or ATCO O_2-absorber-Standa industry, France):

$$Fe\,(OH)_2 + 1/4\ O_2 + 1/2\ H_2O \rightarrow Fe(OH)_3$$

Multisorb Technologies (USA, Freshpax® and Freshmax®) and Bioka Ltd. (Finland) commercially produce other O_2 absorber sachets, which can be found in the packages for many foods such as meat products (e.g. smoked ham and salami). This type of sachet is also used for the conservation of large pieces of meat to limit the growth of spoilage microorganisms such as *Pseudomonas* before cutting up the meat.

Close-fitting packages like vacuum pack for meats are examples where the headspace is very small and oxygen permeation could be a reason for the decrease in quality. It is in such circumstances that oxygen-scavenging plastic films are particularly useful (Rooney, 2000). In parallel to O_2 scavengers, due to a potential microbial development, CO_2-generators are increasingly envisaged in packaging materials used to extend the shelf life of meat products.

1.2. CO_2 generators

Concerning O_2 scavengers, this type of active packaging is frequently associated with MAP systems. For meat preservation, CO_2-generators are mainly used because of their inhibitory activity against a range of aerobic bacteria and fungi. Among oxygen, CO_2, and N_2, which are most often used in MAP systems, CO_2 is the only gas with a direct antimicrobial effect, resulting in an increased lag phase and generation time during the logarithmic phase of growth. As mentioned in Table 1, the commercial systems, such as the CO_2 emitting Verifrais™ package manufactured by Codimer (France) or the CO_2 generators/O_2 scavengers Ageless® G (Mitsubishi gas Chemical Co., Japan) and FreshPax® M (Multisorb Technologies Inc., USA) have been used to extend the shelf life of fresh meats. Such emitting/scavenging systems are based on either ferrous carbonate or a mixture of ascorbic acid and sodium bicarbonate. The inhibitory action of CO_2 has differential effects on microorganisms. Thus, whereas aerobic bacteria as *Pseudomonas* are inhibited by moderate to high levels of CO_2 (10–20%), microorganisms as lactic acid bacteria can be stimulated by CO_2. Furthermore, pathogens such as *Clostridium perfringens*, *Clostridium botulinum* and *L. monocytogenes* are minimally affected by CO_2 levels lower than 50%. However there is concern that by inhibiting spoilage microorganisms, a food product may be made to appear edible while containing a high quantity of pathogens that have multiplied due to a lack of indigenous competition (Yingyuad et al., 2006). Moreover Lovenklev, Artin, Hagberg, Borch, Holst and Radstrom (2004) showed a higher production of *C. botulinum* toxin with high concentration of CO_2 while a decrease in the growth rate was observed. For most applications in meat and poultry preservation, high CO_2 levels (10–80%) are desirable because these high levels inhibit surface microbial growth and thereby extend shelf-life (Vermeiren et al., 1999).

1.3. Chlorine dioxide generators

Chlorine dioxide can exist in gaseous, liquid or solid state. Its efficiency against bacteria, fungi and viruses can be delivered from a solid state, called Microspheres (Bernard Technologies, USA), through the interaction of moisture to produce a controlled and sustained release of chlorine dioxide in gaseous form. According to the company, no residue is left, nor is the food product contained in the packaging tainted in any way. Sustained and controlled release of chlorine dioxide is related to exposure to humidity greater than 80% and light. The result is a high activity against a broad spectrum of microorganisms including actively growing vegetative cells and spores. Microsphere powder can be delivered from sachets previously incorporated in the packaging. The sustained and controlled release of chlorine dioxide from the Microspheres can be varied from peak delivery of 1 ppm–100 ppm for periods of days to 6 weeks. Applications for this technology are just beginning to unfold in the food industry to reduce food safety risks for meat, poultry, fish, dairy, confectionery, and baked goods.

2. Bioactive agents merely dispersed in the packaging

Among some commercial systems based on bioactive compounds directly incorporated into the packaging, an alternative to gaseous generator sachets is the incorporation of the scavenger into the packaging structure itself (e.g. Cryovac OS2000® systems, Sealed Air Corporation, USA; $ZERO_2$, CSIRO-FSA, Australia). The Cryovac® OS System was based on the incorporation of an oxygen-scavenging organic compound into a polymer for use as a layer in a laminated packaging film. The scavenger is designed to be activated by ultraviolet light just before filling and sealing on the packaging line. In MAP systems, with less than 2% initial oxygen levels, Cryovac®OS films can reduce oxygen to less than 0.1% typically in 3 days. The $ZERO_2^{TM}$ from CSIRO was also based on a UV light-activated oxygen- scavenging polymer. It should be noted that the speed and capacity of O_2-scavenging films are considerably low compared with iron-based-O_2-scavenger sachets. The O_2-scavenging film of Ageless-OMAC (Mitsubishi Gas Chemical Co., Japan) is activated by heat. Today, it is used in particular for the preservation of pasteurized cooked rice and could potentially be used for meat preservation (Table 1).

Another good example of commercial systems, is the AgION® antimicrobials which are based on the use of silver, a powerful antimicrobial metal ion, as the active ingredient (Table 1). AgION® compounds are compatible with a wide range of polymers and they will not volatilize during processing. AgION® antimicrobial products can be compounded into bulk polymers, incorporated into fibers during spinning, and mixed into a variety of coatings. Another example is Triclosan (2,4,4′-Trichloro-2′-hydroxydiphenyl-ether), the antimicrobial substance used by Microban (UK). For more than 20 years this chemical compound has been used effectively in personal hygiene products such as toothpaste, mouthwash, deodorant and soap, as well as an antibacterial agent in the hospital environment. This protection is achieved by combining Triclosan with any of the major polymers (e.g., PE, PP, PVC). The Triclosan fits into the empty spaces of the polymer and migrates to the surface to start its work against any developing bacteria. During washing, the molecules closest to the surface are cleaned away but are immediately replaced by other protective molecules. Kerry et al. (2006) reported that a 1% triclosan film could have a strong antimicrobial effect on *L. monocytogenes* in *in vitro* assays whereas the film did not effectively reduce spoilage bacteria and *L. monocytogenes* growth on refrigerated vacuum packaged chicken breasts stored at 7 °C. In the mid-1990s, Freund (Japan) combined an oxygen absorber with an inhibitive agent to produce Negamold, used for fresh fish, meat, delicatessen products, milk products and cheeses.

Such systems are mainly elaborated by adding the antimicrobial compound to the extruder when the film or coextrusion is produced. A disadvantage is that the high temperatures and shearing associated with the extrusion process can deteriorate the antimicrobial additives. If this category of bioactive films has not made it into the supermarket in larger numbers, it is not through any lack of research. Around the world, a range of agents has been selected with the aim of prolonging shelf-life and ensuring food safety. But this is one area where those in R&D may be left feeling that the commercial and regulatory world still has some catching up to do. Different antimicrobial molecules, such as bacteriocins, enzymes, chelatants and

Table 2
Selected bioactive compounds directly incorporated into the packaging for potential applications of meat preservation

Active component	Polymer carrier	Example of tested substrate Target strain	References
Bacteriocin			
Nisin	Cellulose films, PE PE	Ham Beef carcass tissue	Scannell et al. (2000), Siragusa et al. (1999)
	HPMC	Culture media – *Listeria monocytogenes*	Sebti and Coma (2002)
Lacticin	Cellulose film, PE	Ham	
Pediocin	Cellulose	Cooked meats	Ming et al. (1997)
Enzymes			
Glucose oxidase	Alginate	Fish	Field et al. (1986)
Bioactive polymer			
Chitosan	Chitosan	Cooked ham	Ouattara, Simard, Piette, Bégin, and Holley (2000)
Chitosan	Chitosan	Culture media – *Listeria monocytogenes*	Coma et al. (2002)
Organic acids			
Lactic acid	Alginate	Beef muscle	Siragusa and Dickson (1992)
Propionic acid	Chitosan	Cooked ham	Ouattara et al. (2000)
Others			
Tocopherol	LDPE	Beef	Moore et al. (2000)
Triclosan	Plastic matrix	Food borne pathogenic bacteria and bacteria associated with meat surface	Cutter (1999)

organic acids (i.e. propionic and sorbic acids), included in different films, have been tested to suppress pathogenic or saprophyte bacteria growth in meat product preservation (Table 2). For the systems described here and with a non-volatile biocide, a contact between the food and the package is obviously necessary and a migration process from the packaging materials is expected. Therefore, potential food applications include in particular vacuum-packed products (Devliedhere, Vermeiren, and Debevere, 2004).

2.1. Bacteriocins

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. Numerous bacteriocins have been characterized. Although some bacteriocins, such as pediocin PA-1 and lacticin 3147, have been developed for possible approval and use, nisin remains the most commercially important bacteriocin because of its relatively long history of safe use and documented effectiveness against important Gram-positive foodborne pathogens and spoilage agents. Indeed, as mentioned by Siragusa, Cutter, and Willett (1999), nisin, produced by *Lactococcus lactis* (recognized as GRAS by the United States Food and Drug Administration) and pediocin, produced by the meat fermentation starter culture bacterium *Pediococcus acidilactici*, have been demonstrated to be active against *L. monocytogenes* and other Gram-positive bacterial pathogens on meat surfaces when applied. An example of the bioactive properties of nisin potentially combined with organic acid and directly studied on sliced pork bologna is proposed by Samelis et al. (2005). As reported by Millette, Le Tien, Smoragiewicz, and Lacroix (2007), nisin is now known to form poration complexes in target cell membranes through a multi-step process that included binding of the C-terminal via electrostatic interaction. The N-terminal part of nisin is then inserted into the lipid phase of the bilayer resulting in the rapid efflux of small cytoplasmic compounds and finally to the cell death. Siragusa et al. (1999) have incorporated nisin in a liquid form, into a polyethylene-based plastic film that was used to vacuum-pack beef carcasses. They demonstrated the retention of nisin activity when incorporated into the plastic formulation and also that the conditions used to produce the film did not eliminate the antimicrobial activity of nisin. Moreover, after 20 days of storage, the microbial load on meat was lower than samples treated with nisin solution. As mentioned, nisin retained activity against *Lactobacillus helveticus* and *Brochothrix thermosphacta* inoculated in carcass surface tissue sections. An initial reduction of 2-log10 cycles of *B. thermosphacta* was observed with nisin-impregnated packaged beef within the first 2 days of storage at 4 °C. After 20 days of refrigerated storage at 4 or 12 °C (to simulate temperature abuse), *B. thermosphacta* populations from nisin-impregnated plastic-wrapped samples were significantly less than controls without nisin.

Growth inhibition of *L. monocytogenes* and other pathogen strains potentially found in meat products was often studied (Coma, Sebti, Deschamps, and Pichavant, 2001; Degnan, Buyong, and Luchansky, 1993; Ming and Daeschel, 1993; Stevens, Sheldon, Klapes, and Klaenhammer, 1991). Examples are the use of nisin, pediocin AcH and enterocins *A* and *B* in meat and meat products (Aymerich, Garriga, Ylla, Vallier, and Monfort, 2000; Gill and Holley, 1998; Murray and Richard, 1997), and pediocin AcH and sakacin *P* in chicken (Goff, Bhunia, and Johnson, 1996; Katla et al., 2002). Large variations in the degree of inhibition were observed in these studies. Marcos et al. (2007a) studied the capacity of enterocin, produced by *Enterococcus faecium*, for controlling *L. monocytogenes* growth in cooked ham. Indeed, cooked ham submitted to a thermal treatment high enough to eliminate pathogens but is subsequently exposed to the environment during peeling, slicing and repackaging operations, leading to a potential post-processing contamination. The bacteriocin was included in alginate, zein or polyvinyl alcohol-based biopackagings.

These authors showed that a very effective treatment during 6 °C storage was a vacuum-packaging with alginate films containing 2000 AU cm^{-2} of enterocins. The reduction of pathogen strains in cooked ham could still be improved by combining both technologies. For example, in another study, the same authors showed that it was necessary to combine this type of antimicrobial packaging with a high pressure processing to achieve a reduction of inoculated levels of listerial strains at 6 °C and during 60 days of storage (Marcos, Aymerich, Monfort, and Garriga, 2007b). Palmitoylated alginate could be also used to entrap enzymes such as nisin. Millette et al. (2007) studied the entrapment of nisin in modified alginate matrices to protect the bacteriocin against deleterious agents in fresh beef. They evaluated the potential of this system to control *S. aureus* on round beef steak during storage at 4 °C. The authors observed that nisin entrapped in palmitoylated alginate films allowed the release and the protection of nisin activity during storage. Results demonstrated that a concentration of 1000 IU ml^{-1} of nisin in film reduced by 1.86 log CFU cm^{-2} the level of *S. aureus* on steak surface after 7 days of storage.

The activity of bacteriocins in foods has been the subject of only a few studies. In these cases the activity was considerably reduced, or not detectable, after some days of storage (Goff et al., 1996; Katla et al., 2002; Murray and Richard, 1997). Bacteriocins are amphiphilic peptides susceptible to adsorption on food ingredients and proteolytic degradation, which may limit their use as preservation agents. Indeed, Aasen et al. (2003), have demonstrated the influence of different crucial 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 to the muscle protein, but the activity of the protein-bound bacteriocin still remains to be assessed. Proteolytic activity causes degradation of sakacin P, and probably other bacteriocins in non-heat-treated foods, but the losses can be compensated by increased dosages. Fat may inactivate the bacteriocin in liquid food and forcemeat (Roller et al., 2002). Factors that may influence the recovery and efficiency of bacteriocins are binding to meat components, partitioning into polar or non-polar food components, and conditions that destabilize the biological activity, like proteolytic degradation or oxidation (Daeschel, Mc Guire, and Al-Makhlafi, 1992; Murray and Richard, 1997). Reduced activity or recovery of bacteriocins in foods with high fat content were shown for nisin (Bell and Lacy, 1986; Davies and Adams, 1994) and acidocin CH5 (Chumchalová, Josephsen, and Plocková, 1998). Bacteriocins may also adsorb to proteins in the food matrix by ionic or hydrophobic bonds. These kinds of interactions and their effect on the inhibition efficiency have been studied less than the influence of fat. However Murray and Richard (1997) have demonstrated, for example, that protein binding might cause a significant reduction in free bacteriocin in foods. The addition of casein reduced the activity of sakacin P, curvacin and nisin in synthetic media. The degradation attributed to proteolytic activity was demonstrated for pediocin AcH and nisin in raw pork meat (Murray and Richard, 1997). However, as reported by Helander, Nurmiaho-Lassila, Ahvenainen, Rhoades, and Roller (2001) or Carneiro de Melo, Cassar, and Miles (1998), substances lacking inherent toxicity yet causing increases in bacterial outer membrane permeability, could find applications in food protection, as they would sensitize harmful Gram-negative bacteria to other potentially inhibitory substances by faciliting their entry into the bacterial cells. For example, the resistance of Gram-negative bacteria to the biocidal action of lysozyme diminished when the outer membrane was perturbed with EDTA. Apart from direct bactericidal or bacteriostatic activity, chitosan's ability to disrupt the permeability barrier of the outer membrane in Gram-negative bacteria expands the applicability of chitosan as an antimicrobial substance in foods.

2.2. Spices and essential oils

Spices are rich in phenolic compounds, such as flavonoïds and phenolic acids, which exhibit a wide range of biological effects, including antioxidant and antimicrobial properties (Oussalah et al., 2004; Suppalku, Miltz, Sonneveld, and Bigger, 2003; Matan et al., 2006; Han et al., 2007). Direct incorporation of essential oils to food such as meat products will result in the immediate reduction of the bacterial population but may alter the sensory characteristics of added food. This incorporation of essential oils to edible films may be particularly interesting and Seydim and Sarikus (2006) showed that oregano and garlic oil were, for example, effective in whey protein-based films against *S. aureus*, *S. enteritidis*, *L. monocytogenes, E. coli* and *Lactobacillus plantarum*. Basil (*Ocimum basilicum*) is a popular culinary herb and its essential oils have been used extensively for many years in the flavoring of confectionary and baked goods, condiments, sausages and meats, and so on (Suppalku et al., 2003). According to these authors, basil essential oil has a potential use in food preservation, especially in conjunction with technologies of antimicrobial packages for food products. Further research on the antimicrobial activity of the main components is required to evaluate its usefulness in the shelf life extension of packaged foods such as meat and poultry. Nadarajah, Han, and Holley (2005b) studied the capacity of glucosinolates naturally present in non-deheated mustard flour could serve as a source of allyl and other isothiocyanates in sufficient quantity to kill *E. coli* 0157:H7 inoculated in ground beef. They showed that it is possible to use mustard flour at levels of 5–10% to eliminate this pathogen strain from fresh ground beef. Lacroix, Chiasson, Borsa, and Ouattara (2004) and Ouattara, Giroux, Smoragiewicz, Saucier, and Lacroix (2002) evaluated the combined effect of gamma irradiation and application of cross-linked film coatings containing spice powders on microbial growth related to ground beef contamination. Cross-linked films based on caseinate and whey protein isolate were combined with thyme, rosemary, and sage spice. Ha et al. (2001) reported the effect of grapefruit seed extract, incorporated at 0.5–1% by co-extrusion process in multilayered polyethylene films, on the microbial population of fresh beef. These films reduced the growth of aerobic and coliform bacteria in minced beef wrapped with film and stored at 3 °C for up to 18 days.

Further research is needed to determine whether natural plant extracts could act as an antimicrobial agent, as an odor/flavor enhancer in packaged foods, and as a component in antimicrobial packages.

2.3. Enzymes

Due to health concerns, producers are now particularly interested in the use of biopreservatives such as antimicrobial enzymes in packaging. Gill and Holley (2000) used lysozyme, a 14.6 kDa single peptide protein produced by many animals including man, which would exhibit enzymatic activity against the peptidoglycan of the cell wall of both Gram-positive and Gram-negative bacteria, to limit bacterial growth in meat. Previously, these authors showed that a combination treatment of lysozyme, nisin and EDTA might be effective in controlling the growth of spoilage and safety bacteria of cured meat products (Gill and Holley, 1998). However, according to Han (2005), in most cases the lysozyme would be not effective against Gram-negative bacteria. Güçbilmez, Yemenicioğlu, and Arslanoğlu (2007) also reported that Gram-negative bacteria are protected due to their outer membrane surrounding the peptidoglycan. Indeed, lyzozyme exhibits antimicrobial activity by splitting the bonds between *N*-acetylmuramic acid and *N*-acetylglucosamine of the peptidoglycan in the cell wall of bacteria. The destabilization of the outer membrane by EDTA, potentially used in combination, could increase the activity on Gram-negative bacteria. These authors produced zein-based films effective on *E. coli*.

Moreover, the specificity of enzymes should be considered carefully, since antimicrobial activity is very sensitive to its environment and substrates. Han (2005) gives the example of the activity of lyzozyme, which can be significantly affected by temperature and pH. Moreover, in most cases, these biocides were directly incorporated into meat products (e.g., ham or Bologna-based sausages), but may be combined into a packaging material.

2.4. Preservatives and additives

The most common chemical antimicrobials used by researchers are the various organic acids, because their efficiency is generally well understood and cost effective. Sorbic acid and its more soluble salts are widely used as preservatives in various food products. Good solubility, stability and ease of manufacture make potassium sorbate the most widely used form in food systems. In addition, some authors have shown that, during storage at 10 °C, acetic acid, lactic acid or potassium benzoate leads to a reduction of *L. monocytogenes* populations in smoked sausage by 0.4–1.5 log CFU cm^{-2} (Geornaras et al., 2006). As specified by Björkroth (2005) the growth of microorganisms in marinated meat products has been considered to be suppressed by the acidic pH and the use of sorbates and benzoates. However, it seems that marinating may not necessarily have an effect on the survival of enteric pathogens, like *Campylobacter*, which could be due to the buffering capacity of meat. Moreover, as mentioned by Han (2005), organic acids have characteristic sensitivities to micro-organisms. Indeed, sorbic acids and sorbates are very strong antifungal agents while their antibacterial activities are not effective. Therefore mixtures of organic acids have a wider antimicrobial spectrum and stronger activity than a single organic acid. Ouattara et al. (2000) evaluated the feasibility of using acetic or propionic acids included in a chitosan matrix that was designed to slowly release the bacterial inhibitor. This system was studied to improve the preservation of vacuum-packed processed meats during refrigerated storage. They observed that the growth of *Enterobacteriaceae* and *Serratia liquefaciens* was delayed or completely inhibited as a result of film application.

Using again the example of Triclosan, Cutter (1999) showed that material containing 1500 ppm of this ether inhibited the growth of bacterial strains associated with meat alterations, such as *B. thermosphacta*, *S. aureus*, *Shigella*, *S. typhimurium*, and so on. As specified by Quintavalla and Vicini (2002), the use of Triclosan for food contact applications has been recently allowed in EU countries by the Scientific Committee for Food, in the tenth additional list of monomers and additives for food contact materials,with a quantitative restriction on migration of 5 mg kg^{-1} of foods.

For non-volatile bioactive agents, the bioactivity of these categories of films is based on the diffusion of the biocide into the food. Thus, to develop these packaging systems, knowledge on the diffusivity of bioactive substances is needed. In order to produce an antimicrobial food packaging system, Choi et al. (2005) determined the diffusivity of potassium sorbate incorporated into a K-carrageenan film and investigated the effects of pH and temperature on this diffusivity.

Numerous studies concerning the antimicrobial activity of packaging materials have been reported and different methods of antimicrobial activity determination have been used. It is therefore difficult to compare the results of these studies because of substantial variations in the bioactive compounds included in the matrix, packaging matrix, test microorganisms, and test methods. There is a need for the development and validation of standard methods to accurately determine the efficiency of bioactive packaging on the preservation of meat products.

Moreover, a lot of information is available on the diffusion of various molecules in food such as water in drying operations or NaCl in cheese (Simal, Sanchez, Bon, Femenia, and Rossello, 2001; Tütüncü and Labuza,

1996) but, comparatively, little data could be found on the diffusivity of molecules such as bioactive substances in foods or model gel systems, as, sorbic acid diffusivity in agar gels (Giannakopoulos and Guilbert, 1986a, 1986b) or diffusion of sucrose in gellan gels (Bayarri, Rivas, Costell, and Duran, 2001). Mattisson, Roger, Jönsson, Axelsson, and Zacchi (2000) studied the influence of the gel matrix on lysozyme and myoglobine diffusion. Modeling bioactive substances diffusion is crucial to understand or to modulate film activity and to investigate which type of food could be protected efficiently using these systems of active films. Several methods have already been used by several authors who worked on different solute diffusion in food (Giannakopoulos and Guilbert, 1986a, 1986b). Sebti, Carnet-Ripoche, Blanc, Saurel, and Coma (2003) studied the diffusion of nisin in model gel, and a simple model using Fick's second law was developed to determine the apparent diffusion coefficient of nisin in agarose gel. The diffusive process of nisin in agarose model gel was verified, taking into account possible factors influencing the diffusion rates such as nisin concentration, temperature and time of diffusion. For these bioactive packages based on migration phenomenon, further studies are necessary to adapt antimicrobial activity for various meat-based food applications.

3. Bioactive agents coated on the surface of the packaging material

An alternative to the incorporation of antimicrobial compounds during extrusion is to apply the antimicrobial additives as a coating. This has the advantage of placing the specific antimicrobial additive in a controlled manner without subjecting it to high temperature or shearing forces. In addition, the coating can be applied at a later step, minimizing the exposure of the product to contamination.

The coating can serve as a carrier for antimicrobial compounds in order to maintain high concentrations of preservatives on the surface of foods. Bioactive activity may be based on migration or release by evaporation in the headspace (Table 3). For the systems using non-volatile agents and where the bioactivity is based on a migration of the antimicrobial substances, silver-substituted zeolite has been developed in Japan as the most common antimicrobial agent associated with plastics. Zeolite, which has some of its surface atoms replaced by silver, is laminated as a thin layer (3–6 μm) on the surface of the food contact polymer and appears to release silver ions. Silver ions, which inhibit a wide range of metabolic enzymes, have strong antimicrobial activity with a broad spectrum (Quintavalla & Vicini, 2002).

Bacteriocins could be coated or adsorbed to polymer surfaces. Examples include nisin/methylcellulose coatings for polyethylene films and nisin coatings for poultry based on an adsorption of nisin on polyethylene, ethylene vinyl acetate, polypropylene, polyamide, polyester, acrylics and polyvinyl chloride (Chen and Williams, 2005). Ming, Weber, Ayres, and Sandine (1997) applied bacteriocins to the inner surface of plastic vacuum-packaging bags. They reported, using the coated materials with nisin and pediocin, inhibition of *L. monocytogenes* growth on ham, turkey breast meat and beef under refrigerated conditions. Polyvinyl chloride or polyethylene films coated with nisin were effective in reducing *S. typhimurium* growth on the surface of fresh broiler skin (Natrajan and Sheldon, 2000).

As previously mentioned, because most food packaging systems consist of (1) the packaging material, (2) the food, and (3) the headspace in the package, antimicrobial agents may be initially coated on the packaging materials to be released through evaporation in the headspace. The latter can be achieved with essential oils that are volatile and are regarded as "natural" alternatives to chemical preservatives. In addition, their use in foods meets the current demands of consumers for mildly processed or natural products. According to Skandamis and Nychas (2002), their application in active packaging can be of great importance, even if their practical application is limited due to flavor considerations, as well as their effectiveness which is moderated due, in particular, to interaction with food ingredients. Skandamis and Nychas (2002) evaluated the efficiency of volatile compounds of essential oils in

Table 3
Selected bioactive compounds coated on the surface of packaging materials

Active component	Polymer carrier	Example of tested substrate – target strain	Process to release the biocide	References
Bacteriocin				
Nisin	Silicon coating	Beef tissue	*Migration*	Daeschel et al. (1992)
Nisin/pediocin	Plastic vacuum package	*Listeria monocytogenes* on ham Turkey	*Migration*	Ming et al. (1997)
Thyme, rosemary and sage spice	Cross-linked caseinate and whey protein film	Ground beef	*Migration*	Lacroix et al. (2004)
Oregan, pigments	Milk protein films	*Pseudomonas*, *E. coli* 0157. H7 on beef muscle	*Migration*	Oussalah, caillet, salmieri, Saucier, and Lacroix (2004)
Essential oil				
Volatile compounds associated to modified atmosphere packaging	Paper immersed into essential oil extracts	Meat samples	*Evaporation in the head space*	Skandamis and Nychas (2002)

combination with the use of MAP conditions. From paper immersed in pure essential oil extract and placed within the packaging but not in contact with the meat, these authors showed longer shelf life of meat samples supplemented with volatile compounds of oregano essential oil (*Origanum vulgare*). Volatile compounds of this essential oil affected both the growth and the metabolic activity of microorganisms of meat stored in a modified atmosphere. However, such inhibition is not as strong as that due to the contact of pure essential oil with microorganisms, when it is added directly onto the surface of meat.

As a result, further research is required to establish the parameters for optimal antimicrobial efficiency, adhesion on packaging support, or the desorption procedure from the materials. Such parameters as plastic formulation, levels of antimicrobial agents, biocide purity and varying plastic composition will be the focus of further studies. Food composition, as previously mentioned, will necessarily have consequences on the biocide efficiency.

4. Antimicrobial macromolecules with film-forming properties

4.1. Naturally antimicrobial polymer exhibiting film-forming properties

Macromolecules such as chitosan, which exhibit an antifungal and antibacterial activity, also show abilities to retain included antimicrobial substances (Bégin and Van Calsteren, 1999; Coma et al., 2002; Jeon, Kamil, and Shahidi, 2002; Muzzarelli et al., 1990; Ouattara et al., 2000; Shahidi, Arackchi, and Jeon, 1999; Wang, Du, Luo, Lin, and Kennedy, 2007). Chitosan consists of polymer composed principally of 1,4 linked 2-amino-2-deoxy-β-D-glucose and, although more active against spoilage yeasts, it also inhibits some Gram-negative and particularly Gram-positive bacteria. As reported by Helander et al. (2001), the polycationic structure of chitosan can be expected to interact with the predominantly anionic components of the bacteria surface (lipopolysaccharides, proteins). This binding leads to a disruption of the integrity of the outer membrane, resulting in loss of the barrier function. Chitosan has recently been affirmed as GRAS by the US FDA (2001), thus removing some of the regulatory restrictions on its use in foods. Chitosan coating can serve, at the same time, as a carrier. Ouattara et al. (1997) evaluated the feasibility of using an incorporation of acetic and propionic acid into a chitosan matrix with or without the addition of lauric acid or cinnamaldehyde. The various films were tested against indigenous lactic acid bacteria and *Enterobacteriaceae*, and against *Lactobacillus sakei* or *Serratia liquefaciens*, surface- inoculated onto meat products. These authors showed that whereas lactic acid bacteria were not affected by the antimicrobial films, the growth of *Enterobacteriaceae* and *S. liquefaciens* was delayed or completely inhibited as a result of film application. However, improvements are necessary before the concept can be developed into a successful technology, and better antimicrobial agents have to be found, which would be active against a broader range of bacteria including the lactic acid bacteria responsible for the spoilage of refrigerated vacuum-packaged processed meats. Moreover, a better carrier than chitosan has to be found to slow the release of antimicrobial agents. Wang et al. (2007) obtained an increase of the antibacterial activity of chitosan-based film against *S. aureus* or *E. coli* growth, by the association of the aminopolysaccharide with rectorite nanoclays, potentially used for meat preservation. They explained that chitosan around the layered silicate may have more chance to inhibit the growth of bacteria due notably to the increase of the positive charge density in each unit volume by the chitosan chains aggregating between the rectorite interlayer.

Antimicrobial efficiency demonstrated in the laboratory is not always proven in foods, due to the highly reactive nature of the polycationic chitosan, which interacts with proteins, fats and other anionic substances in foods. Sagoo, Board, and Roller (2002) showed that treatment with chitosan increases the shelf-life of raw sausages stored at chilled temperatures from 7 to 15 days. Today, the growth of Gram-negative bacteria, particularly *Pseudomonas* and *Enterobacteriaceae*, is repressed in sulphited sausages. Sulphites have a long history of safe use in meat products. However, exposure to sulphites has been linked to the aggravation of asthmatic and other respiratory problems in some sensitive individuals. In general, spoilage yeasts were more sensitive than Gram-positive bacteria, which were in turn, more sensitive than Gram-negative bacteria to the biocidal action of chitosan. These authors concluded that the antilisterial action of chitosan was particularly notable and could potentially be exploited in chilled foods.

The effect of chitosan in meat preservation was also studied by Darmadji and Izumimoto (1994), including microbiological, chemical, sensory and color qualities. These authors showed that 0.01% of chitosan inhibited the growth of some spoilage bacteria such as *Bacillus subtilis*, *E. coli*, *Pseudomonas fragi* and *S. aureus*. In meat, during incubation at 30 °C for 48 h or storage at 4 °C for 10 days, 0.5–1% chitosan inhibited the growth of spoilage bacteria, reduced lipid oxidation and resulted in better sensory attributes. These authors reported that chitosan at a concentration of 1% reduced bacterial counts by an average of 1-2 log CFU g^{-1} in minced beef patties stored at 4 °C for 10 days. Both studies used chitosan as bioactive preservative and not as a bioactive packaging.

4.2. Surface-bounded bioactive agents

In contrast to naturally antimicrobial polymers, some bioactive materials have been produced by modifying the surface composition of the polymer. Cohen et al. (1995) showed a conversion of amide to amine groups of nylon by irradiation. Not only electron irradiation or UV treatments can be used to modify film surfaces: plasma treatments are under development as well.

Moreover, it is also possible to produce bioactive properties by binding an agent to the surface of the package. This requires a molecular structure large enough to retain activity on the microbial cell wall even though bound to the plastic. According to Quintavalla and Vicini (2002), such agents are likely to be limited to enzymes or other antimicrobial proteins. These functional groups were immobilized on the surface, which prevents the migration of biocides in the food. Moreover, because of organisms normally sensitive to bacteriocin *in vitro* are not necessarily sensitive to bacteriocin in meat system, some authors immobilized the antimicrobial peptides directly on films. Indeed, enzymes immobilization to insoluble polymer matrices could provide stability to these enzymes (Millette et al., 2007).

Scannell et al. (2000) studied the immobilization of different bacteriocins on polyethylene/polyamide materials. They showed that the immobilized nisin maintained its activity for 3 months under refrigerated storage and at room temperature. A reduction of the lactic acid bacteria in ham was observed. However, a decrease in the activity of the bioactive agent when immobilized could be occur. To protect nisin from denaturation from sensitive antimicrobials substances and enzyme in meat products, Millette et al. (2007) elaborated nisin covalently linked to activate alginate beads. The alginate activation was carried out with sodium periodate to generate neighbouring dialdehydes residues. These dialdehydes react with amine groups of the nisin forming a Schiff's-base linkage. After 14 days of storage, 1.77 and 2.21 log CFU g^{-1} *S. aureus* reductions were respectively observed in ground beef samples containing beads with 500 IU g^{-1} nisin or nisin solution at the same concentration. Even if further research is necessary to improve the activity of the linked nisin, this chemical bound between nisin and alginate based films could be an interesting way to produce surface-bounded bioactive packagings.

5. Bioactive edible coating

Another possibility is to incorporate the antimicrobial compound into an edible coating, applied by dipping or spraying onto the food. Selection of the incorporated active agents is limited to edible compounds, because they have to be consumed with the coating layers and foods together. The aforementioned chitosan-based films whether associated with other bioactive compounds (bacteriocins, organic acids, etc.) or not, may also be included in these packaging concepts. According to Gennadios et al. (1997), edible coatings can improve the quality of fresh, frozen and processed meat and poultry products by, for instance, delaying moisture loss, reducing lipid oxidation and discoloration, enhancing product appearance and functioning as carrier of food additives. Yingyuad et al. (2006) studied the effect of chitosan coating and vacuum packaging on the quality and shelf-life of retail packaged grilled pork during refrigerated storage. As already mentioned, despite the extended shelf-life of refrigerated products stored under vacuum packaging, there is concern about the survival and growth of microaerophilic psychotrophic pathogens and these conditions also favor the growth of lactic acid bacteria, which are the dominant bacteria associated with cooked meat spoilage, resulting in a slimy appearance. These authors showed a significant effect of the chitosan coating on the total viable count compared to the uncoated product, providing an interesting active system when associated with vacuum packaging.

Cellulose derivatives such as hydroxy propyl methyl cellulose (HPMC) is a promising raw material for edible coatings associated with antimicrobial entities. HPMC based coatings associated with nisin, where the bioactivity was based on the release of nisin previously incorporated into the film-forming solution, was studied. The effectiveness of bacteriocin protective cultures has mainly been studied in meats (Jacobsen, Koch, Gravesen, and Knochel, 2002; Katla et al., 2002). Agar coatings associated with nisin on fresh poultry have been studied by Natrajan and Sheldon (1995). Substantial reductions in *S. typhimurium* growth were recorded after storage at 4 °C for 96 h. Ming et al. (1997) observed a total inhibition of the *L. monocytogenes* growth on ham, turkey meat and beef when bacteriocins were associated with a cellulose-based matrix. As reported by Quintavalla and Vicini (2002), the commercial application of this technology is described in US patent 5,573,797 assigned to Viskase Co. The package is a film, such as regenerated cellulose, containing a heat resistant *Pediococcus*-derived bacteriocin in a synergistic combination with a chelating agent to inhibit listerial strains.

The potential association of a chitosan-based packaging with bacteriocin could be interesting against strains, which are common meat product contaminants (Li, Kennedy, Peng, Yie, and Xie, 2006). Although this system was only studied as a preservative agent by Roller et al. (2002), using a chitosan–carnocin based combination and a chitosan-sulphite combination, results have shown potential applications for edible films.

Siragusa et al. (1999) investigated the potential of decontamination of raw beef by application of organic acids immobilized in calcium alginate gels. Immobilized lactic or acetic acid resulted in a significantly greater reduction of *L. monocytogenes* growth, compared to acid treatment without an edible polymer.

Oussalah et al. (2004) evaluated the ability of a milk protein-based edible film containing 1% essential oils of oregano, pimento or a mixture of both spices to control *Pseudomonas* spp. and *E. coli* H0157:H7 growth on surface-inoculated beef muscle. The use of film containing essential oils significantly reduced the microorganism level in meat when compared to meat samples coated with free-essential oil film and meat without film during seven days of storage. These authors also showed that both bacteria seem to use milk protein-based film in the absence of essential oils as a substrate to sustain their growth. Moreover, films containing oregano essential oil were the most effective against the growth of both bacteria whereas pimento-based films presented the highest antioxidant activity. Finally, the films allowed a progressive release of phenolic compounds during storage. After seven days, the film remained effective. Thus, the use of edible films containing essential oils as a preservation method for meat is promising. However, further research must be conducted to control the diffusion rate of the bioactive compounds to the meat surface during storage.

6. Conclusion

Within the available arsenal of preservation techniques, the food industry is increasingly investigating the replacement of traditional food preservation techniques (intense heat treatments, salting, acidification, drying and chemical preservation) by new preservation technologies. The most investigated of the latter are non-thermal inactivation processes where active packaging holds a considerable place. In spite of intensive research efforts and investment, very few of these new preservation methods have until now been implemented by the food industry.

One approach to extend the storage and shelf life of fresh meats is to introduce antimicrobials – preferably those that occur naturally – to the surface of the meat (Aymeric et al., 2000; Cutter and Siragusa, 1997; Gill and Holley, 1998; Nattress, Yost, and Baker, 2001). Often antimicrobials are evaluated in an *in vitro* system especially against bacteria associated with meat spoilage (i.e. *B. thermosphacta*, *Carnobacterium*, etc.) or pathogen strains (*L. monocytogenes, Salmonella*, etc.).

Because the microbial contamination of meat products occurs primarily at the surface, due to post-processing handling, the use of packaging films containing antimicrobial agents could be more efficient, by slow migration to the food surface, thus helping to maintain high concentrations where they are needed.

According to Devlieghere et al. (2004), two aspects that are crucial for the practical application of some biocides are often overlooked: (1) the changes in the organoleptic and texture properties of the food when added, and (2) the interaction of these compounds with food ingredients and the influence of this interaction on its efficiency. For instance, these authors have shown that moderate concentrations of NaCl, present in many food products, are often already responsible for the inhibition of the antimicrobial activity of chitosan (Devliedhere, Vermeulen, and Debevere, 2002). The pH of food influences the ionization (dissociation/association) of most active chemicals, and could change the antimicrobial activity of organic acids and their salts. Moreover, each food has its own characteristic microflora. As reported by Quintavalla and Vicini (2002), the release kinetics of antimicrobial agents have to be designed to maintain the concentration above the critical inhibitory concentration with respect to the contaminating microorganisms that are likely to be present. Concerning EC legislation aspects, the European Commission is planning to propose a directive allowing new forms of food packaging to be introduced in the European market and a new directive would allow the introduction of active and intelligent packaging. The proposed directive would set basic safety and labeling guidelines for active and intelligent packaging, but more detailed rules would be needed for more sophisticated systems. The directive would also include traceability requirements (Anonymous, 2001).

In conclusion, despite the extended shelf-life of refrigerated products stored under vacuum- packed or MAP conditions, there is an increasing concern about the growth and survival of microaerophilic psychotrophic pathogens. Thus, additional methods should be used to ensure the safety of such products. Smart, interactive and active packaging are terms that have been used to describe the innovative concept of package structures.

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