Nisin as a food preservative:

Part 2: Antimicrobial polymer materials containing nisin

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Abstract

Nisin is the only bacteriocin approved as a food preservative because of its antibacterial effectiveness and its negligible toxicity for humans. Typical problems encountered when nisin is directly added to foods are mainly fat adsorption leading to activity loss, heterogeneous distribution in the food matrix, inactivation by proteolytic enzymes, and emergence of resistance in normally sensitive bacteria strains. To overcome these problems, nisin can be immobilized in solid matrices that must act as diffusional barriers and allow controlling its release rate. This strategy allows maintaining a just sufficient nisin concentration at the food surface. The design of such antimicrobial materials must consider both bacterial growth kinetics but also nisin release kinetics. In this review, nisin incorporation in polymer based materials will be discussed and special emphasis will be on the applications and properties of antimicrobial food packaging containing this bacteriocin.

Keywords: Nisin; Antimicrobial packaging; Activation process; Biopolymers; Synthetic polymers; Effectiveness evaluation.

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1. Introduction

The rapid loss of nisin activity in some foods is an important factor limiting its effectiveness as food preservative. This activity loss is mainly due to its interactions with food components like proteins and fat particles. Moreover, following the direct addition of nisin on the surface of crude meat products, a rapid reduction of its inhibitory activity due to its enzymatic degradation has been observed (Rose et al., 1999). Therefore, current research works aim at improving nisin stability and efficiency by its incorporation in microcapsules, liposomes, or packaging films.

These antimicrobial materials can reduce microbial growth at the food surfaces in direct contact with them: they can release nisin at the food surface inhibiting or killing foodborne pathogenic or food spoilage microorganisms that can potentially contaminate food products. The interest for these active packaging has strongly increased during the last decade as a result from the change of consumer eating patterns: the consumption of ready-to-eat (RTE) foods stored at low temperature to preserve their freshness has increased. Unfortunately, there has also been an increase in the frequency of health hazards associated with the consumption of RTE foods (Tournas, 2005). Antimicrobial molecules that usually incorporated in packaging films are enzymes, bacteriocins, essential oils, organic acids, or other chemical conservatives (Appendini and Hotchkiss, 2002). However, contamination by *Listeria* is of special concern because of its psychrotrophic nature and the threat it represents for public health (Donnelly, 2001). Besides the respect of good manufacturing practices, antimicrobial packaging containing preservatives which are effective against *Listeria monocytogenes*, such as nisin, and released in the superficial zone

of food products, that is the zone in which post-process contaminations occur, are thus of particular interest.

(Bio)-polymer films can be used to enclose nisin and act as a diffusion barrier to limit and control the diffusion rate and to ensure a constant nisin concentration at the food surface. Currently, general research trends in the field of antimicrobial packaging aim to associate antimicrobial and biodegradable characters. As a rule, antimicrobial packaging aim to prolong the lag phase and/or lower microorganismsô growth rate on the food surfaces. The incorporation of an antimicrobial agent into a food packaging system can take several strategies. **Figure 1** shows that an active compound can be adsorbed to the internal or external side of a packaging material, incorporated into the packaging, or also chemically immobilized in the internal surface of a package. According to Han (2000), several factors must be considered in the design or modeling of an antimicrobial film or package: chemical nature of films/coatings, process conditions and residual antimicrobial activity, properties of both antimicrobial agents and foods, storage temperature, mass transfer coefficients, and physical properties of packaging materials.

When nisin is incorporated in antimicrobial packaging, its release kinetic is the main parameter to be controlled. Kinetics of this release should be estimated by measuring nisin release rate in a food simulant or by measuring the effectiveness of microbial growth inhibition and the increase of the product shelf life. These kinetic parameters depend mainly on the physico-chemical characteristics of the film. The strategy for producing a package containing nisin depends on the desired release properties to the food surface. Rapid nisin release to food surface can significantly reduce packaging effectiveness due to the nisin diffusion in food matrix which causes the use of large nisin amounts. On the other hand, if the nisin release rate is too

slow, inhibitory concentration can not be reached, and consequently, growth of food spoilage or foodborne pathogenic microorganisms in the food product can occur.

2. Importance of nisin immobilization

Direct nisin incorporation in foods is appropriate when the microbial growth can take place on both the surface and inside the food matrix. However, the main deterioration cause is microbial growth on the food surface induced by food processing and the use of this method implies the addition of excessive and not optimized nisin amounts. Antimicrobial polymer matrices can be an alternative to overcome these problems by using small nisin amounts. Food microbiological stability can thus be improved by maintaining a constant high concentration of the antimicrobial agent on the food surface. Particularly, antimicrobial packaging can therefore be used to extend food shelf life or reduce the contamination risk during the storage period. The nisin release control can help to optimize the effectiveness of this antimicrobial agent by maintaining nisin concentration above the minimal inhibitory concentrations (MIC) of food spoilage or foodborne pathogenic microorganisms in the superficial zone of foods throughout their whole shelf life period. Nisin diffusion rate from film to food depends on three main parameters: (i) film properties (polymer(s) nature, temperature, method, plasticizer ...), (ii) food characteristics (pH, water activity, ions, ...), and (iii) storage conditions (temperature, relative humidity, duration ...).

Nisin or commercial preparations containing nisin are added to food packaging through several strategies which can be combined. The advantages and limitations of each strategy are not always clearly given. **Table 1** gathers the different strategies performed to immobilize nisin

and their limitations. When nisin is adsorbed or only ñdepositedò on a film surface, it is rapidly released from the surface which can be a dramatic drawback for food preservation. On the contrary, when nisin is located in the polymer bulk, its release can be kinetically controlled by the polymer properties (diffusivity or solubility barrier) and nisin can be delivered to food for much longer contact time, the solid support acting as a reservoir. For example, to underline the importance of these different release methods, Chi-Zhang et al. (2004) studied the effectiveness of three methods of nisin application for the preservation of food systems and the control of *Listeria monocytogenes*: (i) instantaneous nisin addition in the medium to simulate nisin production by lactic acid bacteria in foods, (ii) slow addition of a nisin solution in the medium using a pump to simulate the slow nisin release from packaging materials, and (iii) a combination of both delivery modes. The authors found that in the presence of a nisin excess, *Listeria monocytogenes* instantly developed a resistance to this bacteriocin, whereas when nisin was added slowly, only a temporary tolerance has been observed and *Listeria* cells became sensitive to nisin. The authors concluded that nisin antimicrobial effectiveness strongly depends on its delivery method.

3. Nisin immobilization in beads and gels

Nisin immobilization in beads and gels and differences in efficacy between free and entrapped nisin has been widely studied for direct food contact (Cutter and Siragusa, 1996; Fang and Tsai, 2003; Lu et al. 2010). **Table 2** gives some examples of the use of beads and gels containing nisin for food preservation. Encapsulation matrices act as a reservoir that delivers nisin molecules into the food providing sustained release through concentration gradient. When encapsulating, it is important to use a matrix compatible with nisin but also with the food to

preserve. The most commonly used polymer for nisin immobilization in beads or gels is sodium alginate because this polymer does not affect its antimicrobial activity (Wan et al., 1997). Several methods have been proposed for nisin immobilization into alginate or gelatin beads (Ku and Kyung, 2007; Millette et al., 2007), gels (Fang and Tsai, 2003), and fiber mats (Dheraprasart et al., 2009). Nisin has been also incorporated into a solid matrix of calcium alginate and then ground to form microcapsules (150 microns) (Wan et al., 1997). This strategy has helped to protect the antimicrobial agent against proteolytic enzymes and to control its release increasing thus its effectiveness.

Cutter and Siragusa (1997) studied the effect of nisin embedded in calcium alginate gels on the growth of *Brochothrix thermosphacta* in ground beef. They showed that this strategy helps to protect against unwanted germs when meat is ground. This protection was shown to be greater than that obtained by direct application of the bacteriocin on the meat surface. These results were mainly interpreted in terms of nisin protection against enzymatic degradation and adsorption to proteins and lipid particles present in meat. Nisin is generally not active against Gram-negative bacteria, however, Fang and Tsai (2003) have encapsulated nisin with EDTA, acetic acid, or potassium sorbate in calcium alginate matrices and obtained results showed that these binary systems increase the efficacy to inhibit the pathogenic bacteria *Escherichia coli* O157:H7 at 10 °C. This antimicrobial activity improvement was not observed when the temperature rises to 30 °C or when nisin was immobilized alone. In another study, nisin immobilization in alginate matrices has led to significantly inhibit *Staphylococcus aureus* on the surface of ground or sliced beef (Millette et al., 2007). Nisin was also successfully encapsulated by spray-drying in a few published articles mainly in order to study the factors influencing its release kinetics (Xiao and

Zhong, 2011; Xiao et al. 2011). Finally, it is important to note that, compared to liposome entrapment, nisin encapsulation in biopolymers such as alginate provides more stable and efficient antimicrobial systems (Wan et al., 1997).

4. Activation of polymeric film surfaces by nisin

Nisin is a surface-active molecule that can bind easily to various molecules which makes it suitable for adsorption to solid surfaces such as food packaging films. For polymer film funcionnalization, nisin (or commercial nisin preparations) is solubilized in acidic aqueous solution (i) without any polymeric component, the non filmogenic solution is spread on the film surface, or the plastic film immersed in this nisin based solution or (ii) in the presence of another polymer (or polymers blends) in order to perform a filmogenic solutions to be coated on a plastic film. According to this method, plastic synthetic films can be used: coating of polyethylene films with nisin/methylcellulose solutions and nisin adsorption on films of polyethylene, ethylene vinyl acetate, polypropylene, polyamide, polyester, acrylic, and polyvinyl chloride are some typical examples (Appendini and Hotchkiss, 2002). These films often require a surface treatment which is necessary to wet the polymer with an aqueous solution for adherence purposes.

LDPE (low-density polyethylene) has good processing properties and is widely used in food packaging. Grower et al., (2004) have performed nisin based solutions made of soluble polymers blends to coat at room temperature the surface of polyethylene films (LDPE) with the aim to release nisin and inhibit *Listeria monocytogenes* growth. LDPE films coated with different nisin concentrations (10 000 and 2 500 IU mL^{-1}) have been mainly analyzed for their physicochemical properties. Main results showed that tensile strength of these activated films

increased when incorporated nisin concentration increased, which also corresponded to increasing the film thickness. Nisin concentration (10 000 and 2 500 IU mL^{-1}) had no significant effect on water vapor permeability of activated films. Franklin et al., (2004) studied *Listeria monocytogenes* inhibition on the surface of individually packaged hot dogs with a plastic packaging film containing nisin. They observed that the packaging film coated with a methylcellulose/hydroxypropyl methylcellulose-based coating solutions containing 10 000 and 7 500 IU mL^{-1} nisin causes a significant decrease in the number of *Listeria monocytogenes* cells on the surface of hot dogs by more than 2 log CFU (colony-forming unit) per package after 60 storage days.

Leung et al. (2003) studied the influence of surface energy on the nisin adsorption to the surface of 5 commercial films (LDPE, LLDPE, 2 ethylene acrylic acid copolymers and ethylene vinyl alcohol (EVOH) copolymer) after immersion in aqueous nisin solutions. Parameters like nisin concentration, contact time, pH, and temperature during the coating step were studied. The authors determined a combination of factors providing the most effective nisin adsorption: film having a low surface energy (LLDPE), high nisin concentration solutions, pH 3.0, and low temperature (no nisin adsorption has been observed at 80 °C).

Nisin adsorption (aqueous solutions formulated with EDTA) on different polyethylene films has also been studied by atomic force microscopy (La Storia et al., 2008). All LDPE substrates underwent a Corona treatment before being coated in order to be effectively wetted by the aqueous antimicrobial solution. Obtained results showed that coated nisin is not uniform but agglomerated on the film surface probably trapped by EDTA salts crystals. In this study, the authors managed to establish a relationship between nisin distribution on the film surface and

antimicrobial efficacy of these films against the strain *Listeria monocytogenes* V7. Mauriello et al. (2005) tested the antimicrobial activity of nisin coated LDPE based food packaging film (from nisin aqueous solution) to inhibit *Micrococcus luteus* ATCC 10240 strain. Experiments resulted in a strong inhibition of this bacteria, used as an indicator of milk quality during storage, after 24 h at 25 °C, but only a slight reduction was observed at 4 °C. This antimicrobial package was able to delay microbial growth in fresh, pasteurized, and UHT milk. The authors also concluded that these antimicrobial films are effective against the tested strain and that nisin release is dependent on temperature and pH.

In another study, Scannel et al. (2000) have prepared antimicrobial films by immersion of cellulose paper and plastic films (polyethylene/polyamide bilayer films) in aqueous nisin solutions containing citric acid (0.01 N). After air drying, the antimicrobial activity of these films was evaluated by diffusion test in agar gel. Results showed that these films can reduce by more than 2 log the number of *Listeria innocua* cells and 1.5 log the number of *Staphylococcus aureus* ones in ham or cheese packaged under vacuum. Luchansky and Call (2004) evaluated the ability of cellulose films coated with nisin to control *Listeria monocytogenes* inoculated on the surface of commercially prepared frankfurters. The pathogen number decreased by 0.88 log CFU per unit after 15 days storage but then increased remarkably after 60 days storage at low temperature. Antimicrobial papers coated with nisin and chitosan beforehand dissolved in vinyl acetate-ethylene co-polymer have been prepared and their antimicrobial activities and migration were evaluated in water, milk, and orange juice (Lee et al., 2003). The paper coated with nisin alone was effective against *Listeria monocytogenes* and that coated with chitosan alone was effective

in inhibiting *Escherichia coli O157:H7*. The authors concluded that the combination of the two preservatives in the coating could improve food stability against both bacterial strains.

Nisin absorption by a cellophane film was studied to determine the optimum temperature and concentration for this process (Guerra et al., 2005). Measuring the nisin absorption kinetics has shown that it is done in two steps, a fast one followed by a slow one. In fact, the second adsorption step was slow because of the unavailability of binding sites for nisin which can subsequently form a second layer. According to this study, nisin sorption was highest at 8 °C and activated films were effective in reducing total aerobic bacteria on ground meat surface by 1.5 log after 12 days at 4 °C. To finish this part, we can also note that Polyvinyl chloride (PVC) is an amorphous or weakly crystalline polymer, rigid, and having good barrier properties but rarely used in food industry. PVC films have been also coated with a solution containing 100 µg mL^{-1} of nisin to inhibit *Salmonella typhimurium* on fresh broiler (Natrajan and Sheldon, 2000). More recently, a polyamide/polyethylene co-extruded film was coated with a polyvinyldichloride (PVDC) lacquer containing nisin and natamycin (Hanusová et al., 2010). These films were effective in inhibiting *Penicillim expansum, Fusarium culmorum, Lactobacillus helveticus*, and *Listeria ivanovii*.

In general, packaging polymer film properties (mainly hydrophobicity) play an important role in the adsorption or absorption of nisin. In addition, the relationship between the ad/absorbed nisin amount and the film antimicrobial activity is not always linear because of nisin-film interactions (Appendini and Hotchkiss, 2002). For example, it has shown, by using circular dichroism spectroscopy, that polyethylene oxide chains confer conformational stability to nisin (Tai et al., 2008).

5. Nisin incorporation in food packaging films

5.1. Some helpful factors to develop packaging films containing nisin

5.1.1. Food properties

Food composition (carbohydrates, proteins, lipids ...), physicochemical (pH, water activity, oxygen partial pressure, temperature, ionic strength ...), and biological (enzymes ...) properties result in different environmental conditions for both bacteria growth and nisin activity. For example, low pH promotes acidophilic bacteria growth and improves nisin solubility and activity. Similarly, water activity may affect nisin solubility and its chemical stability but also the selection on packaged food surface of microflora whose growth is dependent on water activity. Although nisin is a peptide known to be thermostable, storage temperature of packaged food must also be well controlled because it influences bacteria growth but can also modify their sensitivity to nisin action.

5.1.2. Polymer properties

Polymer properties such as solubility, polarity, mechanical strength, crystallinity, permeability, toxicity ... are important to consider. Films functional properties are influenced by the polymer molecular nature, but also by the compounds added to modulate film properties such as plasticizers or cross-linking agents. Nisin must be incorporated in advance in the packaging materials and intend to subsequently migrate into the food through diffusion and partitioning. Nisin incorporated in a polymer film is generally dispersed in the amorphous regions of the polymer matrix. When the antimicrobial is used in high concentrations, these amorphous areas may be saturated and nisin can interfere with the polymer-polymer interactions in the crystalline

regions (Han, 2003). When they are in contact with food, these films should not only show the properties of flexibility and permeability to facilitate nisin diffusion but they must also resist to breakage and abrasion. Nisin is more generally incorporated in biopolymers than in synthetic films. One of the most important characteristics of biopolymers is their ability to form a dense network due to hydrogen bonding. However, films based on biological polymers have low oxygen permeability, but high permeability to water vapor. To reduce this water sensivity, some authors have proposed the inclusion of lipids in films (Carnet Ripoche et al., 2006) or the biopolymer structure modification by crosslinking (Sebti et al., 2003).

5.1.3. Which process for nisin incorporated film production?

The method of nisin incorporation into the film and the packaging manufacturing process must be also considered and their effects on nisin inactivation should be studied. Antimicrobial packaging containing nisin can be obtained by adding nisin to the packaging materials during manufacture. Regarding the methodology used to obtain films, a very large number of studies have used the casting technique, and few have used plastic processing like extrusion.

For solvent casting, polymer(s) are dissolved in a solvent such as water, alcohol, dilute acid solutions, or mixtures of solvents. In some cases it is necessary to heat or adjust the pH of the suspension to dissolve the polymer. The addition of plasticizers is generally necessary to form self standing films with acceptable mechanical properties. The most widely used plasticizer is glycerol because of its efficiency, stability, and its compatibility with hydrophilic polymer chains. Once polymers have been dissolved, nisin is added, usually in an aqueous hydrochloric acid solution (in order to have maximum solubility). The next step is to remove the solvent by

evaporation. The drying rate (time/temperature) determines the film structural characteristics and must be optimized in preliminary studies.

During extrusion process, a partial or total inactivation may result because of the undergone high temperature and shear forces. These physical parameters, in addition to the residence time in the extruder must be controlled in order to predict the residual antimicrobial activity. Effects of the use of plasticizers and chemical solvents as well as printing and drying operations must be well quantified. Nisin antimicrobial activity can be affected during the film processing and storage conditions as well as ageing of the active packaging lead to a residual activity which is the actual nisin activity measured in the final package after all manufacturing operations. **Table 3** gives some examples of polymer based packaging containing different nisin concentrations and more details about nisin incorporation in films and their uses will be reviewed in the next section.

5.2. Polysaccharide based films containing nisin

Polysaccharides based films containing nisin are made mainly from cellulose ethers, starch, chitosan and alginate. Polysaccharides can produce homogeneous and transparent films but with moderate mechanical properties and high water sensitivity.

5.2.1. Cellulose derivatives

Cellulose is a crystalline glucose polymer that is water insoluble. Chemical substitution of hydroxyl groups gives rise to ionic (carboxymethyl cellulose, CMC) and nonionic (methylcellulose (MC); hydroxypropylcellulose, (HPC); hydroxypropylmethylcellulose (HPMC)) cellulose ethers. Films based on cellulose derivatives are flexible, transparent,

mechanically strong, and resistant to fats and oils. Coma et al., (2001) incorporated nisin into cellulose films based on HPMC by adding nisin to the film-forming solution. The inhibitory effect of this film has been shown against *Listeria innocua* and *Staphylococcus aureus*. In contrast, the addition of stearic acid to improve the film barrier properties to water vapor decreased the nisin inhibitory activity. This behavior was explained by the existence of electrostatic interactions between stearic acid and nisin which fixes it in the film and reduced its effectiveness. Recently, the effect of nisin and content of glycerol used as a plasticizer, on HPMC films obtained by casting was investigated (Imran et al., 2010). In this study, the authors used a commercial nisin preparation, Nisaplin®. The presence of salt and denatured proteins in this preparation had greatly affected the transparency, thickness, and water sorption of HPMC films. As attempted, the plasticizer improves films elasticity but alters their permeability and tensile strength. The authors also show that glycerol has no effect on the nisin release and hence on the inhibition zone diameters measured on agar gel containing an indicator bacteria strain. These films were primarily effective against strains of *Listeria*. Sebti et al., (2007) prepared films from HPMC film forming solutions containing nisin (250 µg mL^{-1}). HPMC films were transparent whereas nisin incorporation induced film bleaching. Measurements of tensile strength and elongation at break showed that nisin incorporation significantly alter these mechanical properties.

HPMC films crosslinked with citric acid and containing nisin have been shown to be active against *Micrococcus luteus* (Sebti et al., 2003). However, it has been demonstrated in this work that the crosslinking conditions render nisin inactive by denaturing it or by binding it irreversibly to HPMC when nisin is added before the crosslinking process. Packaging films

containing nisin were also prepared by mixing methylcellulose (MC) and HPMC (ratio MC/HPMC: 70/30%) and by using polyethylene glycol 400 as plasticizer (Cooksey, 2005).The first step of this work was the determination of the minimum inhibitory concentration of a *Listeria monocytogenes* strain (156 IU mL^{-1}). Only films obtained from film forming solutions containing nisin concentration well above the MIC have reduced significantly the number of bacteria in solution and in vacuum-packed hot dog. However, the author concluded also that nisin incorporation reduced its effectiveness compared to that of free nisin.

Nguyen et al., (2008) have proposed films containing nisin and based on bacterial cellulose produced by *Gluconacetobacter xylinus* K3 in corn steep liquor based medium containing mannitol. Activation of these cellulosic films with nisin reduced the *Listeria monocytogenes* number by 2 log CFU g^{-1} in frankfurters after 14 days storage (Nguyen et al., 2008). The antimicrobial activity of a combination of nisin and lacticin in cellulose films has been tested on cheese slices and ham. The resulting films reduced the cell number by more than 2 log for *Listeria innocua* in these two foods and nearly 1.5 log for *Staphylococcus aureus* in cheese and 2.8 log in ham (Scannell et al., 2000). Other cellulose based antimicrobial packages containing nisin were developed for use as inserts placed between portions of sliced products such as cheese. Scannel et al., (2000) have included films containing nisin between slices of Cheddar cheese packaged in modified atmosphere which has dramatically reduced populations of *Listeria innocua* and *Staphylococcus aureus*.

5.2.2. Starches

Starches are polymers of glucose units which, according to their source, differ by amylose (linear) and amylopectin (branched) proportions. Starch can be used to produce

biodegradable packaging films with an acceptable cost. However, some properties such as mechanical strength and water vapor permeability, must be improved before their use as food packaging. In particular, films based on rich amylose starch are flexible, oxygen impermeable, and heat-sealable (Gennadios et al., 1997). Sanjurjo et al. (2006) have prepared *tapioca* starch films plasticized with glycerol and containing nisin. After starch gelatinization at ~ 70-75 °C, nisin was added and the mixture was cast on a glass plate and dried at 50 °C. Obtained films have been shown to significantly inhibit *Listeria innocua* through the gradual release and despite nisin activity loss.

5.2.3. Alginate

Alginate is a linear copolymer composed of 1,4-ɓ-D-mannuronic acid and Ŭ-L-guluronic acid residues. Alginate jells in the presence of divalent cations like calcium and zinc as a result of an "egg box" structure formation. In fact, the reaction with divalent ions is so instantaneous that casting for films preparation is difficult. For these reasons, two-stage procedures have been developed to obtain alginate films by casting (Rhim, 2004). The first step is to spread and dry an alginate solution, followed by immersion in a calcium chloride solution or, alternatively, spraying the pre-formed film with a calcium solution.

Cutter and Siragusa (1996) observed that the direct nisin application at beef carcass surface can not protect it effectively against *Brochothrix thermosphacta* bacteria proliferation especially during long storage in spite of nisin stability at low temperatures. To improve the effectiveness of this treatment, the authors immobilized nisin in alginate gel. The control of nisin release reduced the *Brochothrix thermosphacta* by 3 log CFU cm^{-2} after 7 days of storage while the direct application of nisin solutions on carcass led to an increase of 2 log CFU cm^{-2} during

the same period. Incorporation of nisin in alginate gel was subsequently used to inhibit *Brochothrix thermosphacta* growth in ground beef (Cutter and Siragusa, 1997). Alginate has been also modified to produce palmitoylated alginate that was used to immobilize nisin and inhibit a *Staphylococcus aureus* strain that contaminates beefs (Millette et al., 2007). These hydrophobic and water vapor resistant films have been made from gel forming solutions containing 3% palmitoylated alginate, 1.2% polycaprolactone, 2% glycerol, and 0.5% whey protein isolate. Obtained gel forming solutions containing 500 or 1 000 IU mL^{-1} jelled in the presence of $CaCl_2$ and have reduced the tested *Staphylococcus aureus* population by 0.91 or 1.86 CFU cm^{-2}, respectively.

5.2.4. Chitosan

Chitosan is the most abundant natural polymer in nature after cellulose. It is produced by deacetylation of chitin, a major component of the exoskeleton of arthropods such as crustaceans and insects. Chitosan is a cationic polysaccharide having good film-forming and intrinsic antimicrobial properties (Sebti et al., 2005). This polymer has also been used as a carrier for incorporating nisin in order to improve more its antimicrobial activity (Pranoto et al., 2005). According to this study, only films containing a nisin concentration above 50 000 IU g^{-1} could significantly inhibit *Staphylococcus aureus*, *Listeria monocytogenes*, and *Bacillus cereus* while maintaining good mechanical and optical properties. However, the nisin incorporation did not improve the chitosan intrinsic antimicrobial properties against *Bacillus subtilis* (Vartiainen et al., 2004).

5.3. Protein based films containing nisin

Proteins ability to form films and coatings depends strongly on their molecular characteristics: molecular weight, conformation, flexibility, electrical properties (charge versus pH or ionic strength), and thermal stability. In general, the first step in film formation procedure is heat treatment in order to denature the protein, followed by casting and solvent evaporation. Protein based films generally have satisfying gas barrier and mechanical properties compared to polysaccharides based ones because proteins have a structure based on a combination of amino acids which gives a wider range functional properties. However, like most biopolymers, high water vapor permeability and moisture sensitivity are the main defects to be considered when proteins will be used to produce packaging films. These moderate functional properties of protein based films can be improved by using specific treatments such as irradiation with ɔ rays that cause cross-linking between protein chains (Ouattara et al., 2002). Ko et al., (2001) studied the correlation between protein based films surface hydrophobicity and antimicrobial activity of nisin incorporated into these films. Proteins with different hydrophobicities were tested: whey protein isolates, soy protein isolates, egg albumen, and wheat gluten. The authors concluded that films with high hydrophobicity values under acidic conditions exert the highest inhibitory effect against *Listeria monocytogenes*.

The combination of nisin with lysozyme and their incorporation in soy protein and corn zein films have led to significant lysis of *Lactobacillus Plantarum* cells (Padgett et al., 1998). In this study, addition of the chelator EDTA also allowed the inhibition of *Escherichia coli*. Comparison of film manufacturing techniques (casting or heat-press) shows a better release of the antimicrobials in the case of films obtained by casting. Indeed, cold cast films were more porous and more effective in inhibiting *Lactobacillus plantarum* and *Escherichia coli* on agar

gels. Dawson et al., (2002) have developed soy protein based films plasticized with glycerol and containing lauric acid (8 wt%) and a commercial preparation containing 2.5 wt% of pure nisin (4 wt%). Films were formed by press heating at 140 °C for 2.5 min. These films had reduced by more than 6 log *Listeria monocytogenes* cell number after 8 h contact. Hoffman et al., (2001) reported that the incorporation of lauric acid, EDTA and nisin in films made from corn zein can protect effectively against the growth of *Listeria monocytogenes* and *Salmonella enteritidis*. The incorporated lauric acid reduced the number of *Listeria monocytogenes* by 4 log after 48 h exposure, while the combination of the three antimicrobial molecules induced a decrease of more than 8 log the number of the same bacteria. Concerning *Salmonella enteritidis* bacteria, these films showed only a bacteriostatic activity.

Soy protein isolates based films containing nisin (10 000 IU g^{-1}) in combination with grape seed extract and EDTA have been developed (Sivarooban et al., 2008). These films were able to reduce *Listeria monocytogenes* population by 2.9 log CFU mL^{-1}, that of *Escherichia coli* by 1.8 log CFU mL^{-1} and that of *Salmonella typhimurium* by 0.6 log CFU mL^{-1}. The addition of these antimicrobial agents significantly altered thickness, mechanical, and optical properties of films. Moreover, interactions between nisin and soy protein amino acids can affect its retention and its release to the food matrix (Sivarooban et al., 2008).

Casting method was used to produce sodium caseinate based films containing nisin and plasticized with sorbitol (Kristo et al., 2008). Results showed that nisin incorporation does not alter water absorption capacity and water vapor permeability of caseinate films. Microbiological tests showed that these films are more effective in inhibiting *Listeria monocytogenes* than those containing potassium sorbate and sodium lactate. Cao-Hoang et al. (2010) have also obtained

sodium caseinate films plasticized with sorbitol. In this study a commercial nisin preparation (2.5 wt% pure nisin) was added to the film-forming solution before casting to obtain films containing 6.25 mg g^{-1} (or 1 000 IU cm^{-2}) of pure nisin. These antimicrobial films were tested in cheese inoculated with a *Listeria innocua* strain and results showed that film contact with cheese reduced the population of this strain by 1.1 log CFU g^{-1} after one week at 4 °C. This reduction was lower when cells are far from the cheese surface (depth inoculation).

In whey protein based films, the combined use of 50 IU mL^{-1} nisin with 3 % malic acid or citric acid exerted a synergistic antilisterial effect (Pintado et al., 2009). Probably, membrane pores opened by nisin helped acids penetrate the bacteria cell. Films based on whey protein isolate plasticized with sorbitol and containing nisin were also produced by casting (Rossi-Márquez et al., 2009). The authors justify the use of sorbitol instead of glycerol by the stability of mechanical properties of whey protein isolate /sorbitol films during storage. These films containing 473 IU cm^{-2} nisin have reduced the population of *Brochothrix thermosphacta* by 4 log on ham surface after incubation at 4 °C for 8 days. Concerning nisin diffusivity from whey protein isolate films, results showed that the release is favored at low pH, but it does not change significantly when temperature increases from 5 to 10 °C. These results were interpreted in terms of weak interactions between nisin and whey protein at low pH values due to their both electrostatic positive charges under acidic conditions.

The effect of the obtaining protein films method (cast or heat pressed films) and that of the nisin diffusion temperature (5-45 °C) were studied for films made from corn zein and wheat gluten (Teerakarn et al., 2002). Zein is a water insoluble prolamine protein consisting of roughly one-third hydrophilic (i.e., glutamine) and two-thirds hydrophobic amino acid residues, which is

closely related to its unique aqueous-alcohol solubility and film forming ability. For the cast method, proteins were mixed with an aqueous ethanol solution, glycerol, and glacial acetic acid (except for wheat gluten), and nisin powder (2.5% pure nisin) and then casted, and dried at room temperature for 24 h. For the heat press method, mixtures (protein, glycerol, and nisin) were pressed at 125 °C and 15 000 lb for 60 s (corn zein) and 140 °C and 20 000 lb for 90 s (wheat gluten). For these films, the authors found that nisin diffusion depends on temperature according to the Arrhenius model which proves the absence of morphological changes when temperature rises from 5 to 45 °C (Teerakarn et al., 2002). In another study (Dawson et al., 2003), it was demonstrated that nisin activity in protein films depends not only on the protein type, but also on the film forming method (casting or heat-pressing). According to this study, wheat gluten retains better nisin activity than corn zein and it was also shown that casting better protects nisin antimicrobial activity. However, this activity retention is low because the best obtained retention was for wheat gluten casted films and did not exceed 15.8% of the original activity.

5.4. Synthetic polymer based films containing nisin

Synthetic polymers or "plastics" are manufactured by reacting monomers or petrol derivatives under controlled conditions. Films based on synthetic polymers have good mechanical and barrier properties. The main polymers which have been used as antimictobial films are polyamide, polyethylene, polypropylene, and polyvinyl chloride.

5.4.1. Polyethylene

Polyethylene (PE) is an apolar polymer polymerized from ethylene repeating units whose mechanical properties are strongly dependent on the size and type of ramifications, crystallinity,

and molecular weight. Generally, PEs are classified according to density and branching degree into HDPE (high-density polyethylene), LDPE (low-density polyethylene) and LLDPE (linear low-density polyethylene). These polymers are not water soluble and films based on it are obtained by casting from chemical solvent solutions or by thermomechanical processing. Solvents, temperature, and shearing used during film production can seriously damage nisin activity, which explains the small number of articles on this topic.

Cutter et al., (2001) incorporated nisin in LDPE films containing polyethylene oxide (PEO) or EDTA to enhance antimicrobial activity against a *Brochothrix thermosphacta* strain. The authors used a single screw extruder at a temperature of 120-125 °C, a screw rotational speed of 20 rpm and a retention time in the extruder of about 7 min. Obtained films were used for beef vacuum packaging initially inoculated with 3.5 log CFU cm^{-2}. After 21 days storage at 4 °C, the bacteria number was reduced to 4.12 log CFU cm^{-2} for films containing EDTA and 0.30 log CFU cm^{-2} for films containing PEO. The authors explain these results by the fact that PEO polymer is hygroscopic, which accelerate nisin release from the film. Nisin was previously incorporated by the same research team in LDPE films to protect beef carcass against *Brochothrix thermosphacta* contamination (Siragusa et al., 1999). Results have showed that after 20 days storage, the meat microbial load was lower than that treated with a nisin solution. In this study nisin was incorporated in LDPE films by extrusion, and has not migrated into distilled water at room temperature, but it has migrated into a saline solution containing a surfactant, Tween 20. Also, heat treatment has also allowed the nisin release from the film (Siragusa et al., 1999). Thus, authors have concluded that nisin does not have chemical bonds with the film polymer. In a more recent study poly-ethylene-co-vinyl acetate films containing nisin were

prepared by a single screw extruder equipped with a film blowing unit (Nostro et al., 2010). Although the high temperature used during the extrusion process (120 °C), the prepared films were effective in reducing the biofilm forming ability of three *Listeria monocytogenes*, *Staphylococcus aureus*, and *Staphylococcus epidermidis* strains. Later, this same team showed that nisin can be effectively incorporated into poly-ethylene-co-vinyl acetate films at temperatures values higher than 160 °C (Scaffaro et al., 2011). They explained this thermal nisin stability by the short residence time (60 to 90 s). In addition to the antimicrobial activity preservation during the extrusion process, the authors showed in another work that nisin is able to stabilize the polymer film against photo-oxidative degradation by UV irradiation (Scaffaro et al., 2012). This protective effect of nisin was attributed to the progressive photo-oxidation of lanthionine groups and the presence of NaCl in the nisin used formulation.

5.4.2. Polypropylene

Polypropylene (PP) is a linear and semi-crystalline apolar polymer stiffer than PE. Jofré et al., (2008) evaluated the effectiveness of antimicrobial packaging films based on polypropylene and polyamide in combination with a high hydrostatic pressure treatment (400 MPa, 10 min, 17 °C) to control *Salmonella* sp. in sliced cooked ham inoculated with this bacteria. Compared to several antimicrobial agents, results showed that the pathogen agent elimination can be achieved by combining high pressure and nisin. Therefore, according to these authors, the use of an antimicrobial packaging containing nisin, high pressure, and chilled storage appear to be an effective combination for safe long term storage of foods like ready-to-eat products.

5.4.3. Polylactic acid

Polylactic acid (PLA) is a biodegradable semi-polar polymer made up of lactic acid chains and produced by starch fermentation and lactic acid condensation. PLA has good tensile strength, is suitable for oil rich foods, and is sealable at low temperatures (Krishnamurthy et al., 2004). The coating of meat pieces with film-forming solution containing 2% PLA, 2% lactic acid, and 400 IU mL^{-1} of nisin has reduced significantly *Listeria monocytogenes* population after storage at 4 °C for 42 days (Ariyapitipun et al., 2000). In another study, Jin and Zhang (2008) have prepared antimicrobial films as follows: PLA was mixed with a commercial nisin preparation (2.5 wt%) and then dispersed in methylene chloride. Films were obtained by casting and solvent evaporation. Films of 150 microns thickness containing 6.25 mg g^{-1} (which is equivalent to 0.04 mg cm^{-2}) of pure nisin were obtained. Microscopic observations showed that nisin particles (and particles of milk protein and sodium chloride) were distributed within the film but also on the surface. The authors suggested that surface nisin particles result, upon contact film/food, in a rapid release of the antimicrobial agent whereas nisin particles located inside will ensure slow release.

Jin (2010) has developed glass bottles coated with PLA films formulated with nisin for the preservation of skim milk and liquid egg white. This study showed that the coating substantially inactivates *Listeria monocytogenes* cells at 4 and 10 °C, which suggests that this strategy can be applied to various liquid food products. Manufacture of antimicrobial PLA based films containing nisin is limited by the high melting temperature of the polymer (~ 160 °C) because nisin is inactivated at temperatures above 120 °C. Liu et al. (2009) have proposed a simple method to produce PLA films containing active nisin after the extrusion step. In summary, the authors coextruded PLA with a plasticizer (lactic acid, lactide, or glycerol

triacetate) at 160 °C. After PLA melting, the mixture is cooled to 120 °C, nisin was added, and the extrusion is continued. The authors suggest that these films are significantly active against *Listeria monocytogenes*. During PLA extrusion, it was also demonstrated that pectin addition improves nisin thermostability and preserves its antimicrobial activity (Liu et al., 2009). The authors used atomic force microscopy to show that nisin forms a complex with pectin improving its thermal stability.

5.4.4. Polyvinylalcohol

Polyvinylalcohol (PVOH) is a polymer that was used to modulate nisin release kinetics by controlling the crosslinking degree in the film (Buonocore et al., 2003). *Micrococcus lysodeikticus*, *Alicyclobacillus acidoterrestris*, and *Saccharomyces cerevisiae* were used to test the antimicrobial effectiveness of released nisin. Results showed that nisin release kinetics can be correlated with the polymer crosslinking degree (Buonocore et al., 2004). In another study, antimicrobial packaging of fermented sausages with PVOH films containing nisin induced a pronounced reduction of *L. monocytogenes* counts during refrigerated storage for 90 days (Marcos et al., 2013).

5.4.5. Poly(butylene adipate-co-terephthalate)

Poly(butylene adipate-*co*-terephthalate) (PBAT) is an aliphatic-aromatic copolyester, which is completely biodegradable by microbial lipases (Herrera et al., 2002). Recently, antimicrobial films containing different nisin concentrations (0 ï 5 000 IU cm^{-2}) were obtained by casting a PBAT film forming chloroform solution containing Nisaplin® powder (Bastarrachea et al., 2010). Because solvent selection during films manufacture by casting is important for

maintaining the antimicrobial agent activity, it should be noted in this context that chloroform does not affect nisin activity. Indeed, during nisin extraction on an industrial scale, nisin is often recovered from culture media using chloroform without altering its antimicrobial activity (Burianek and Yousef, 2000). Nisin incorporation had not affect film elongation at break, but significantly altered the elastic modulus, tensile strength, and some thermal properties such as enthalpies of crystallization and melting and crystallization temperature. The environmental scanning electron microscopy highlighted the pores formation in PBAT films containing nisin. Despite these changes, the authors suggest that the oxygen permeability and water vapor permeability are not affected by nisin incorporation (Bastarrachea et al., 2010). Nisin diffusion rate from PBAT films to distilled water was higher compared to nisin diffusion from other tested films. The authors suggested that the proposed PBAT film can be used in a multilayer structure to better control the nisin release (Bastarrachea et al., 2010).

5.5. Polymer blend based films containing nisin

Cha et al. (2003) have developed films containing nisin and based on a mixture of polyethylene (PE), methylcellulose (MC), hydroxypropylmethyl cellulose (HPMC), and ə-carrageenan. The flat diffusion method was used for released nisin quantification using *Micrococcus luteus* ATCC 10240 strain as microorganism test. Results showed that films containing 5 wt% of MC had the highest antimicrobial activity which, according to the authors, could be due to differences in chemical interactions between nisin and biopolymers. Nisin was also incorporated into films formed from a film-forming solution containing a mixture of MC, HPMC, and polyethylene glycol (PEG) (Neetoo et al., 2008). Antimicrobial films were formed by casting and their antimicrobial activity was tested on samples of smoked salmon. Results

showed that films containing 2 000 IU cm^{-2} are capable of reducing *Listeria monocytogenes* population by 3.9 log CFU cm^{-2} after 56 days at 4 °C or 49 days at 10 °C. The authors also showed that film effectiveness depends significantly on the nisin incorporated amount. Ye et al. (2008) have developed antimicrobial films based on a mixture of chitosan and HPMC. The film-forming solution was prepared by mixing 13 parts of a chitosan solution (2%) and 2 parts of a HPMC solution (3%). After addition of several antimicrobial agents including nisin (500 IU cm^{-2}), films were obtained by coating a plastic film. In this work, the formulation for inhibiting *Listeria monocytogenes* growth on the surface of cold-smoked salmon stored under vacuum has been determined. This strategy was used also by the same research team to coat plastic films with a cellulose based carrier solution containing nisin at a concentration of 1 000 IU cm^{-2} (Neetoo et al., 2007). In this study, the authors showed that, by using the same coating solution containing cellulose and nisin, plastic film type (used as a support) did not have any effect on the antimicrobial activity of the nisin coated films.

Li et al., (2006) have developed mixed films of glucomannan and chitosan containing nisin (42 000 IU g^{-1}). These films showed good mechanical and physical properties but also good antimicrobial properties against *Staphylococcus aureus*, *Listeria monocytogenes*, and *Bacillus cereus*. Glucomannan was also used in combination with gellan gum to prepare antimicrobial films containing nisin (Xu et al., 2007). These films showed antimicrobial activity against *Staphylococcus aureus* that increased with increasing gellan gum content. The authors have highlighted the hydrogen interactions between the two polymers and concluded that the nisin release kinetics could be related to these interactions.

Extruded composite films based on PLA and pectin containing nisin (500 IU mg^{-1}) were obtained by Jin et al., (2009). After extrusion of the two polymers mixture, film activation was performed by immersion in a nisin solution at pH 2. After nisin diffusion in films, they are rinsed and dried. Microbiological tests showed that obtained films can reduce the *Listeria monocytogenes* number by 4.5 and 3.7 log mL^{-1} in liquid egg white and orange juice, respectively. In contrast, pectin addition caused a decrease in film mechanical properties. Pectin, a natural hygroscopic water soluble polymer was used in this work to facilitate nisin incorporation into the film and control its release when contacting the food matrix.

5.6. Multilayer films containing nisin

Antimicrobial peptides, like nisin, could be combined with Layer-by-layer assembly (LBL) to develop a new class of biodegradable, edible, and antimicrobial films, coatings, and related products (Guiga et al., 2009). Guiga et al. (2010) have developed multilayer films containing nisin and based on ethylcellulose / HPMC / ethyl cellulose (**Figure 2**) which showed significant antimicrobial activity. The authors suggested that multilayer films with non hydrosoluble outer layers, formed by ethylcellulose, could be a potential means of controlling nisin release from antimicrobial packaging. Indeed, the inner HPMC layer was used as a hydrophilic reservoir for nisin and the two outer layers of ethylcellulose as a diffusion barrier in direct contact with food. In the design of these multilayer films, it was necessary to use a surfactant that ensures adhesion between the different layers.

6. Evaluation of packaging films containing nisin

6.1. Antimicrobial properties

Antimicrobial activity of packaging films containing nisin can be measured by classic microbiological experiments. Food packaging can be tested on bacteria inoculated on the surface or in depth. Bacteriaôs counting after incubation allows determining the lag phase duration, growth rate during the exponential phase, and bacteria maximum number reached at the stationary phase. These indicators can then be compared with those obtained under the same conditions for a food unpackaged or packaged in a conventional packaging. Thus, reduction of microbial growth can be correlated with the food shelf life increasing and the improvement of its microbiological quality.

In addition to this method based on the direct application of a package containing nisin in a food model, other methods may be used. Indeed, the following two methods: (i) agar plate methods and (ii) minimally inhibitory concentrations (MIC) based ones are the commonly used strategies to evaluate the activity of free antimicrobial agents. The MIC method may for example be used to compare the antimicrobial activity of a film containing nisin to free nisin one. MIC method consists on seeding a series of tubes containing a culture medium with a target microorganism and adding film pieces containing various nisin concentrations. Tubes are then incubated for a given duration and microbial growth assessed using a spectrophotometer, for example (turbidity). When this method is used, results must mention some film properties (length, width, thickness, plasticizer used ...). **Table 4** gives some examples of nisin minimal inhibitory concentrations for common food contaminating bacteria that can help to formulate active films containing nisin.

Concerning the agar plate test, a piece of antimicrobial film with known dimensions is deposited on an agar solid medium containing the target microorganism. The medium is then

incubated at the microorganism optimum growth temperature until growth is visible. The nisin diffusion from the film to the medium causes a growth lack around the film (clear zone) (**Figure 3**). Typically, the inhibition zone diameter is directly proportional to the nisin amount migrated from film to food. This method simulates what can happen when the film containing nisin is in contact with contaminated food. The agar well diffusion method proposed by Barefoot and Klaenhammer (1983) was used by Cha et al., (2003) to find a relationship between nisin concentration and inhibition diameter. The following relationship was proposed for the inhibition of the strain *Micrococcus luteus* ATCC 10240:

$$Y = (X - 0.611)^{0.5000}$$

where X is log nisin units and Y is the inhibition diameter.

This test is usually applied as a preliminary step to determine whether nisin is available to act as antimicrobial in the film matrix. In such tests, diffusion depends only on the film structure and leads to a conclusion about the film ability to inhibit microbial growth.

Surface inoculation test is another test widely used to determine the number of bacteria on the surface of a film disc in contact with a semi-solid agar as a food product model. This test is useful to simulate surface contamination. Results may suggest what happens when microbial contamination occurs and gives an idea about the capacity of the barrier film to avoid external contamination. If tests in real conditions are desired, film containing nisin can be directly applied to the food surface, the effectiveness will be evaluated later by the count of inoculated or indigenous microbial population during the food storage (Martins et al., 2010).

6.2. Physicochemical properties

Nisin incorporation can affect other film characteristics such as water vapor and gas permeability, elasticity, optical and surface properties. These changes are to be considered in the development of antimicrobial films (Appendini and Hotchkiss, 2002). Several physical tests can be applied to characterize mechanical and barrier properties of antimicrobial films containing nisin. Tensile mechanical properties like Young's modulus, tensile strength, and elongation at break, as well as water vapor permeability are commonly reported (Guiga et al., 2010). More details about this method can be found elsewhere (Kristo et al., 2007). The film resistance to water, determined from water sorption isotherms, is an important parameter for film uses for packaging moist foods. Low water solubility is often desired except in the case of edible films that will be consumed at the same time as the packaged food. Film oxygen permeability can provide an idea on gas exchange that may occur between the packed food and the outside environment through the antimicrobial packaging. This oxygen permeability is often measured according to ASTM (1988). Since then, other methods have been proposed and that we can find the details in literature (Ayranci and Tunc, 2003). Oxygen permeability of packaging containing nisin is an important parameter to control because it can influence oxygen concentration in the space between food and package and promote aerobic bacteria growth. The following section gives some examples of physicochemical characterization of polymer films containing nisin.

It was shown that the interactions between nisin and proteins based films change in particular the mechanical properties and permeability to water vapor (Ko et al., 2001). On the other hand, nisin incorporation did not increase the thickness of films based on whey proteins, but the thickness of films based on soy protein and wheat gluten were increased by nisin addition. In another work, in films based on soy protein, increased puncture resistance was

observed when 2 050 IU nisin per g of proteins were added (Eswaranandam et al., 2004). In films made with corn zein, more than 12 000 IU mL^{-1} of the film forming solution caused a significant increase in tensile strength (Ku and Kyung, 2007). These results are consistent with those of Ko et al., (2001) for films based on whey protein isolate containing 6 000 IU of nisin per g of film. According to these authors, nisin caused a rearrangement of disulfide and hydrophobic bonds and increased protein-protein interactions which could be the cause of improved mechanical properties. However, in the case of soy protein isolate based films, nisin incorporation did not alter their tensile strength. According to Ko et al., (2001), this different behavior was related to the fact that soy proteins are less hydrophobic than whey ones and, therefore, nisin effect on the interactions between protein chains is low hence there are no significant differences in tensile strength with or without nisin. Papadokostaki et al., (1998) have highlighted the relationship between the polymer film structure and the active molecule release kinetic. For example, ɔ irradiation or heat, can increase the crosslinking degree of proteins in films and thereby improve their mechanical and functional properties. These structural changes also improve films ability to control the nisin release.

7. Conclusions

Incorporation of nisin in polymer films can enhance its antimicrobial activity in two main ways: (i) only the necessary nisin amount is used, and (ii) nisin is protected against the food constituents able to inactivate it. However, when developing films containing nisin, several factors must be considered: (i) manufacturing conditions (temperature, shear, solventé) should not alter the incorporated nisin activity, (ii) food properties (pH, water activity, storage temperature, physical state, ...) (iii) chemical interactions between nisin and film forming

polymers that have a large effect on nisin diffusivity, (iv) polymer hygrothermal properties (chain mobility, permeability, elongation, stiffness, ...) may be modified by nisin incorporation, and (v) also the production cost. On the other hand and whatever the method of making films containing nisin (by incorporation or coating), direct contact between the activated packaging and the packaged food is necessary for the system effectiveness and the nisin migration into the food matrix. Indeed, an intimate contact between food and packaging materials is required, and consequently the potential applications of these packages are intended especially for vacuum-packed foods and skin-packaged products such as meat, cheese, fish, ..

Particularly, for films made from synthetic plastics, nisin is added by adsorption on the surface because it is not always possible to directly incorporate it into the film matrix. Indeed, the use of extrusion or heat press leads to nisin thermal inactivation, while the use of the casting method requires selecting the correct volatile solvent that can dissolve the polymer without altering the nisin biological activity. For these reasons, nisin incorporation and release have been mainly studied in films based on water soluble biopolymers, mainly proteins and polysaccharides. Unfortunately, the use of biopolymer based films for food packaging is limited by their poor barrier properties and low mechanical properties. For this reason, natural polymers must be blended with synthetic ones and the current trend is to develop nano-composite polymers containing all the desired properties. The application of these hybrid systems could be a promising alternative for nisin incorporation and the control of its release. Studies on the activity loss during film manufacture by extrusion, the effect of plasticizers on nisin retention, and nisin effect on polymer films morphology will be interesting. More work on understanding

and modeling the kinetics of nisin release from films will be particularly important to develop effective antimicrobial films.

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Table 1. Different strategies for nisin immobilization and their limitations.

Nisin location	Immobilization strategy	Limitations
At the surface	Grafting	Chemical grafting to perform, no migration, and surface activity.
In the bulk	From a formulated aqueous for dispersion or solution	Fast release and surface pre-treatments apolar polymer are requested.
	Encapsulated in solid or gel beads which can be further added to food or food packaging.	Nisin stability with temperature/drying.
	Embedded in a monolayer film (added to a hydrosoluble polymer and casted or extruded)	Nisin stability with temperature, sensitivity to water.
	Multilayer packaging.	Nisin stability with temperature, sensitivity to water, and compatibilizing agents.

Table 2. Some examples of immobilized nisin uses in food applications

Food	Immobilization matrix	Target	References
Reconstituted Skim milk	Ca-alginate beads	*Lactobacillus curvatus*	Wan *et al.* (1997)
Ground beef	Ca-alginate gels	*Brochothrix Thermosphacta*	Cutter & Siragusa (1997)
Ground beef	Ca-alginate gels	*Escherichia coli*	Fang & Tsai (2003)
Beef slices or ground beef	Palmitoylated alginate beads/gels	*Staphylococcus aureus*	Millette *et al.* (2007)

Table 3. Polymer packaging containing nisin

Polymer	Concentration	Food/Simulant	References
Soy protein isolate, corn zein films	----	Culture media	Padgett *et al.* (1998)
Polylactic acid	400 IU mL^{-1} of PLA solution (2%)	Raw bee	Ariyapitipun *et al.* (2000)
Soy protein isolate or whey protein isolate	6 000 IU g^{-1} of dry film	Culture media	Ko *et al.* (2001)
Soy protein	40 000 IU g^{-1} of dry film	Turkey bologna	Dawson *et al.* (2002)
Corn zein	70 000 IU g^{-1} of dry film	Water	Teerakarn *et al.* (2002)
Cast corn zein	81 000 IU g^{-1} of dry film	Water	Dawson *et al.* (2003)
Cast wheat gluten	110 000 IU g^{-1} of dry film	Water	Dawson *et al.* (2003)
Heat-pressed corn zein	43 000 IU g^{-1} of dry film	Water	Dawson *et al.* (2003)
Heat-pressed wheat gluten	49 000 IU g^{-1} of dry film	Water	Dawson *et al.* (2003)
Methylcellulose	2 500-10 000 IU cm^{-2}	Culture media	Grower *et al.* (2004)
HPMC	2 500-10 000 IU cm^{-2}	Culture media	Grower *et al.* (2004)
Chitosan	51 000 IU g^{-1} of film	Culture media	Pranoto *et al.* (2005)
Glucomannan-Chitosan	42 000 IU g^{-1} of film	Culture media	Li *et al.* (2006)

Tapioca starch	881-2 204 IU cm^{-2}	Tryptic soy broths	Sanjurjo *et al.* (2006)
Polylactic acid	250 000 IU g^{-1} or 1600 IU cm^{-2} of film	Orange juice	Jin & Zhang (2008)
LDPE	2 000 IU cm^{-2}	Cold-smoked salmon	Neeto *et al.* (2008)
Soy protein isolate	10 000 IU g^{-1}	Culture media	Sivarooban *et al.* (2008)
Chitosan-coated plastic film	500 IU cm^{-2}	Cold-smoked salmon	Ye *et al.* (2008)
Pectin/Polylactic acid composite films	500 IU mg-1 of films	BHI media, orange juice, liquid egg white	Jin *et al.* (2009)
PBAT	1 000-5 000 IU cm^{-2}	Tryptic soy broth	Bastarrachea *et al.* (2010)
Sodium caseinate	1 000 IU cm^{-2}	Cheese	Cao-Hoang *et al.* (2010)

Table 4. Examples of nisin minimal inhibitory concentration (MIC) for common food contaminating bacteria

Bacteria	**MIC (IU mL^{-1})**	**Conditions**	**References**
Bacillus cereus	16	BHI medium, 21 °C	Abee *et al.* (1995)
Lactobacillus brevis	16	BHI medium, 21 °C	
Lactobacillus plantarum	16	BHI medium, 21 °C	
Brochothrix thermosphacta	0.4	BHI medium, 21 °C	
Pediococcus acidilactici	0.4	BHI medium, 21 °C	
Listeria innocua	32	BHI medium, 21 °C	
Listeria monocytogenes	16	BHI medium, 21 °C	
Listeria monocytogenes	32	BHI medium, 30 °C	
Bacillus cereus	32	Milk, 21 °C	
Listeria monocytogenes	16	Milk, 21 °C	
Listeria monocytogenes	48	Milk, 30 °C	
Lactococcus lactis	3.2	-----	Chan *et al.* (1996)
Micrococcus luteus	2.8	-----	
Listeria monocytogenes	156	Culture media	Cooksey (2005)

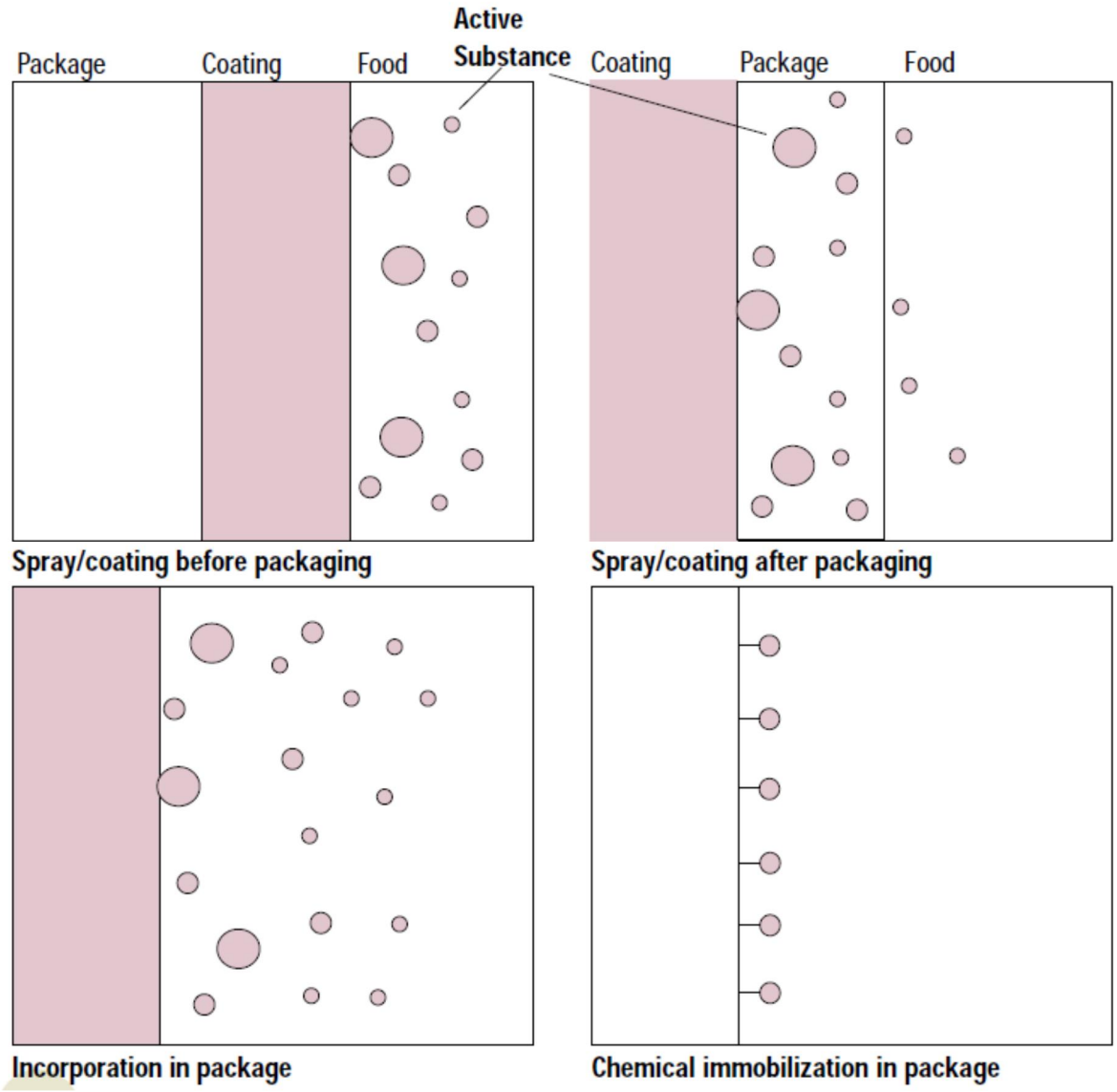

Figure 1. Strategies for the immobilization of active substances giving different antimicrobial packaging systems (Han, 2000).

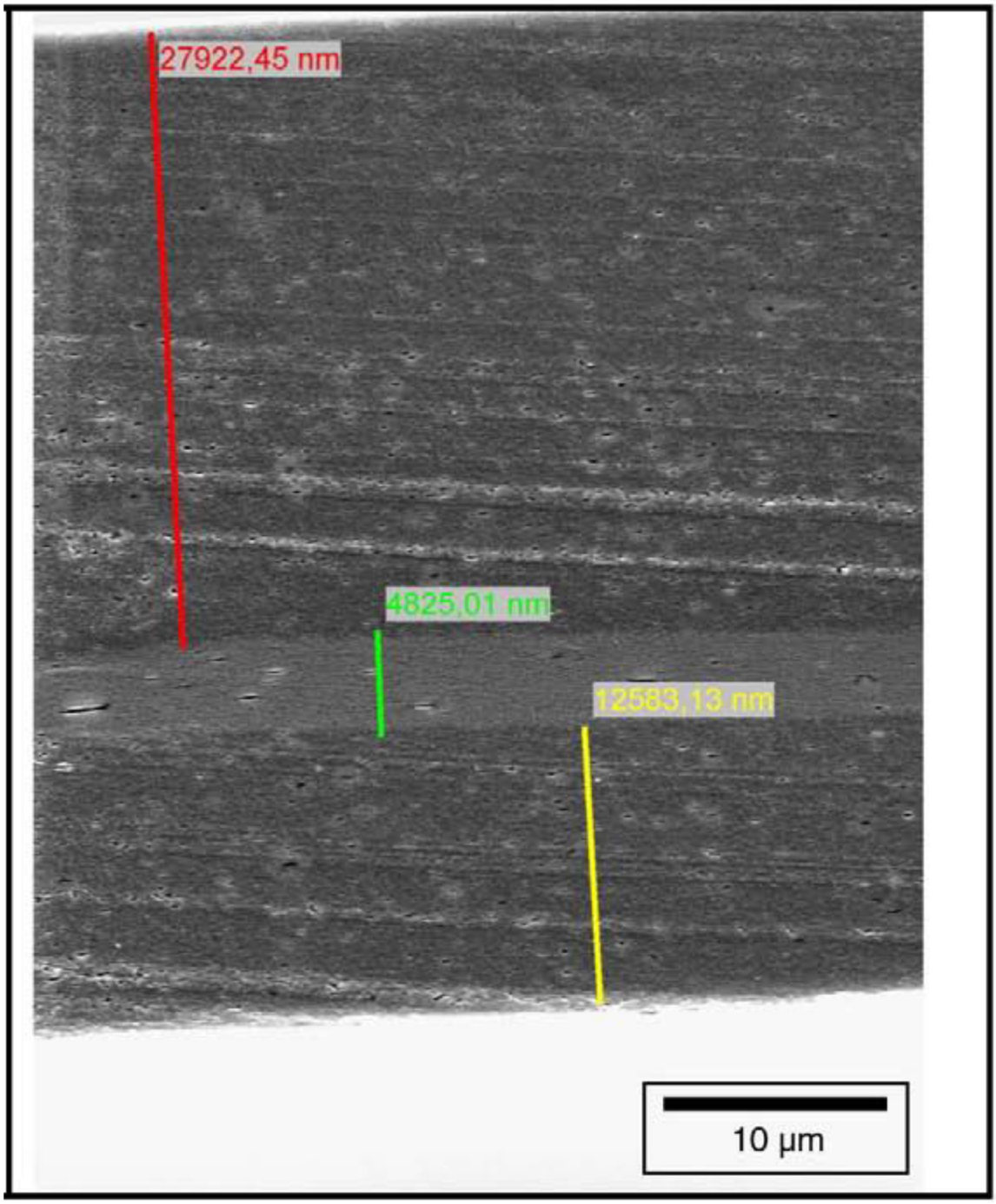

Figure 2. Observation of ethylcellulose / HPMC / ethyl cellulose three-layer films containing nisin by scanning electron microscopy (SEM) (Guiga *et al.* 2010).

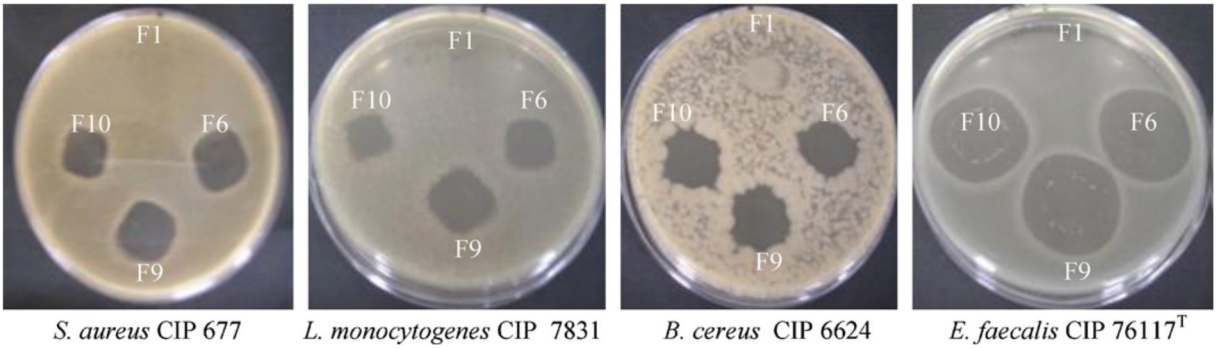

Figure 3. Inhibition zone of nisin-based active films against bacteria of food origin. (**F1** =HPMC film, **F6** =HPMC+ 10^4 IU Nisaplin®, **F9** =HPMC+ 30% glycerol + 10^4 IU Nisaplin®, **F10** =HPMC+ 50% glycerol + 10^4 IU Nisaplin®). (Nisaplin® is a commercial preparation of nisin) (Imran, El-Fahmy, Revol-Junelles, & Desobry, 2010).

