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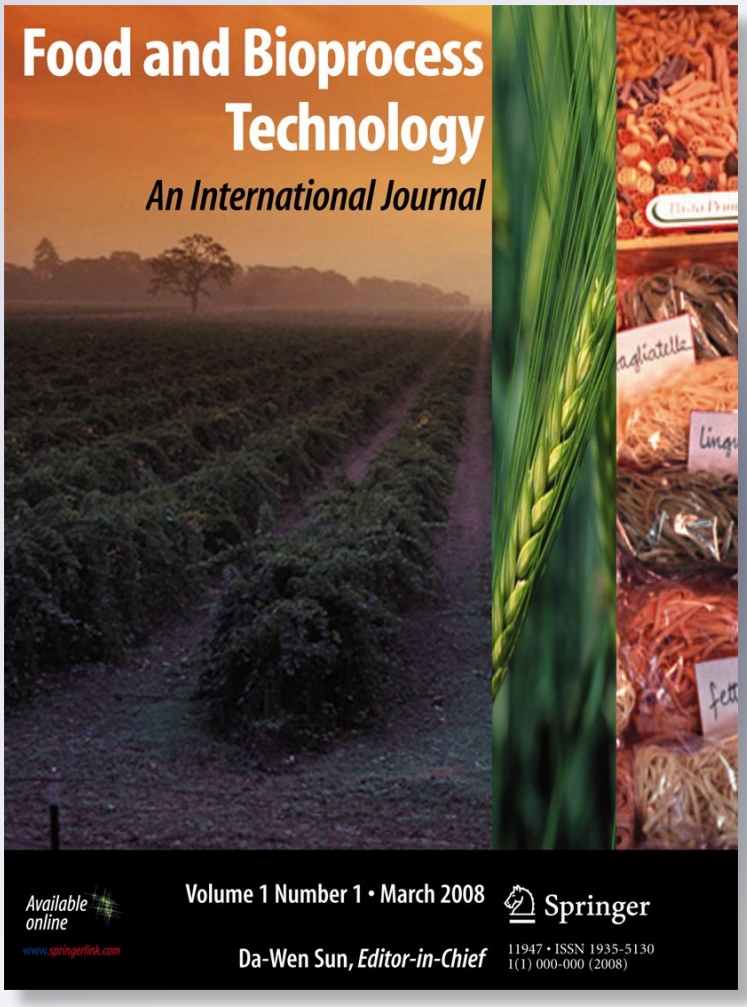

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REVIEW PAPER

Development of Edible Films and Coatings with Antimicrobial Activity

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Abstract Over the last years, considerable research has been conducted to develop and apply edible films and coatings made from a variety of agricultural commodities and/or wastes of food product industrialization. Such biopolymers include polysaccharides, proteins, and their blends. These materials present the possibility of being carriers of different additives, such as antimicrobial, antioxidant, nutraceuticals, and flavorings agents. In particular, the use of edibles films and coatings containing antimicrobials has demonstrated to be a useful tool as a stress factor to protect foodstuff against spoilage flora and to decrease the risk of pathogen growth. The more commonly antimicrobials used are organic acids, chitosan, nisin, the lactoperoxidase system, and some plant extracts and their essential oils. For the selection of an antimicrobial, it must be considered the effectiveness against the target microorganism and also the possible interactions among the antimicrobial, the film-forming biopolymer, and other food components present. These interactions can modify the antimicrobial activity and the characteristics of the film being these key factors for the development of antimicrobial films and coatings. The main objective of this article is to review the bibliography of the last years concerning the main hydrocolloids and antimicrobials used for developing edible films and coatings, the methods used to evaluate the antimicrobial activity, the applications and the legislation concerning edible films and coatings. Also, the different strategies related to the modification of structural characteristics and the future trends in the development are discussed. The information update will help to improve the design, development, and application of edible films and coatings tending to increase the safety and quality of food products and to prepare for food legislation changes that might be necessary while identifying future trends concerning a better functionality of edible films thought as a stress factor for lengthening shelf life of food products.

Keywords Edible films and coatings · Antimicrobials · Hydrocolloids · Preservation · Packaging · Antimicrobial activity

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Introduction

Packaging materials protect food from surrounding challenges. Changes in industrial procedures like the introduction of combined techniques for the obtention of medium- and high-moisture food products, the research on the application of emergent stress factors like high pressures, the development of convenient food products with longer shelf life, and the changing in retailing practices and/or in way of life have promoted the development of new and/or improved packaging materials. Consumer demands for more natural foods, and also for environmental protection, catalyzed, during past decades, the development of new packaging materials. Active, intelligent, and edible packaging is emerging factors of all this background.

Edible coatings and films do not pretend to replace traditional packaging materials but to provide an additional stress factor to be applied for food preservation; they can

also help to reduce the cost and also the amount of traditional packaging used. They can control moisture, gases, and lipid migration and can be supporters of additives and nutrients. For their formulation, there can be used polysaccharides, proteins, and lipids and they must result neutral with respect to color and flavor. An important component is the plasticizer which enhances flexibility and extensibility (McHugh and Krochta 1994). Edible films are intended to lengthen shelf life and also to respond to consumer demand for even more natural products and for the lower contamination of the environment.

Solvents primarily used for edible films production comprise water, ethanol, or a combination of both. The control of temperature during edible films and coatings elaboration is important because high temperatures enhance solvent evaporation during drying producing a structure not enough continuous and cohesive. Anyhow, if extrusion is the technique applied, high pressures and temperatures are common (Flores et al. 2010) and the process must be modulated to assure good barrier and mechanical properties for the film. Cohesiveness is related to chemical nature and structure of the polymer used, presence of additives like crosslinking agents and to ambient conditions during film formation. The increase of film structural cohesion results in a decrease of flexibility, porosity and permeability to gases, water vapor, and solutes.

Mainly during last decade, research concerning edible films and coatings as supporters of antimicrobials has increased. This application of edible films can be used to exert a highly localized functional effect without increasing excessively the global concentration of the additive in the food (Giannakopoulos and Guilbert 1986) or to produce the gradual liberation of the antimicrobial to the food giving origin to what is named technically as "active packaging" (Chang et al. 2000) or to protect the additive from the interaction with other food components or from ambient conditions which can promote its destruction or inactivation (Rojas-Graü et al. 2009). It is important to remark that film-forming conditions and film composition affect additive migration and, as a consequence, its effectiveness. Films have been studied as natamycin and potassium sorbate supporters (Flores et al. 2007a; Franssen et al. 2002), for the slow liberation of lysozime and nisin (Buonocore et al. 2003; Sanjurjo et al. 2006), for the development of films carrying sorbate or benzoate (Chen et al. 1996; Flores et al. 2007a); and for the slow liberation of propylparaben (Chung et al. 2001). The effect of polymer functionalization (Sousa et al. 2009), of characteristics of the food (Flores et al. 2007b), and of interaction between different film components (Vásconez et al. 2009) on antimicrobial liberation and effectiveness have been also studied. It is known that many natural substances, specifically phenolic structures like catechin, present in vegetal tissues, have antimicrobial activity (Araya et al. 2006) and the use and functionality of edible films as supporters of these substances have also begun to be explored (Ku et al. 2008).

Weiss et al. (2006) stated that a nanolaminate is a very thin film (1–100 nm) which can have different uses in food industry like, for example, the modulation of additives and nutrients which are supported on the film. According to Teixeira (2007), in the frame of thin films and coatings based in nanotechnology, nanoscale effects can be used to design systems with optimized or improved properties that are of ample interest for food, health, or biomedical industries. Although the legislation concerning these subjects is not fully developed, nanotechnology presents interesting possibilities in the area of food industry and particularly in edible film development. de Azeredo HMC (2009) published a very interesting review concerning studies of nanocomposites for food packaging applications but edibility was not a goal of the developments therein reported.

Marudova et al. (2005) studied the deposition of alternating layers of pectin and chitosan on a solid surface, observing the effect of pH on the adhesiveness and thickness and also the influence of the multilayered structure on film functionality. The effect of different film architectures (Guiga et al. 2010) on nisin activity has also been reported. The combination of polysaccharides and/or proteins with lipids in blends, emulsions, or multilayer structures have been also studied for controlling edible film properties (Phan The et al. 2002; Cho et al. 2002).

The main objective of this article is to review the bibliography of the last decade concerning the use of edible films and coatings as carriers of food antimicrobials. This information update will help to improve the safety and quality of food products, to prepare for food legislation changes that might be necessary and to identify future trends concerning a better functionality of edible films thought as a stress factor for lengthening shelf life of food products.

Hydrocolloids Used to Constitute Edible Films and Coatings

Elaboration of edible films and coatings has been possible thanks to the filmogenic capacity of natural biopolymers. Hydrocolloids have good aptitude to form a continuous and cohesive matrix with adequate mechanical properties (Bourtoom 2009; Bourtoom 2008). Such ability is related to the chemical structure of these compounds, which allows the association through hydrogen bonding of their polymeric chains. The literature reports that the most common biopolymers used for antimicrobial film elaboration are polysaccharides (single or blend of several types), proteins (single or mixtures from different sources), and blends of

carbohydrates and proteins. Although lipids such as waxes and fatty acids are mainly used to constitute edible coatings, they do not have a suitable stand-alone filmmaking nature. For this reason, lipids are often supported on a polysaccharide matrix to provide a film with mechanical strength (Bourtoom 2009). Lipids are incorporated to hydrocolloid-based films formulation to improve their water barrier characteristics or change their visual appearance (Karbowiak et al. 2007; Maftoonazad et al. 2007a).

Regarding methodology to obtain edible films, a very high number of papers used casting technique, being less reported other methods like high pressure, extrusion, spread coating, or coacervation (Flores et al. 2010). In a first step, the material must be properly dispersed and/or dissolved into a solvent like water, alcohol, diluted acids solutions, or mixtures of solvents. In some cases, it is necessary to heat or adjust the pH of the slurry containing the hydrocolloids in order to dissolve the macromolecule (Vargas et al. 2008). The addition of substances with plasticizing properties is, in general, a must to provide the films with good mechanical behavior in terms of flexibility. The plasticizer most used is glycerol because of its better stability and compatibility with hydrophilic biopolymeric chains in comparison with sorbitol, polyethylene glycol (PEG), and sugars (Fernández Cervera et al. 2004). Once the hydrocolloids were dispersed, it is possible to add other substances, like antimicrobials, antioxidants, flavorings, and colorants, to the film-forming solution in order to confer the desired functional property to the film or coating.

The removal of the solvent in excess is the following step. The drying rate and environmental conditions will determine the final thickness and structural characteristics of the resultant films. In these sense, a very-well controlled drying process should be performed.

As was previously mentioned, one of the most important characteristics of biopolymers is their ability to constitute a resistant network. As well, it is very desirable that films have selective barrier properties to several gases. It has been reported the very low oxygen permeability of edible films, but it is also known that hydrocolloid-based films possess high water vapor permeability (WVP; Buonocore et al. 2005). Many efforts have been made by the scientist to overcome this shortcoming. In general, lipid addition was the strategy selected for the majority of the researchers to reduce the water vapor transmission rate (Anker et al. 2001; Ayranci and Tunc 2003; García et al. 2000). Another possibility to reduce the interaction with water molecules is the modification of polymer structure by crosslinking reaction, photocrosslinking, gamma-irradiation, or reaction with polyvalent ions (Delville et al. 2003; Le Tien et al. 2000; Marques et al. 2006; Rhim 2004).

There can be found in literature different type of physical assays to characterize mechanical and barrier properties of edible films. In order to test edible films behavior as a packaging material, quasi-static tests applying big deformations (tension or puncture) are performed on the materials till breaking off. Mechanical parameters as Young modulus, tensile strength, and strain at break are commonly reported (Chillo et al. 2008; Lim et al. 2010). Another important property is the WVP of the films which is mainly determined in accordance with the ASTM E-96 static method. This standard method is recommended for thin sheet plastic materials and has been adapted for the new hydrophilic films (Flores et al. 2007a; Shen et al. 2010).

The resistance of films to water, determined by the solubility in water test, is critical for the potential application of films. Sometimes, high water solubility is desired. This is the case when the film or coating will be consumed simultaneously with the food. However, in other technological situations such as packaging application of films, a low solubility in water molecules is extremely necessary.

As a consequence of the poor water vapor resistance and lower mechanical strength in comparison with synthetic polymers, edible films have still limited application in food packaging.

Some recent examples of the use of hydrocolloids in edible films are resumed in Table 1. Mechanical parameters and WVP are included into the table for comparison. In the following, the most usual filmmaking materials and their most relevant characteristics are mentioned.

Polysaccharides

Polysaccharides render transparent and homogeneous edible films with moderate mechanical properties. However, the application of these films is limited by their water solubility and poor WVP. To solve this shortcoming, the blending with different biopolymers (Xu et al. 2005), the addition of hydrophobic materials such as oils or waxes (Anker et al. 2001; Ayranci and Tunc 2003; García et al. 2000), or chemical modification of polymer structure (Marques et al. 2006) have been proposed.

Cellulose and Derivatives

Cellulose is the structural material of plant cell walls and it is composed of linear chains of (1→4)-β-D-glucopyranosyl units. Chemical substitution of some hydroxyl groups along the chain gives origin to ionic (carboxymethylcellulose, CMC) and nonionic cellulose ethers (methylcellulose, MC; hydroxypropylcellulose, HPC; hydroxypropyl methylcellulose, HPMC). Cellulose derivatives films are tough, flexible, totally transparent, and highly sensible to water presence but resistant to fats and oils (Lin and Zhao 2007; Vargas et al. 2008). Crosslinking treatments can be used to

Table 1 Hydrocolloids used in edible films elaboration and films mechanical properties and water vapor permeability

Hydrocolloid	Concentration in the film-forming solution	Plasticizer	Mechanical and permeability properties			Reference
			Tensile strength (MPa)	Strain at break (%)	Water vapor permeability (g mm m^{-2} day^{-1} kPa^{-1})	
Cellulose ethers	HPC 3% *w/w*	–	16–18	60–110	0.043–0.056 (0/50–23 °C)[a]	Belalia et al. 2008
	HPMC 6% *w/v*	Glycerol	20–43	26–41	3.6–89.9 (0/100–38 °C)	Imran et al. 2010
	HPMC 1–6% *w/w*	PEG	3–86	1.9–60.9	4.1–13.7 (0/50–23 °C)	Sebti et al. 2007
	MC 1.5–6% *w/w*	PEG	17–44	14–97	2–10 (0/52–25 °C)	Turhan and Sahbaz 2004
Starches	Tapioca 5% *w/w*	Glycerol	0.16–2.3[b]	70	54–139 (0/70–25 °C)	Flores et al. 2007a
	Cassava 5% *w/w*	Sugars	1.0–4.7	39–164	1.5–7.2 (0/75–23 °C)	Kechichian et al. 2010
	Sweet potato 4% *w/w*	Glycerol	8–43	0.6–3.2	14–86 (0/75–23 °C)	Shen et al. 2010
Seaweed extracts	κ-Carrageenan 0.1-1% *w/w*	–	57	7	–	Lafargue et al. 2007
	Alginate 1% *w/w*	Glycerol	39–66	2.7–4.8	18.7–30.9	Pranoto et al. 2005b
	Alginate 1.5% *w/v*	Glycerol	23–160	2.2–32.2	7–14 (0/75–25 °C)	Zactiti and Kieckbusch 2005
Gums	Locus bean 0.9% *w/v*	PEG	4–40	0.5–10	–	Aydinli et al. 2004
	Gellan 0.5% *w/v*	Glycerol	–	–	18–23 (100/33–25 °C)	Tapia et al. 2007
Pectin	Pectin citric fruit 2.5–5.8 mg/cm^{2}[c]	–	13–25	0.8–1.2	1.6–4.7 (0/84–25 °C)	Giancone 2006
Chitosan	2% *w/v*	Glycerol	5.5–21.3	7–43	4.8–7.3 (0/75–25 °C)	Hosseini et al. 2009
	1% *w/w*	–	7–12	11–17	60–138 (100/59–5 °C)	Vargas et al. 2009
	1.5% *w/w*	–	18–106	5–20	14–80 (100/50–25 °C)	Zivanovic, et al. 2005
Proteins	Sodium caseinate 4% *w/w*	Sorbitol	2–77	2–130	85–564 (100/53–25 °C)	Kristo et al. 2008
	Sodium caseinate 5% *w/w*	Glycerol	3.4–4.1	78–125	–	Mendes de Souza et al. 2010
	Soy protein isolate 10% *w/w*	Glycerol	4.7–10.7	–	–	Sivarooban et al. 2008.
	Whey protein isolate 5% *w/w*	Sorbitol	4–16	1–10	204–264 (100/53–25 °C)	Zinoviadou et al. 2009
Blends	Whey protein/HPMC	Glycerol	4–61	16–112	–	Brindle and Krochta 2008
	Starch/chitosan	Glycerol	0.36–19.7	61–152	0.15–3.3 (0/70–25 °C)	Chillo et al. 2008
	Pectin/fish skin gelatin or soybean flour protein	Glycerol	17–99	1.7–6.4	98–226[d] (0/95–22 °C)	Liu et al. 2007
	Sago starch/sodium alginate	Glycerol	13–16	3.7–13.2	20.7–34.6 (0/52–30 °C)	Maizura et al. 2007

– data not specified

[a] Measurement conditions: relative humidity (RH%) on bottom and top sides of film and temperature (RH% bottom/RH% top—°C)

[b] Tensile strength at a deformation of 70%

[c] Concentration expressed on film weight

[d] Water vapor transmission rate (g m^{-2} day^{-1})

decrease the water solubility of cellulose ethers (Coma et al. 2003).

Sebti et al. (2007) prepared edible films based on HPMC incorporating or not nisin (250 μg/mL). HPMC films were transparent whereas nisin incorporation induced a twofold lightness parameter increase and, consequently, involved whiter films. Measurements of tensile strength, as well as ultimate elongation, showed that HPMC films were elastic and flexible. However, additive incorporation induced less elastic and more plastic films. According to Belalia et al. (2008), HPC-based films showed a plastic deformation, with 90% elongation at break (ε_r) and a tensile strength (σ_r) of 18 MPa, but films were totally soluble in water. Ayranci and Tunc (2003) determined the water vapor and CO_2 transmissions of MC-based edible films with varying amounts of the fatty acids, stearic acid, palmitic acid, and lauric acid; the results were compared with those obtained for a film without added fatty acid. In general, it was observed that WVP values decreased with increasing fatty acid content whereas CO_2 transmission parameters depended on the type of fatty acid incorporated. Turhan and Sahbaz (2004) studied the effect of film-forming solutions composition on the physical properties of the films. In these work, the WVP, σ_r, ε_r, adsorption capacity, and soluble matter (%) were investigated in MC films plasticized by PEG. The WVP of films was determined to be 0.232×10^{-10}–1.160×10^{-10} g/ms Pa, σ_r took values between 17 and 44 N/mm^2 and ε_r between 14% and 97%,

depending on composition. Film formation was affected by MC concentration, ethanol presence in the solution, and the presence of PEG. Incorporation of PEGs of increasing molecular weights to the polymer matrix increased both WVP and ε_r, and decreased σ_r. Increase in PEG400 concentration had similar effects. Solubility studies indicated that MC films were water-soluble and PEG containing samples had higher solubilities. Results suggest that mainly hydrogen bonding between PEG and MC or blocking effect in the case of high molecular weight PEGs determined the film properties. Moreira et al. (2009) analyzed the effect of a CMC coating during drying process on the quality of butternut squash slices and observed a slight improvement in weight loss and ascorbic acid retention in comparison with control samples without any coating. In addition, Wambura et al. (2008) analyzed the effects of a CMC-based edible coating containing rosemary and tea extracts on the reduction of lipid oxidative rancidity. Reduction in oxidation of 66.1% and 10.4% was observed for samples roasted and coated with CMC films formulated with extracts of rosemary and tea, respectively, as compared to uncoated sample.

Starches and Derivatives

Starch granules contain two types of polymeric molecules: amylose, a linear chain of (1→4)-α-D-glucopyranosyl units and amylopectin, a larger molecule which has a backbone of amylose and is highly branched with side units of D-glucopyranosyl linked by α-1,6-glycosidic bonds. Amylose has excellent film-forming ability rendering strong, isotropic, odorless, tasteless, and colorless films. By addition of an adequate plasticizer it is possible to obtain films with adequate mechanical properties, especially in films elaborated from high amylose starches. However, the film mechanical behavior could be affected by the tendency of starch systems to retrogradate, when double helices of amylose and amylopectin form a physically crosslinked network and starches-based materials become more rigid (Famá et al. 2006).

Flores et al. (2007a) analyzed the effect of different gelatinization and drying techniques on physical properties of tapioca starch edible films containing sorbates. Lower gelatinization and drying rates rendered films with the highest tensile stress, elastic modulus, and crystalline degree. On the other hand, faster gelatinization and drying reduced mechanical and water vapor barrier properties, since a more amorphous structure of film matrix were obtained. Sweet potato starch was also used to constitute antimicrobial and biodegradable films by incorporation of potassium sorbate or chitosan (Shen et al. 2010). It was reported that potassium sorbate incorporation retarded the crystallinity development of the films and that hydrogen bonds were formed between chitosan and starch, affecting in an opposite way the gas permeability and mechanical properties of sweet-starch-based films. In order to decrease the effect of water on film structure, Demirgöz et al. (2000) elaborated films based on the material obtained from the crosslinking of corn starch and cellulose acetate. The obtained films showed reduced ability to water sorption and slower degradation rate in aqueous media in relation to native starch films. Similarly, Marques et al. (2006) verified that films based on crosslinked cassava starch were less soluble in water (9–16%) than native starch films.

Seaweed Extracts

Alginates are the principal biomacromolecule extracted from brown seaweeds. They are sodium salts of alginic acid, which is a linear (1→4) linked polyuronic acid containing poly-β-D-manopyranosyluronic acid (M) blocks, poly-α-L-gulo pyranosyluronic acid (G) blocks and M–G blocks containing both polyuronic acids. Alginates form strong and quite brittle films with poor water resistance. However, alginates have a unique ability to react irreversibly with polyvalent metal cations, in particular calcium ions, to produce water insoluble polymers. Calcium has the aptitude to crosslink alginate by binding with G block; therefore, different proportions of G produce films with different water resistance (Olivas et al. 2008). In fact, the reaction with calcium ions is so instantaneous that casting to make films is difficult (Rhim 2004; Roger et al. 2006). For these reasons, two-steps procedures were developed for obtaining casted films. The first step consists in the casting of a partially dry alginate film solution, followed by an immersion into a calcium chloride solution or, alternatively, spraying the pre-formed film with the calcium solution (Carulo and Kieckbusch 2005; Pranoto et al. 2005b).

Zactiti and Kieckbusch (2005) examined the potassium sorbate permeability behavior in sodium alginate films crosslinked with different Ca^{2+} concentrations using a diffusion cell. Solutions of different potassium sorbate concentrations (150 to 1,050 mg/L) in contact with the films increased the permeability constant of the preservative, reflecting modifications of the film polymeric structure. An increase of the degree of crosslinking decreased the potassium sorbate permeability constant when the concentration of the crosslinking solution used was raised from 2% to 7%. Alginate films presented a drastic decrease in water solubility after crosslinking using calcium chloride solutions. The crosslinked Ca^{2+} films showed high tensile strength and low elongation. The authors recommended a careful control of the reticulation density in order to guarantee an adequate release of the active agent.

Carrageenans are extracted material from red seaweed. They are a complex mixture of several water-soluble

galactose polymers. There are three principal carrageenans fractions which differ in sulfate ester content and distribution of 3,6-anhydro-α-D-galactopyranosyl residues. The κ-Carrageenan is the one with less negative charges per disaccharide having excellent properties to form gel and films. When compared with λ- and ι-carrageenan, κ-carrageenan films exhibited the highest tensile strength (Seol et al. 2009). Edible films based on carrageenans are not commonly reported in literature. However, more information is available in relation with the application of carrageenans as a coating. Lafargue et al. (2007) studied the mechanical and calorimetric properties of κ-Carrageenan cast films and its mixtures with acid hydrolyzed hydroxypropylated pea starch. The results showed that film properties of the blends were inferior to those of the films with κ-Carrageenan alone. κ-Carrageenan films displayed a fragile behavior (rupture in the elastic domain) while starch films exhibited a ductile behavior (rupture in the plastic domain). Regarding coating application, the study of Bico et al. (2009) can be mentioned where fresh-cut bananas were coated with a 0.5% *w*/*w* carrageenan solution in combination with a chemical impregnation (0.5% ascorbic acid, 2% calcium chloride, and 0.75% cysteine) and controlled atmosphere (3% O_2 and 10% CO_2). The study suggested that dipping into chemical solution combined with carrageenan coating, plus storage under controlled atmosphere, could be a good method to preserve fresh-cut bananas for 5 days at 5 °C. In another work, the effect of carrageenan edible coatings (0.5 g/100 mL) in combination with antibrowning agents on minimally processed apple slices was studied during storage at 3 °C for 2 week (Lee et al. 2003). Edible coating was effective as semi-permeable barrier against air to control initial respiration rate of apple slices and, in combination with antibrowning agents, also showed positive sensory analysis results and beneficial reduction of microbial levels.

Gums

This polysaccharide family includes exudate gums (arabic, tragacanth, and karaya), seed gums (locust bean and guar), and microbial fermentation gums (xanthan and gellan). They have been studied as coating material or as an edible film component in combination with starches (Flores et al. 2010; Soares et al. 2005; Veiga-Santos et al. 2005). Chemical structure of gums is complex: some of them are anionic (i.e., xanthan gum) and on hydrolysis yield D-galacturonic acid, L-rhamnose, L-arabinose, D-galactose, D-xylose, L-fucose, D-mannose, or D-glucose.

The mechanical and light transmittance behavior of locust bean gum in relation with the amount and molecular weight of polyethylene glycol was examined by Aydinli et al. (2004). Haze increased while the luminous transmittance and total light transmittance values decreased with both the quantity and molecular weight of PEG.

Rojas-Graü et al. (2008) applied alginate and gellan-based edible coatings to extend the shelf life of fresh-cut Fuji apples packed in trays with a plastic film. The coating was successful for prolonging the shelf life of Fuji apple wedges by 2 weeks of storage compared with the control apple slices which showed shelf life of less than 4 days. In another study, Cerqueira et al. (2010) coated a commercial semi-hard cheese with a galactomannan coating and observed that the cheese shelf life was improved as the coating decreased the O_2 consumption and the CO_2 production rates, enhancing the weight and appearance. The authors finally remarked that coating can be used to incorporate natural preservatives to reduce post contamination.

Pectins

Pectic substances occur widely in land plants and are polymers mainly composed of (1→4) α-D-galactopyranosyluronic acid units naturally esterified with methanol. According to their content of methyl esters or degree of esterification (DE), pectins are divided in high-methoxyl (HM, DE>50%) or low-methoxyl (LM, DE<50%). The DE has a decisive effect on pectin solubility and gelation properties.

Literature related to antimicrobial films and coatings based on pectin substances is quite scarce. It can be mentioned the study of Giancone (2006) who elaborated and physically characterized films with increasing pectin content. Mechanical measurements demonstrated that films became stiff and not much flexible as pectin amount increased. In another recent work, the authors concluded that although the film structure was unaffected by pectin surface density (ρ_s), WVP increased with the rise in ρ_s while oxygen and carbon dioxide permeabilities decreased (Giancone et al. 2009).

Maftoonazad et al. (2007b) evaluated the protective effect of pectin-based edible emulsion coating on activity and disease severity of *Lasiodiplodia theobromae* in avocados. The results revealed that coated fruits sustained a significantly slower rate of disease spread and respiration rate. In addition, quality changes (texture and color) were much lower in coated fruits as compared with the control. Thus, the pectin-based coating was effective in controlling the spread and severity of stem end rot in avocados.

Chitosan

Chitosan is a natural carbohydrate polymer derived by deacetylation of chitin [poly-β-(1→4)-*N*-acetyl-D-glucosamine] which is a major component of the shells of crustacean such as crab, shrimp, and crawfish. It is a high

molecular weight cationic polysaccharide that exhibits antibacterial (Fernandez-Saiz et al. 2009; Zivanovic et al. 2005) and antifungal activity (Ziani et al. 2009) as well as film-forming properties (Arvanitoyannis 2008; Sebti et al. 2005). Numerous information has been reported about chitosan potential to act as a food preservative, function that was evaluated either on the basis of in vitro trials or through direct application of chitosan on real complex matrix foods (Durango et al. 2006; Han et al. 2004; Park et al. 2004; Ribeiro et al. 2007; Vásconez et al. 2009). Chitosan-based films have good mechanical properties and selective gas permeabilities (CO_2 and O_2). However, the high water vapor permeability limits their application. In order to overcome the poor water barrier properties, Vargas et al. (2009) prepared high molecular weight chitosan films and added different concentrations of oleic acid to film formulation. The authors observed that the higher the oleic acid content, the lower the WVP and the moisture sorption capacity. However, the addition of oleic acid into the chitosan matrix led to a significant decrease in the tensile strength, elongation at break, and elastic modulus of the composite films. The changes observed could be explained in terms of the film microstructure.

Due to the good film-forming capacity of chitosan, it was extensively used to protect, improve quality and extend the shelf life of fresh and processed foods. In these sense, single chitosan coating was successfully applied on silver carp (Fan et al. 2009) and ready-to-eat roast beef (Beverlya et al. 2008); chitosan coatings enriched with cinnamon oil retained the good quality characteristics and extended the shelf life during the refrigerated storage of rainbow trout (Ojagh et al. 2010); modified atmosphere packaging in combination with chitosan edible coating maintained quality and enhanced phenolic content in carrot sticks (Simões et al. 2009) and coatings based on high molecular weight chitosan alone (Han et al. 2005) or combined with oleic acid extended strawberry shelf life (Vargas et al. 2006).

Proteins

The ability of different proteins to form films and coatings is highly dependent on their molecular characteristics: molecular weight, conformations, electrical properties (charge vs pH), flexibilities, and thermal stabilities (Vargas et al. 2008). In general, film formation involves heat treatment to denature the protein, followed by solvent evaporation (casting). Protein-based films could have impressive gas barrier properties and mechanical properties compared with those prepared from polysaccharides and fat-based films, since proteins have a unique structure which confers a wider range of functional properties, especially a high intermolecular binding potential. However, the poor water vapor resistance limits their application. Fortunately, improvement of protein film properties could be attained by modifying the properties of protein by chemical and enzymatic methods, combining them with hydrophobic materials or some polymers, or using a physical method (Bourtoom 2009).

Milk proteins are some of the most common source of proteins used to obtain films and coatings. Casein and whey proteins, the main milk protein fractions (80% and 20%, respectively), have acquired particular interest since they can provide a high nutritional added value and good taste in addition to their barrier and filmogenic properties.

Kristo et al. (2008) physically characterized sodium caseinate films used as carriers of preservatives (sodium lactate, potassium sorbate, and nisin). The results clearly demonstrated that the antimicrobial agents, especially potassium sorbate, might substantially alter the thermo-mechanical and water vapor barrier properties of the films. Recently, Mendes de Souza et al. (2010) studied the behavior of lysozyme incorporated to a modified (using chemical or biochemical crosslinkers) sodium caseinate film. Results showed that active caseinate films modified by pH and glyoxal efficiently retarded the release of lysozyme, being a promising way to extend antimicrobial effects during food storage, enhancing food safety.

Antimicrobial films were prepared by incorporating different levels of oregano oil (0.5%, 1.0%, and 1.5% *w/w* in the film-forming solution) into sorbitol-plasticized WPI films (Zinoviadou et al. 2009). Results showed that the moisture uptake behavior and the WVP were not affected by the addition of oregano oil, however, a decrease of Young modulus and maximum tensile strength accompanied with an increase in elongation at break were observed with increasing oil concentration. In another work, Ozdemir and Floros (2008) proposed an optima combination of protein, sorbitol, beeswax, and potassium sorbate concentrations in whey protein films in terms of the most convenient WVP and organoleptic properties. The authors reported that beeswax was the most important factor influencing the stickiness and appearance of the films. Mixture proportions of protein= 0.53, sorbitol=0.38, beeswax=0.08, and potassium sorbate= 0.01 would yield an edible film with minimum stickiness, WVP$\leq$9 g mm m^{-2} h^{-1} kPa^{-1}, water solubility$\geq$39%, and appearance score$\geq$80.

The properties of edible films based on gelatin from different sources (bovine hides and tuna skins) and with oregano or rosemary extract addition were studied by Gómez-Estaca et al. (2009), with the goal of determining how inherent gelatin characteristics may affect interaction of the gelatin with the polyphenols. The conclusions were that the bovine-hide gelatin reacted only slightly with the polyphenols as shown by the electrophoretic profile. However, mechanical properties, water solubility, and

WVP were practically unchanged. On the other hand, the tuna skin gelatin did evidence some interactions with the polyphenols, decreasing deformability and increasing water solubility. Opacity increased irrespective of gelatin origin and plant extract type and concentration.

Blends

Edible films and coatings may consist of a blend of polysaccharides, protein, and/or lipids. The combination between polymers to form films could be from proteins and carbohydrates, proteins and lipids, and carbohydrates and lipids. This approach enables to utilize the distinct functional characteristics of each compound (Bourtoom 2008). According to literature, the main objective of producing films from biopolymer blends is to improve the permeability characteristics or mechanical properties as dictated by the need of a specific application.

The association among the polymers can be achieved through blending, extruding, laminating, or coating with other polymers with desirable properties. Blending is an easier and effective way to prepare associated polymeric materials (Zhong and Xia 2008).

The addition of another film-forming agent was used to improve the mechanical properties of chitosan-based films. Addition of polysaccharides such as tapioca starch (Vásconez et al. 2009), hydroxypropyl methylcellulose (de Moura et al. 2009) and proteins such as round scad (Artharn et al. 2009) or whey proteins (Ferreira et al. 2009) or gelatin (Arvanitoyannis et al. 1998) has been proposed with that purpose.

Starch-based edible films are widely proposed as a protective barrier for foods, but their application is limited by its high water solubility and brittleness. In order to overcome these shortcomings, Xu et al. (2005) blended waxy and regular corn starch with chitosan. It was observed that the blended films had increasing tensile strengths and elongation at break and decreasing water vapor transmission rates with increasing starch to chitosan ratios. In addition, the crystalline structure of chitosan was depressed and the amino group peak in IR spectrum of chitosan molecule shifted from 1,578 to 1,584 cm^{-1} with the incorporation of starch, suggesting that these two film-forming components were compatible and that an interaction existed between them.

In other studies, the individual and interactive effects of glycerol and chitosan on tapioca starch-based edible film properties were investigated (Chillo et al. 2008). Tests were run to determine film-forming solution apparent viscosity as well as mechanical properties and WVP of the films. It was observed that all film-forming solutions exhibited pseudoplastic behavior. From the mechanical point of view, chitosan had a positive effect on the films. With reference to WVP data, the chitosan addition improved the barrier property. It could be concluded that the concentration of chitosan led to changes in tapioca starch edible film properties, potentially affecting film performance. Similarly, Flores et al. (2010) analyzed the influence of different levels of potassium sorbate and xanthan gum on mechanical and antimicrobial properties of tapioca-starch-based films obtained by extrusion. The gum produced a reinforcing effect on the films and also enhanced solubility in water. The analysis also revealed significant interactions between components in the mixture and that potassium sorbate was available to act as an antimicrobial agent.

Another useful hydrocolloid mixture was proposed by Brindle and Krochta (2008). The authors blended WPI and HPMC using different combinations and at different conditions. The results indicated that while WPI:Gly and HPMC films were transparent, blend films were translucent, indicating some degree of immiscibility and/or WPI–HPMC aggregated domains in the blend films. The WPI:Gly-HPMC films were stronger than WPI:Gly films and more flexible and stretchable than HPMC films, with films becoming stiffer, stronger, and less stretchable as the concentration of HPMC increased. Overall, HPMC had no effect on oxygen permeability through the polymer network. WPI–HPMC blend films had a desirable combination of mechanical and oxygen barrier properties, as a result of hydrogen bonds, hydrophobic interactions, and disulfide bond crosslinking in the blended polymer network.

Blends of biopolymers can also be used in coating formulations. Rojas-Graü et al. (2008) analyzed the effect of alginate and gellan-based edible coatings on the shelf life of fresh-cut Fuji apples. The results suggested that the application of the edible coatings retarded the microbiological deterioration of fresh-cut apples and prolonged the shelf life of Fuji apple wedges by 2 weeks of storage compared with the control apple slices which showed a considerable cut surface browning and tissue softening from the very early days of storage, limiting their shelf life to less than 4 days.

Antimicrobials Used in Edible Films and Coatings

Many antimicrobials are proposed to be used in the formulation of edible films and coatings in order to inhibit the spoilage flora and to decrease the risk of pathogens. There is a trend to select the antimicrobials from natural sources and to use generally recognized as safe (GRAS) compounds so as to satisfy consumer demands for healthy foods, free of chemical additives (Devlieghere et al. 2004). The more commonly antimicrobials used are organic acids, the polysaccharide chitosan, some polypeptides as nisin, the lactoperoxidase system, and some plant extracts and its essential oils among others.

For the selection of an antimicrobial, it must be considered the effectiveness against the target microorganism and the possible interactions among the antimicrobial, the film-forming biopolymer, and others food components present. These interactions can modify the antimicrobial activity and the characteristics of the film being these key factors for the development of antimicrobial films and coatings.

Characteristics of the most common antimicrobial agents are briefly discussed in relation to their use in edible films and coatings.

Organic Acids

Organic acids such as lactic, acetic, malic, and citric acids, among others, are present in the composition of many foods and are widely used for preservation. The antimicrobial activity is based in pH reduction, disruption of substrate transport, and reduction of proton motive force.

Acidulants

The most common acidulant agents are acetic, lactic, and malic acids. They are obtained by fermentation and are effective against the main pathogen bacteria encountered in foods (Samelis and Sofos 2003).

Organic acids have been used as acidulants in edible films made from carbohydrate, proteins, and chitosan as can be seen in Table 2. Also they are used to enhance the antimicrobial activity of lipophilic acids.

Incorporation of organic acids can promote changes in film characteristics and physical properties such as thickness, puncture strength, and WVP. This trend is partially related to the fact that acids may exert a plasticizing effect on films since they possess hydroxyl groups that can take part in polymer–polymer interactions by developing hydrogen bonds (Cagri et al. 2001).

Changes induced by organic acids have been extensively studied in films using proteins as biopolymer (Eswaranandam et al. 2004; Kristo et al. 2008; Pintado et al. 2009; Zinoviadou et al. 2010).

In nisin–soy protein films, incorporation of organic acids modified the thickness of films and the effect depended on acid used and on its concentration. Tartaric and citric acids produced films with greater thickness than lactic and malic acids. This trend was related to the higher molecular weight of citric and tartaric compared with malic and lactic acids (Eswaranandam et al. 2004). Concentrations of acids within the range of 0.9% to 1.8% *w*/*w* produced films with greater thickness. The authors postulated that, at the levels mentioned, the aggregates form in the film-forming solution by the low net charge of proteins contributes to the greater thickness of films. Puncture strength of films is a useful parameter to evaluate the mechanical strength of the film, it is expected that films with higher strength will have greater mechanical properties. In nisin–soy protein-based films, organic acids modified puncture strength according to the nature of acid and its concentration. Lactic and malic acids increased puncture strength of films up to 1.8% *w*/*w* of acid. However, tartaric and citric acids decreased the puncture strength (Eswaranandam et al. 2004). Probably, the different effect of acids was related to the differential influence that they could exert on protein–protein interactions.

In whey-protein-based films containing 3% *w*/*w* of glycerol as plasticizer, the use of 1.5% or 3% of formic, acetic, and fumaric acids or 3% of citric acid produced films of extreme brittleness. In the case of using 1.5% of acetic acid, whey proteins precipitated since pH was close to isoelectric pH of proteins; as a consequence, gels formed were thick and could not form films (Pintado et al. 2009).

The type and concentration of organic acid modified the mechanical properties of films. In general, an increase in lactic or malic acid from 1.5% to 3.0% *w*/*w* promoted an increase in elongation and a decrease in tensile stress (Pintado et al. 2009).

Addition of sodium lactate (1.0–1.5% *w*/*w*) to whey protein isolate film-forming solution containing sorbitol as plasticizer increased water uptake and water vapor permeability of the films and decreased maximum tensile strength and Young modulus (Zinoviadou et al. 2010). Similar results were observed for the effect of sodium lactate in pectin, alginate, and caseinate films (Kristo et al. 2008; Parris et al. 1995).

From the point of view of the antimicrobial activity, organic acids exert different effects depending on the type of acid, its concentration, the systems composition, the environmental conditions, and the target microorganism. As a consequence, it is not possible to predict the inhibitory activity taking into account the data obtained from in vitro experiments using culture media. Some examples are commented so as to illustrate the different trends observed.

In whey-protein-based films, citric and malic acids at 3% *w*/*w* showed a greater inhibitory activity against *Listeria monocytogenes* than lactic acid at the same level (Pintado et al. 2009). According to the authors, the lower pK_a of citric and malic acids allowed a greater decrease in pH than did lactic acid.

In soy-protein-based films, addition of organic acids controlled the growth of *L. monocytogenes*, *Escherichia coli*, and *Salmonella gaminara.* Inclusion of lactic and malic acids at a 2.6% *w*/*w* decreased *S. gaminara* population by three log cycles; in contrast, citric acid caused a reduction of only one log cycle. This trend was linked with the fact that malic and lactic acids can easily enter into bacterial cells as a result of having a lower molecular weight than citric acid. Incorporation of nisin did

Table 2 Antimicrobials used in films and coatings showing antimicrobial activity in vitro studies

Antimicrobial and concentration in the film-forming solution	Hydrocolloid	Microorganism target	Assay performed	Effect observed on microorganisms	Reference
Acidulants					
Citric, lactic, malic, tartaric acids (0.9–1.8–2.6% *w/w*) in combination with nisin (205 IU/g of protein)	Soy protein	*L. monocytogenes*, *E. coli O157:H7*, *S. gaminara*	Film disk agar diffusion assay; total plate count of survivors	*L. monocytogenes* was inhibited by all acids *Salmonella* Malic and tartaric were more effective than citric *E. coli* Citric and tartaric were more effective than malic and lactic	Eswaranandam et al. 2004
Sodium lactate (10% to 40% *w/w*)	Sodium caseinate	*L. monocytogenes*	Plate count of mo population from inoculated agar systems in contact with antimicrobial films	A slight inhibition was observed by addition of 40% *w/w*	Kristo et al. 2008
Malic, citric, lactic acids (3% *w/w*)	Whey protein	*L. monocytogenes*	Film disk agar diffusion assay	Antimicrobial activity in increasing order: lactic < citric < malic	Pintado et al. 2009
Lipophilic acids					
Sorbic acid or paraminobenzoic acid (0.5–0.75–1.0–1.5 *w/v*) At pH 5.2 and 6.8	Whey protein isolate	*L. monocytogenes*, *E. coli O157:H7*, *S. typhimurium*	Film disk agar diffusion assay	*L. monocytogenes* and *E. coli* were inhibited for all levels of both antimicrobials *S. typhimurium*, 0.5% or 0.75% of sorbic acid did not inhibit some strains	Cagri et al. 2001
Potassium sorbate (5% *w/w*) or (1% to 2.5% *w/w*)	Xantham gum and tapioca starch	*Z. bailii*	Plate count of mo population from inoculated film disk in contact with agar plates	A microbiostactic effect was observed; xanthan gum exert a negative effect on inhibition	Flores et al. 2010
Potassium sorbate (15% *w/w*)	Alginate	Total aerobic bacteria	Plate count of microbial population in coated potato samples	A delay in microbial growth was observed	Mitrakas et al. 2008
Potassium sorbate (15% *w/w*)	Sweet potato starch	*E. coli*, *S. aureus*	Film disk agar diffusion assay	*E. coli* growth was inhibited by a 15% *w/w* KS *S. aureus* was not inhibited by KS	Shen et al. 2010
Potassium sorbate (0.05% *w/v*) and chitosan (1% *w/w*)	Tapioca starch	*Z. bailii*, *Lactobacillus* spp.	Plate count of microbial population from inoculated film disk in contact with agar plates	No inhibition of *Lactobacillus* spp. was observed *Z. bailii* population decreased 2 log cycles after 48 h	Vásconez et al. 2009
Nisin					
Nisin (10^4 IU/ml)	Methylcellulose, Hydroxypropyl-Cellulose, κ carragenan, Chitosan	*M. luteus*	Film disk agar diffusion assay	Chitosan-nisin (10.30 IU/g) films showed the strongest antimicrobial activity	Cha et al. 2003
Nisin (12,000 IU/ml)	Gelatin or corn zein	*L. monocytogenes*	Plate count of *Listeria monocytogenes* population from inoculated film disk	*L. monocytogenes* growth was reduced 1.4 log cycle for corn zein film and 0.6 log cycle for gelatin film	Kyoungju and Song 2007
Nisin (42×10^3 to 336×10^3 IU/g of film)	Chitosan–konjac glucomannan	*E. coli*, *S. aureus*, *L. monocytogenes*, *B. cereus*	Film disk agar diffusion assay	Nisin at 42×10^3 IU/g of film exerted antimicrobial activity against *S. aureus*, *L. monocytogenes*, and *B. cereus*	Li et al. 2006

Nisin (50 IU/ml) in combination with organic acids	Whey protein	*L. monocytogenes*	Film disk agar diffusion assay	A synergistic antimicrobial effect of nisin with malic or citric acid was observed	Pintado et al. 2009
Nisin (2,000–3,000–5,000 IU/ml)	Tapioca starch	*L. innocua*	Plate count of microbial population from inoculated film disk in contact with agar plates	*L. innocua* was reduced 4 log cycle during 240 min of contact with a film with 2,205 IU/cm^2	Sanjurjo et al. 2006
Lactoperoxidase system					
Lactoperoxidase system, (1–2–3–4% *w/v*)	Whey protein	*E. coli O157:H7*, *S. enterica*	Plate count of mo population from film disk in contact With surface inoculated agar plates; plate count of mo population from inoculated film disk in contact with agar plates	Both pathogens were inhibited in films	Min et al. 2005a
Lactoperoxidase system (11–17–23–29 mg/g film)[a]	Whey protein	*L. monocytogenes*	Plate count of mo population from film disk in contact With surface inoculated agar plates; plate count of mo population from inoculated film disk in contact with agar plates	*L. monocytogenes* population was significantly reduced	Min et al. 2005b
Lactoperoxidase system (0.1-0.5-1% *w/v*)	Whey protein	*P. commune*	Plate count of mo population from film disk in contact With surface inoculated agar plates; plate count of mo population from inoculated film disk in contact with agar plates	*P. commune* growth was inhibited	Min et al. 2005c
Lactoperoxidase system (0.2 to 0.6 mg/g)	Alginate	*E. coli*, *L. innocua*, *P. fluorescens*	Plate count of bacteria population from inoculated liquid media containing disk of film	Growth of all bacteria was prevented for 6 h using LPS and 0.4 mM H_2O_2 and 4 mM KSCN	Yener et al. 2009
Chitosan					
Chitosan (1% *w/v*)	Chitosan	*S. aureus* *L. monocytogenes* *P. aeruginosa*	Plate count of mo population from film disk in contact with surface inoculated agar plates	*S. aureus* and *L. monocytogenes* surface growth was inhibited by chitosan film; *P. aeruginosa* was not inhibited	Coma et al. 2003
Chitosan (1% *w/v*), thyme, clove and cinnamon essential oils (0.5–1–1.5% *w/v*)	Chitosan	*S. aureus*, *L. monocytogenes*, *P. aeruginosa*, *S. enteriditis*	Film disk agar diffusion assay	Chitosan alone did not inhibit the bacteria, thyme essential oil showed the highest antimicrobial efficacy	Hosseini et al. 2009
Chitosan (1% *w/v*), garlic oil (100 to 400 υ/g of chitosan), potassium sorbate (50 to 200 mg/g of chitosan), nisin (51 to 204 IU/g of chitosan)	Chitosan	*E. coli*, *S. aureus* *L. monocytogenes* *B. cereus* *S. typhimurium*	Film disk agar diffusion assay	Chitosan alone did not inhibit the growth of any bacteria Incorporation of garlic acid or potassium sorbate or inhibited the growth of *S. aureus*, *L. monocytogenes*, and *B. cereus*	Pranoto et al. 2005a
Chitosan (5–10–15% *w/w*)	Sweet potato starch	*E. coli* *S. aureus*	Film disk agar diffusion assay	*E. coli* and *S. aureus* growth was inhibited by chitosan	Shen et al. 2010
Chitosan (1% *w/v*)	Chitosan	*A. niger* *A. alternata* *R. oryzae*	Measurement of radial growth form film disk in contact with surface inoculated agar plate	Molds growth was decreased by films and coatings, effectiveness varied with the type of mold	Ziani et al. 2009

Table 2 (continued)

Antimicrobial and concentration in the film-forming solution	Hydrocolloid	Microorganism target	Assay performed	Effect observed on microorganisms	Reference
Essential oils					
Thyme, clove, and cinnamon essential oils (0.5, 1–1.5% *v*/*v*)	Chitosan	*L. monocytogenes*, *S. aureus*, *S. enteritidis*, *P. aeruginosa*	Film disk agar diffusion assay	Thyme essential oil revealed larger inhibition zones than clove and cinnamon for all bacteria tested	Hosseini, et al. 2009
Rosemary, oreganum, olive, capsicum, garlic, onion, and cranberry oleoresins (1% *w*/*w*)	Chitosan, carboxymethyl cellulose or casein	Squash native microflora (in vitro) *L. monocytogenes* (in vitro)	Film disk agar diffusion assay; mesophilic aerobic bacteria count of coated butternut squash slices	Olive and rosemary oleoresins had meaningful antimicrobial activity in in vitro test	Ponce et al. 2008
		Mesophilic aerobic bacteria (in vivo)		Chitosan coatings enriched with rosemary and olive oleoresins produce a slight antimicrobial effect	
Oregano oil/carvacrol; cinnamon oil/ cinnamaldehyde; and lemongrass oil/citral (0.1–0.5% *w*/*w*)	Alginate–apple puree	*E. coli* O157:H7	Bactericidal activities, BA_{50} values (film-forming solutions) film disk agar diffusion assay	Antimicrobial activities were in the following order: carvacrol > oregano > citral > lemongrass > cinnamaldehyde > cinnamon	Rojas-Graü et al. 2007
Oregano, rosemary and garlic essential oils (1%, 2%, 3%, and 4% *w*/*v*)	Whey protein isolate—Candelilla wax	*L. plantarum* *S. enteritidis* *E. coli* O157:H7 *L. monocytogenes* *S. aureus*	Film disk agar diffusion assay	Oregano oil was most effective at 2% level. Garlic oil was effective only at 3% and 4% level. Rosemary oil did not exhibit antimicrobial activity	Seydim and Sarikus 2007
Oregano oil (0.5–1.5% *w*/*w*)	Whey protein isolate	Native flora: total viable count Lactic acid bacteria *Pseudomonas* spp.	Count of native flora in appropriate media	Significant inhibition of spoilage flora by reducing the specific growth rate of the bacteria	Zinoviadou et al. 2009

[a] Antimicrobial concentration is expressed on film weight

not influence the susceptibility of *S. gaminara* to lactic acid but decreased the susceptibility to malic and tartaric acids (Eswaranandam et al. 2004).

Weak Lipophilic Acids

Weak lipophilic acids have the ability to penetrate the cell membrane when they are in the undissociated form; they act acidifying the cytoplasm and inhibiting the growth. Its antimicrobial action is enhanced by the addition of an acidulant which decreases the pH (Sofos 2000). Within the lipohilic acids, sorbic acid and its potassium salt are the most frequently used in edible films. The potassium salt (KS) and the acid, commonly named as sorbates are usually employed because of their high solubility in water (Sofos 2000). Sorbates inhibit the growth of bacteria, yeast and molds, being more effective against the latter. The antimicrobial action is more pronounced at pH below 5.0 and depends on system composition and environmental conditions. Sorbates suffer an oxidative degradation that depends on pH, a_w, presence of other additives, and conditions of storage and processing (Gerschenson et al. 1995)

Many films and coatings containing sorbates have been developed to inhibit the growth of yeast and bacteria and are intended to be used in different foods systems (Table 2).

Potassium sorbate presence changed the physical properties of films based in different biopolymers such as whey proteins (Cagri et al. 2001) or polysaccharides (Famá et al. 2005; Flores et al. 2007a; Shen et al. 2010; Vásconez et al. 2009).

In particular, the effect of potassium sorbate on physical and mechanical properties of tapioca starch has been evaluated. An increase in WVP was observed when sorbates or other hydrophilic compounds were added to films (Cagri et al. 2001; Flores et al. 2007a; Shen et al. 2010; Vásconez et al. 2009). Addition of 0.05% *w*/*w* of KS on chitosan–tapioca starch films increased WVP from 2.8×10^{-10} g/m Pa s to 6.7×10^{-10} g/m Pa s. Probably, electrostatic and/or hydrogen bonds between KS and chitosan might have prevailed over starch–chitosan interaction; as a consequence, the tapioca starch in KS–chitosan–starch films could have more free HO hydrophilic positions available to interact with water (Vásconez et al. 2009). The same trend was reported by Shen et al. (2010) for sweet potato starch films. In the case of whey protein films, addition of 1% (*w*/*w*) of KS increased the WVP by 40% (Cagri et al. 2001).

Solubility in water is of major importance since it could condition the uses of films in technological applications. Addition of KS to suspensions of tapioca starch–glycerol used for the preparation of films produced an increase of film solubility in water (Flores et al. 2007a). The same trend was observed for sweet potato starch films (Shen et al. 2010). According to Flores et al. (2007a), the presence of the preservative promoted the formation of a less organized starch network, as it was demonstrated by X-ray diffractograms.

In relation to mechanical properties, sorbates as well as other organic acids act as plasticizers and, as expected, they increase the elongation and decrease tensile strength. This behavior was observed in films based in tapioca starch, in sweet potato starch, and in whey protein (Cagri et al. 2001; Flores et al. 2010; 2007a; Shen et al. 2010). In the case of tapioca starch films, mechanical measurements made with the Dynamic Mechanical Thermal Analyzer, showed that sorbate incorporation promoted a 75% decrease in the storage modulus and a 200% increase in the tangent of the phase angle (tan δ) of the film pieces after 2 weeks of storage. Moreover, a continuous decrease in tan δ was observed along 8 weeks storage as well as an increase in the tendency to rupture with time. Aging of starch, which affects moisture content, characteristics of the network and sorbate destruction along storage, were responsible for the changes observed in mechanical properties of the film along 8 weeks. It must be remarked that films without sorbate showed an increase of tan δ along 4 weeks and they presented rupture. The decrease in elasticity and the increase in extensibility due to potassium sorbate presence might avoid chipping and cracking of the film during handling and storage (Famá et al. 2005). But, it must be taken into account that this plasticizer effect also increased gas, water vapor, and solute permeability of the film and could decrease cohesion, having a negative impact on film performance.

Tapioca starch films containing KS have been successfully used as a barrier to prevent external contamination with *Zygosaccharomyces bailii*, spoilage yeast very resistant to the action of preservatives, in model and food systems of high water activity and acid pH (Flores et al. 2007b; Vásconez et al. 2009; Garcia et al. 2008). Effectiveness of KS depended on system composition. Presence of other additives influenced preservative action. Flores et al. (2010) reported that the presence of xanthan gum in tapioca starch–glycerol films exerted a negative effect on potassium sorbate antimicrobial action. This trend was enhanced when the preservative was present in low concentrations.

Nisin

Nisin is a small antimicrobial peptide produced by lactic acid bacteria; it inhibits gram positive bacteria such as *L. monocytogenes* and *Staphylococcus aureus* and gram negative bacteria, when the bacteria cell wall was previously weakened by a permeabilising agent such as EDTA or lysozime. Nisin is generally recognized as safe and is permitted for use in over 50 countries (Delves-Broughton

2005). Its mode of action includes the inhibition of cell wall synthesis and the formation of pores in the cytoplasmic membrane. Nisin has been tested on meat and meat products, dairy foods, and vegetarian foods (Thomas et al. 2000). It has been used in films made by tapioca starch (Sanjurjo et al. 2006), whey protein (Pintado et al. 2009; Ko et al. 2001), sodium caseinate (Kristo et al. 2008), soy protein (Eswaranandam et al. 2004), methylcellulose, and hydroxypropyl methylcellulose (Cha et al. 2003; Coma et al. 2001), corn zein (Kyoungju and Song 2007); and glucomannan (Li et al. 2006). Some examples of uses of nisin in edible films and coatings are shown in Table 2.

Antimicrobial activity of nisin in films and coatings depends on system composition. In hydroxypropyl methylcellulose films, inclusion of stearic acid with the purpose of improving water vapor barrier reduced the inhibitory activity of nisin against *Listeria innocua* and *S. aureus*. This behavior was explained through the existence of electrostatic interactions between stearic acid and nisin that fixed the preservative to the film diminishing its effectiveness (Coma et al. 2001). In whey-protein-based films, the joint use of 50 IU/ml of nisin with 3% of malic or citric acids exerted a synergistic antilisterial effect. Probably, the pores opened in the membrane by nisin helped the acids to penetrate into the bacterial cell membrane. Also, the chelating activity exerted by the acids can improve the performance of nisin (Pintado et al. 2009). However, in the case of soy protein film, addition of 2,050 IU/ml of nisin to 2.6% of citric acid showed no effect on the antilisterial activity exerted by the acid (Eswaranandam et al. 2004).

The presence of nisin modified the physical properties of films. In the case of soy-protein-based films containing organic acids, an increase in puncture strength was observed when 2,050 IU of nisin/g of protein was added (Eswaranandam et al. 2004). In films made with corn zein, addition of 12,000 IU/ml to the film-forming solution caused an increase in tensile strength (Kyoungju and Song 2007). The same was reported by Ko et al. (2001) for films made with whey protein isolate by the incorporation of nisin (6,000 IU/g of film). According to these authors, nisin caused a rearrangement of disulfide and hydrophobic bonds or increased protein–protein interactions. Moreover, electrostatic interactions between nisin and protein molecules may also contribute to increase tensile strength. In soy protein isolate films, incorporation of nisin did not modified tensile strength. According to Ko et al. (2001), this differential behavior was related to the fact that soy proteins posses lower hydrophobicity than whey proteins, and as a consequence, the lower number of potential hydrophobic bounds between nisin and soy protein account for the lack of significant differences in tensile strength.

The effectiveness of nisin in different protein-based films depended on protein characteristics and on pH of the media. Ko et al. (2001) found that nisin in whey protein films was more effective in reducing *Listeria* growth than in wheat gluten films, suggesting that antilisterial activity of nisin was enhanced in hydrophobic films. Also, a greater inhibitory activity against *Listeria* was verified under acidic conditions. The commented results demonstrated that nisin effectiveness is strongly dependent on system characteristics and environmental conditions.

Lactoperoxidase

The lactoperoxidase system (LPS) is a natural antimicrobial present in milk and in mammalians saliva and tears. It presents a broad antimicrobial spectrum since it shows bactericidal effect on Gram (−) bacteria, bacteriostatic effect on Gram (+) bacteria, and antifungal activity (Naidu 2000). The LPS system consists of three components: LPS, thiocyanate, and hydrogen peroxide (H_2O_2). The enzyme catalyzes the oxidation of thiocyanate (SCN–) by the use of H_2O_2 and produces hypothiocyanite (OSCN–) and hypothiocyanous acid (HOSCN). These products inhibit microorganisms by the oxidation of sulphydryl (–SH) groups in their enzyme systems and proteins (Seifu et al. 2005).

Lactoperoxidase and its components have been used in whey protein films (Min et al. 2005a, Min et al. 2005b; Min et al. 2005c) and in alginate films (Yener et al. 2009). Applications of lactoperoxidase system in films and coatings are presented in Table 2.

In alginate films, the incorporation of the LPS system exerted some inhibitory effect on inoculated bacteria. The decreasing order of the resistance of bacteria to LPS system in this film was: *E. coli*, *L. innocua*, and *Pseudomonas fluorescens*. The extension of inhibitory action depended on activity of enzyme and on initial concentrations of H_2O_2 and KSCN (Yener et al. 2009).

In whey protein films and coatings, the incorporation of the LPS system effectively reduced the population of *L. monocytogenes* and inhibited the growth of aerobic microorganisms inoculated before or after the application of films and coatings in agar medium and in smoked salmon (Min et al. 2005b). Moreover, LPS showed strong bactericidal effects against *Salmonella enterica* and *E. coli O157:H7* when the cells were contacted with the LPS-WPI films, whether inoculation was on an agar medium before placement of the film or the inoculation was on the film itself (Min et al. 2005). These results suggest that films containing the LPS system can be used to decrease the risk of contamination with mentioned pathogenic bacteria.

From another point of view, the incorporation of the LPS to alginate films did not modify significantly the mechanical and barrier properties of evaluated films (Yener et al. 2009). However, in a whey protein film, incorporation of LPS promoted a significant reduction in elastic modulus

and in tensile strength when LPS concentration was equal to or higher than 0.15 g of LPS per gram of film (Min et al. 2005a).

Chitosan

Chitosan can be extracted from shell wastes with different deacetylation grades and molecular weights and, therefore, different functional properties and biological activities were observed (No et al. 2007). Its antimicrobial activity is linked to its positively charged amino group which interacts with negatively charged microbial cell membrane promoting an increase in their permeability and causing disruptions that lead to cell death (Ziani et al. 2009). It was demonstrated that chitosan inhibited the growth of many spoilage and pathogenic bacteria and also yeast and molds (No et al. 2007; Roller 2003). Antimicrobial activity depends on the type of chitosan, degree of acetylation, molecular weight, the target microorganism, the pH of the medium, and presence of other additives or food components (Aider 2010).

Examples of the uses of chitosan as a film-forming agent and also as antimicrobial are presented in Table 2.

Several authors have reported that effectiveness of chitosan depended on the application technique; in a coating solution it is more available to act as a preservative than when the preservative is forming the film (Vásconez et al. 2009; Zivanovic et al. 2005). Taking into account the mentioned trend, it is frequent the addition of another antimicrobial agent such as potassium sorbate, nisin, and essential oils, to enhance chitosan antimicrobial action (Hosseini et al. 2009; Pranoto et al. 2005a; Vásconez et al. 2009). Incorporation of other antimicrobials to chitosan films and coatings generally improved antimicrobial activity and also modified physical and mechanical properties of films. Hosseini et al. (2009) reported that addition of thyme, clove, and cinnamon essential oils to chitosan films, in general, inhibited the growth of *L. monocytogenes*, *S. aureus*, *Salmonella enteriditis*, and *Pseudomonas aeruginosa*. Thyme essential oil exhibited the greatest inhibitory action on tested bacteria. In essential-oil-free films, no inhibition of bacterial growth was observed suggesting that chitosan is incapable to diffuse through the agar and pointed out the necessary addition of other antimicrobial in the film. Essential oils also modified physical and mechanical properties of films. In the case of thyme and clove oils, scanning electronic microscopic images of films showed a surface and a cross-section covered with pores. Moreover, tensile strength values were decreased. These changes were attributed to the breakup of the network caused by the essential oils. Similar results were reported by Pranoto et al. (2005a) with chitosan-based films containing garlic acid. Moreover, incorporation of potassium sorbate or nisin also promoted a decrease in tensile strength of chitosan films (Pranoto et al. 2005a).

According to Buonocore et al. (2005) films based on chitosan have lower WVP than films based on others biopolymers such as alginates and casein. Incorporation of other antimicrobials generally increased WVP, probably as a result of extending intermolecular interactions promoting a loosening of the structure. This trend was observed for potassium sorbate addition into chitosan tapioca starch films (Vásconez et al. 2009) and for nisin and potassium sorbate into chitosan films (Pranoto et al. 2005a).

Antimicrobials from Herbs and Spices

Since ancient times, spices and herbs have been added to foods as seasoning additives due to their aromatic properties. In the development of antimicrobial edible films and coatings, essential oils from herbs and spices have been extensively used.

Essential oils (EOs**)** are aromatic oily liquids obtained from individual or integrated plant material: flowers, buds, seeds, leaves, twigs, bark, herbs, wood, fruits, and roots (Burt 2004). EOs are commonly obtained by steam distillation of plants. Chemical composition of EOs is complex and strongly dependent on the part of the plant considered (e.g., seed vs. leaves), the moment of the harvest (before, during, or after flowering), the harvesting season and the geographical sources. Major components in EOs are phenolic substances, which are thought as the responsible of the antimicrobial properties, and many of them are classified as GRAS. However, it has been reported that other minor components have a critical influence in the antimicrobial and antioxidant activity, acting synergistically with other components (Zheng et al. 2009). There is abundant scientific evidence in relation to the effectiveness of EOs fractions of many spices and herbs and their components as antimicrobial, antifungal, and antiviral compounds (Burt 2004; Dorman and Deans 2000; Holley and Patel 2005; Lozina et al. 2006; Moreira et al. 2005; Valero and Francés 2006). Examples of such plants are cassia, clove, garlic, sage, oregano, pimento, thyme, rosemary, lemongrass, scutellaria, and forsythia suspensa. The antimicrobial activity of the EOs can be attributed to their content of monoterpenes that, due to their lipophilic character, act by disrupting the integrity of microbial cytoplasmic membrane, which thus loses its high impermeability for protons and bigger ions. Lipophilic compounds accumulate in the lipid bilayer according to its specific partition coefficient, leading to disruption of the membrane structure (Zhang et al. 2009). Then, membrane functions are compromised, not only as a barrier but also as a matrix for enzymes and as an energy transducer (Liolios et al. 2009). Some disadvantages of EOs are their biological

and chemical instability, reduced solubility in water and poor distribution to target sites. In general, levels of EOs and their compounds necessary to inhibit microbial growth are higher in foods than in culture media. This is, in part, due to interactions between phenolic compounds and some components of the food matrix like proteins and fat. Contrarily, it was reported that the addition of carbohydrates seemed to have no effect on the inhibitory action of the EOs in broth cultures (Bagamboula et al. 2004). Therefore, the incorporation of EOs and oil compounds to edible film formulation can be a novel method in order to improve EOs stability and their bioavailability. At the same time, it can be achieved an enhancement of the antimicrobial applications of films. In these sense, Rojas-Graü et al. (2007) evaluated the antimicrobial activities against *E. coli* O157:H7 of several EOs (oregano, cinnamon, and lemongrass) and oil compounds (carvacrol, cinnamaldehyde, and citral) incorporated in alginate–apple puree edible film. Bactericidal activities, defined as the percent of test compound that kills 50% of the bacteria under the test conditions, were determined for film-forming solution and the data might be used as a guide to select appropriate levels of EOs in film formulation. In addition, the film disk agar diffusion assay was performed as a qualitative test for antimicrobial activity of the films. The results demonstrated that carvacrol exhibited the strongest antimicrobial activity being followed by oregano oil, citral, lemongrass oil, cinnamaldehyde, and cinnamon oil. On the other hand, the authors reported that the presence of plant essential oils did not significantly affect water vapor and oxygen permeabilities (around 5 g mm/kPa h m^2), but significantly reduced the elastic modulus of films (5.7 MPa vs 7.1 MPa in films without EOs). Regarding the performance of some EOs incorporated in edible films, it is important the study of their stability. Du et al. (2008a) determined the destruction of carvacrol, the main constituent of oregano oil, during the preparation and storage of apple-based films made by continuous and batch casting methods. According to HPLC analysis, the relative concentration of carvacrol in cast films increased by 2.2 and 3.2 times the initial concentration in the solution used to cast the films, and carvacrol in the films remained unchanged over a storage period of up to 7 weeks. Furthermore, WVP was reduced, tensile strength was slightly increased while elongation at break had no changes when carvacrol was present in film formulation. In the same work, it was reported that the optimal antimicrobial effects were observed with carvacrol levels of 1.0% added to the initial film preparation against *E. coli* determined by disk inhibition zone assay. In a similar study, Du et al. (2008b) used tomato-puree-based edible films and observed that carvacrol concentration and bactericidal effect remained unchanged over a storage period of up to 98 days at 5 and 25 °C. In this case, carvacrol addition to the tomato puree used to prepare the films increased WVP of films.

According to the literature, EOs and/or its compounds have been added to films and coatings formulations in order to protect different kinds of foods. Oregano oil was also incorporated into antimicrobial whey protein isolate based films which were used to wrap fresh beef samples (Zinoviadou et al. 2009). In order to establish the antimicrobial action against native flora (total viable count, lactic acid bacteria, and *Pseudomonas* spp.) of beef during storage (5 °C), the film was carefully removed and added in the ringer solution to wash off the bacteria that could be attached to its surface. The results showed that the maximum specific growth rate (μ_{max}) of total viable count and *Pseudomonas* were significantly reduced by a factor of two with the use of antimicrobial films (1.5% *w*/*w* oil in the film-forming solution), while the growth of lactic acid bacteria was completely inhibited. However, a decrease of Young modulus, maximum tensile strength, and an increase in elongation at break was observed, while WVP was not affected, by the addition of oregano oil.

Raybaudi-Massilia et al. (2008) studied the effect of malic acid and EOs of cinnamon, palmarosa, and lemongrass as natural antimicrobial substances incorporated into an alginate-based edible coating used for lengthening the shelf life and safety of fresh-cut melon (*Cucumis melo* L.) stored at 5 °C. On the other hand, melon pieces were inoculated with *Salmonella enteritidis* before applying the coating. Results suggested that edible coating was effective to improve shelf life of melon from the microbiological (up to 9.6 days) and physicochemical (>14 days) points of view in comparison with non-coated samples. In addition, the incorporation of EOs or their active compounds into the edible coating prolonged the microbiological shelf life by more than 21 days probably due to an enhanced antimicrobial effect of malic acid + EOs. Significant reductions of *S. enteritidis* population in inoculated coated fresh-cut melon were also achieved. In another study (Gomez-Estaca et al. 2007), oregano or rosemary essential oils incorporated to gelatin-based edible films, in combination with high pressure, showed to be useful for diminishing lipid oxidation and to reduce microbial growth in cold-smoked sardine during chilled storage (5 °C). In fact, neither luminescent bacteria nor *Enterobacteriaceae* were detected in any of the batches. Results could be ascribed to the migration of antioxidant/antimicrobial substances from the film to the muscle.

Methods Used to Evaluate Antmicrobial Activity in Edible Films and Coatings

Numerous studies have been performed to establish the effectiveness of antimicrobials in films and coatings. The

election of the method depends on the purpose of the assay, the nature of the antimicrobial and the characteristics of target microorganisms, among others.

The film disk agar diffusion assay consists of applying a film disk containing the antimicrobial on an inoculated agar plate and after incubation at specific conditions, the diameter of the zone where no growth occurred is measured. This test is generally applied as a screening step to test if the preservative is available to act as antimicrobial in the film matrix (Eswaranandam et al. 2004; Min et al. 2005, a,Min et al. 2005b and Min et al. 2005c; Pintado et al. 2009; Sanjurjo et al. 2006). In this assay, diffusion of antimicrobials from the film disk depends on the size, shape, and polarity of the diffusing molecule, as well as the chemical structure of the film (Cagri et al. 2001). This test is an end point assay and gives information of the ability of the antimicrobial incorporated in the film to inhibit microbial growth at a prefixed time.

The enumeration by plate count of microbial population, at selected times, from inoculated surface agar plates in contact with film disk containing the antimicrobial is a test useful to model wrapping of foods and obtained results may suggest what can happen when the film enters in contact with a contaminated surface (Coma et al. 2003; Kristo et al. 2008; Min et al. 2005a, Min et al. 2005b and Min et al. 2005c)

The film surface inoculation test is another assay frequently performed and consists in the enumeration by plate count of the microbial population inoculated on the surface of a film disk in contact with a semisolid media such as agar that model a certain food product. This assay is useful to simulate surface contamination. Results obtained may suggest what happens when microbial contamination occurs on coatings or films in contact with food and gives an idea of the barrier ability of the film to prevent an external contamination (Flores et al. 2007b; Sanjurjo et al. 2006; Vásconez et al. 2009).

Mentioned methods have been applied in literature for in vitro evaluation of antimicrobial film performance. When the film or coating is applied on the food, the effectiveness is evaluated through the enumeration of indigenous or inoculated microbial population during food storage (Martins et al. 2010; Mitrakas et al. 2008; Moreira et al. 2009; Seol et al. 2009).

It must be remarked that the release of the preservative from a film or coating exerts a great influence on its effectiveness. For some applications, a quick release of the antimicrobial is required to control microbial growth in the food. For the contrary, in other applications, a slow release is required so as to assure a certain level of the preservative at the surface to control the external contamination. The determination of the rate of release together with the evaluation of antimicrobial activity through the time might help to optimize the development of films and coatings as a potential active packaging material.

Applications of Edible Films and Coatings to Foods

Antimicrobial edible films and coatings are used for improving the shelf life of food products without impairing consumer acceptability. They are designed as a stress factor in minimally processed foods in order to prevent surface contamination while providing a gradual release of the active substance (Buonocore et al. 2003).

Antimicrobials alone never provide the "magic bullet" for the prevention of food spoilage and poisoning and it is generally accepted that combination of preservatives with another stress factor is a way to improve food safety. According to this trend, application of edible films and coatings containing antimicrobials is usually made together with other preservation factors in order to improve the quality of food products such as fruits and vegetables, meats, seafoods, and cheese.

Fruits and Vegetables

Fruit and vegetable tissues may remain biologically active, suffering many physiological changes from post-harvest, during storage, and until they are consumed or processed. In addition, operations like washed, sorting, trimming, peeling, slicing, coring, etc., are usually carried out on these products, promoting cell tissue disruption and membrane collapse. Because of their characteristics, fresh or minimally processed fruits and vegetables are very perishable and required of some combined techniques to extend properly the shelf life (Ponce et al. 2008; Robson et al. 2008).

Edible films and coatings have long been known to protect perishable fruits and vegetables from deterioration by retarding dehydration, suppressing respiration, improving textural quality, helping to retain volatile flavor compounds, and reducing microbial growth (Han et al. 2004; Lin and Zhao 2007; Maftoonazad et al. 2007). In addition, they can be used as a vehicle for incorporating functional ingredients, such as antioxidants, flavor, colors, antimicrobial agents, and nutraceuticals (Bifani et al. 2007; Garcia et al. 2008). Some of the functions previously cited are related to their property of being a barrier to moisture loss and having selective gas permeability (Vargas et al. 2008).

The technology for using edible films and coating as carriers of additives to extend the shelf life has been widely explored. Garcia et al. (2008) wrapped pumpkin cylinders with tapioca starch edible films containing potassium sorbate and demonstrated that films could act as a physical barrier to exclude the entrance of microorganisms, provided

a source of preservative available to prevent microbial growth at the surface, and, at the same time, controlled spoilage flora in the pumpkin tissue, since part of the antimicrobial was released to the food. Chien et al. (2007) used chitosan-based coating to improve the quality of mango slices and verified the effectiveness of coating for the inhibition of mesophilic aerobic bacteria. Durango et al. (2006) evaluated the application of a starch–chitosan coating on minimally processed carrots and observed a substantial inhibition of total viable count, lactic acid bacteria, psicrotrophic total coliforms, and yeasts and molds. Edible films have also demonstrated their effectiveness for the organoleptic and nutritional preservation of fresh vegetables. Ayranci and Tunc (2003) reported that methylcellulose coating containing ascorbic, citric, and stearic acids lowered the browning rate and the reduction of vitamin C in mushrooms and cauliflower.

Table 3 summarizes relevant applications of an antimicrobial external edible films or coatings to prevent microbial spoilage.

Meat and Meat Products

Minimally processed ready to eat meats are a potential source of food-borne pathogens such as *Salmonella typhimurium*, *L. monocytogenes*, and *E. coli* O157:H7. Contamination with pathogens may occur during further processing or packaging. It was reported that most outbreaks of contamination are associated with the consumption of meat products (Coma 2008). Edible films and coatings carrying antimicrobials are a promising tool for decreasing the risk of pathogenic bacteria and also for extending product shelf life. In Table 4, some examples of the use of antimicrobial films and coatings are presented. In meat products, application of a coating or film not only is useful as a carrier of the antimicrobial but it can also prevent moisture loss during storage of fresh or frozen meats, hold juices of fresh meat cuts when packed in plastic trays, reduce the rate of rancidity, and restrict volatile flavor loss and the uptake of foreign odors (Quintavalla and Vicini 2002).

Seafood and Seafood Products

The quality of seafood is quickly reduced during storage being chemical and enzymatic reactions the cause of the initial loss of freshness, while microbial spoilage produces the end of the shelf life. For these reasons, the main objective of using edible films and coatings in seafood is to prevent the contamination with spoilage flora. In many cases the target inhibition is *L. monocytogenes* growth which constitutes the major risk in freshly processed cold-smoked salmon (Datta et al. 2008; Ye et al. 2008). On the other hand, another important objective is to avoid oxidative spoilage, in the case of fat specimens, by the use of antioxidant agents, and also to prevent moisture loss (Gomez-Estaca et al. 2007). The most relevant edible films and coating systems developed for preserving seafood are presented in Table 5.

Cheese

Cheese is a complex food product that contains mainly casein, fat, and water. In the case of fresh and semi-hard cheese, microbial stability must be controlled. Edible films and coatings are used mainly to control microbial growth in the surfaces and also to diminish the risk of post-processing contamination with *L. monocytogenes*. Also, the coating or film applied must be able to let gas exchange with the environment in order to maintain cheese quality. Applications of antimicrobial edible films and coatings to cheese are shown in Table 6.

Modification of Structural Characteristics of Edible Films and Coatings

Concerning structural characteristics of edible films, different trends can be observed in the most recent literature. In this sense, Karbowiak et al. (2010) suggested as strategy for obtaining controlled release of antimicrobials within the medium or the product, the use of encapsulation agents like ι-carrageenan while lipids are used for controlling the water barrier.

Guiga et al. (2010) designed nisin-loaded multilayer films based on ethylcellulose/hydroxypropyl methylcellulose/ethylcellulose which showed significant antimicrobial activity. They informed that multilayer films with hydrophobic layers, like those constituted by ethylcellulose, could be a potential way to control nisin release from antimicrobial biopackagings. It is important to remark that ethylcellulose is approved for use in the European Union (EU).

Debeaufort et al. (2000), Cho et al. (2002), and Kristo et al. (2006, 2007) studied the effect of a bilayer structure on sorption, permeability, and thermal properties observing the benefits of the combination of different polysaccharides or protein/polysaccharides or the introduction of lipids and their organization in multilayers on the physicochemical properties.

de Moura et al. (2009) prepared chitosan nanoparticles and incorporated them in a hydroxypropyl methylcellulose matrix. The chitosan nanoparticles tended to occupy the empty spaces in the pores of the HPMC matrix, increasing the collapse of the pores and thereby improving film tensile properties and WVP. The thermal stability of the films increased with addition of nanoparticles. Chang et al. (2010) obtained nanoparticles of about 50–100 nm from chitin. Glycerol-

Table 3 Application of antimicrobial edible films and coatings to improve the quality of fruits and vegetables

Hydrocolloid	Antimicrobial	Fruit/ vegetable	Effect	Reference
Starch/chitosan	Chitosan	Carrot slices	Inhibition of total viable count, lactic acid bacteria, psicrotrophic total coliforms and yeast and mold during storage at 10 °C	Durango et al. 2006
Cassava starch	Potassium sorbate	Pumpkin cylinders	Aerobic mesophiles, lactic acid bacteria, yeasts, and molds growth was prevented	Garcia et al. 2008
Starch or MC/HPMC	Propolis extract	Fresh noodle	Total microorganism count was reduced during 4 weeks at 10 °C	Kim et al. 2005
Alginate	Potassium sorbate	Potato cylinders	Initial microbial load was decrease during refrigerated storage at 5 °C	Mitrakas et al. 2008
Chitosan Casein CMC	Chitosan	Butternut squash	Coating reduced the counts of mesophilic aerobic bacteria	Moreira et al. 2009
Chitosan, carboxymethyl cellulose, and casein	Natural plant extracts	Butternut	Coatings enriched with rosemary and olive oleoresins produced a slight antimicrobial effect against native microflora and *Listeria monocytogenes*	Ponce et al. 2008
Alginate	Cinnamon, palmarosa, and lemongrass	Fresh-cut melon	Native flora growth and *S. enteritidis* population was reduced extending shelf life by more than 21 days	Raybaudi-Massilia et al. 2008
Agar-agar	Chitosan and acetic acid	Garlic	Filamentous fungi and aerobic mesophilic were inhibited during 6 days storage, at 25 °C	Robson et al. 2008
Chitosan	Chitosan	Carrots slices	Native microbial populations were maintained very low	Simões et al. 2009
Hydroxypropyl methylcellulose-lipid	Organic acid salts, parabens	Mandarins	Antifungal action of the coatings was fungistatic rather than fungicidal	Valencia-Chamorro et al. 2009
Chitosan/cassava starch/ gelatin	Chitosan	Mango slices	Inhibition of *Botryodiplodia theobromae* was reduced on fruit surface was observed	Zhong and Xia 2008

plasticized potato starch was combined with nanoparticles to prepare all-natural nanocomposites by casting and evaporation. At low loading levels, there was a good interaction between the filler and matrix, which led to improvements in tensile strength, storage modulus, glass transition temperature, and water vapor barrier properties of the composites.

With the object of obtaining more diverse functionalities of edible films, from 2000 and on, (Averous et al. 2001; Carvalho et al. 2003; Curvelo et al. 2001; Famá et al. 2010) the change in mechanical properties (storage modulus and tension to rupture) of edible films through the inclusion of fibers in the matrix is reported in literature. Mastromatteo et al. (2008) studied the individual and interactive effects of spelt and wheat bran, on the properties of wheat gluten-based edible films. In general, mechanical properties enhanced with bran presence. Yellow Index and b parameter of Hunter scale increased with the bran concentration, whereas the L values decreased.

Table 4 Application of antimicrobial edible films and coatings to improve the quality of meat products

Hydrocolloid	Antimicrobial	Meat product	Effect	Reference
Chitosan	Chitosan	Ready to eat roast beef	*Listeria monocytogenes* growth was controlled by a chitosan coating	Beverlya et al. 2008
Hsian-tsao leaf gum	Green tea extract	Pork slices	*Listeria monocytogenes* and *Staphylococcus aureus* growth was prevented	Chiu et al. 2010
κ-Carragenann	Ovotransferrin EDTA Potassium sorbate	Chicken breast	Total aerobic count decreased by the use of a coating containing ovotransferrin and EDTA	Seol et al. 2009
Soy protein	Nisin Grape seed extract Green tea extract	Turkey frankfurter	*Listeria monocytogenes* growth was prevented	Theivendran et al. 2006
Whey protein	Sodium lactate Polylisine	Fresh beef	Lactic acid bacteria, *Pseudomonas*, and total aerobic bacteria growth was prevented	Zinoviadou et al. 2010

Table 5 Application of antimicrobial edible films and coatings to improve the quality of seafood products

Hydrocolloid	Antimicrobial	Seafood product	Effect	Reference
Calcium alginate	Oyster and hen lisozyme, nisin	Smoked salmon	Microbial growth was delayed	Datta et al. 2008
Chitosan	Chitosan	Sliver carp	Total aerobic mesophiles counts decreased and shelf life was extended to 30 days during frozen storage	Fan et al. 2009
Gelatine, gelatin–chitosan	Oregano and rosemary extracts	Cold-smoked sardine process by high pressure	Microbial growth and lipid oxidation was decreased	Gomez-Estaca et al. 2007
Chitosan	Chitosan	Herring cod	Reduced lipid oxidation, and microbial growth was observed. Moisture loss was prevented	Jeon et al. 2002
Whey protein	Lactoperoxidase system	Cold-smoked salmon	*Listeria monocytogenes* growth was prevented	Min et al. 2005, b
Chitosan	Cinnamon oil	Rainbow trout	Successful inhibition of lipid oxidation and microbial growth was obtained, shelf life was extended to 12 days at 4 °C	Ojagh et al. 2010
Soy and whey protein, carboxy-methyl cellulose	Thyme oil, cynamaldehyde	Cooked shrimp	Microbial growth was delayed	Ouattara et al. 2001
Chitosan, chitosan–starch	Chitosan	Salmon	Microbial growth of aerobic mesophiles and pscrophiles decreased and global quality was extended to 6 days at 2 °C	Vásconez et al. 2009

Legislation Related to Edible Films and Coatings

Edible films supporting antimicrobials can be considered as an active film. Definitions stated in Regulation 1935/2004/EC and in Regulation 450/2009/EC consider that "active materials and articles are intended to extend the shelf life or to maintain or improve the condition of packaged food". They are designed to deliberately incorporate components that would release or absorb substances into or from the packaged food or the environment surrounding the food (Restuccia et al. 2010).

According to legislation and labeling in the USA, edible coatings and films are considered part of the food; as a consequence, their ingredients must comply with the Code of Federal Regulations and be declared on the label under the Federal Food, Drug, and Cosmetic Act (Franssen and Krochta 2003). The EU considers that an edible film is a special active part of the food and, seen from a legal point of view, it is to be regarded as a foodstuff, along with the food packed in the film, having to fulfill the general requirements for food (Fabec et al. 2000). According to Rojas-Graü et al. (2009), another important topic within regulatory status is the presence of allergens because many edible films and coatings are made with or can contain ingredients that could cause allergic reactions such as wheat protein (gluten) or peanut protein. Therefore, the presence of a known allergen on a film or coating on a food must be also clearly stated in the label.

Table 6 Application of antimicrobial edible films and coatings to improve the quality of cheese

Hydrocolloid	Antimicrobial	Cheese	Effect	Reference
Chitosan Galactomannan Agar Corn oil	Chitosan	Semi-hard	Mold growth was prevented and water evaporation was decreased	Cerqueira et al. 2009
Chitosan Galactomannan	Chitosan	Semi-hard regional	Water loss and microbial counts were decreased	Cerqueira et al. 2010
Chitosan	Chitosan	Emmenthal	*Pseudomonas aeruginosa* lag phase was increase and maximum population at the stationary phase was decreased	Coma, Sebti et al. 2003
Galactomannan	Nisin	Ricotta	Growth of *Listeria monocytogenes* was prevented for 7 days at 4 °C	Martins et al. 2010
Casein	Natamycin	Kashar	Mold growth was suppressed for one month	Yildirim et al. 2006

On the other hand, each country has clear regulations regarding the addition of preservatives to food, which often include purity requirements, analytical methodology, labeling, and maximum allowed levels. Therefore, at the moment, under such legislation must be ruled the application of edible films containing preservatives. As a consequence, it is important to remark that the edible film formulation proposed must be adapted in order to ensure a content of preservative in the food that is in accordance with maximum values allowed by food legislation of the country of application.

Economical Aspects Related to Edible Films and Coatings

Edible films and coatings can have an important impact on:

- The agriculture sector by reducing the post-harvest losses
- Industrialized food products making necessary less expensive packaging materials

All these can lower food prices helping to boost exportations.

Anyhow, for an adequate evaluation of the incidence of edible films and coatings on food cost, the following factors must be taken into account:

- The relation cost/benefit.
- The cost of the food product to which it is applied. Depending on this, the value added to the food product through the improvement of its quality or the lengthening of shelf life will be recognized or not by the market.
- The decrease in pollution that the use of the edible film or coating might produce. Damage of environment has a hidden expenditure that must not be overseen.

Of course, to reduce costs, it is recommended to use:

- Hydrocolloids that constitute underused materials
- Macromolecules obtained from left over of the food industry in the country where the use of edible films and coatings is being evaluated

Conclusions

Antimicrobial edible films and coatings are used for improving the shelf life of food products without impairing consumer acceptability. They are designed as a stress factor in order to prevent surface contamination and/or providing a gradual release of the active substance.

The edible film formulation proposed must be adapted in order to ensure a content of preservative in the food that is in accordance with maximum values allowed by food legislation of the country of application.

Characteristics of edible films depend greatly on hydrocolloid used. Polysaccharides rend transparent and homogeneous edible films with moderate mechanical properties. However, the application of these films is limited by their water solubility and poor water vapor permeability. Protein-based films could have impressive gas barrier properties and mechanical properties compared with those prepared from polysaccharides; however, the poor water vapor resistance limits their application. To solve this shortcoming, the blending with different biopolymers, the addition of hydrophobic materials such as oils or waxes or chemical and/or enzymatic modification of polymer can be performed.

Many antimicrobials are proposed to be used in the formulation of edible films, in order to inhibit the spoilage flora and to decrease the risk of pathogens. There is a trend to select the antimicrobials from natural sources and to use generally recognized as safe (GRAS) compounds so as to satisfy consumer demands for healthy foods free of chemical additives. The more commonly antimicrobials used are organic acids, the polysaccharide chitosan, some polypeptides as nisin, the lactoperoxidase system, and some plant extracts and its essential oils among others.

It must be remarked that the release of the preservative from a film or coating exerts a great influence on its effectiveness. As a consequence, the evaluation of the rate of release together with the evaluation of antimicrobial activity through the time will help to optimize the development of films and coatings for lengthening the shelf life of food products.

Future Trends

Edible films and coatings can be considered an additional stress factor for preserving food products, assuring its quality as well as a prolonged shelf life. When they are used for supporting antimicrobials, the stability, the concentration on the surface of the product, the bioavailability, and the gradual release are all important characteristics of the preservative related to its functionality, being prevalent the one that most conceals with the use proposed for the film or coating.

A challenge for the use of edible films and coatings is their compatibility with other emergent stress factors like high pressures, electric fields, ultrasound, microwave radiation, and gamma radiation.

Modifications that can be produced in edible film structure without endangering the safety of the food product might help to achieve target purposes. In this sense:

- The crosslinking of the polymer
- The generation of composites with different fillers

- The use of polysaccharide/protein blends
- The use of hydrophilic biopolymers blended with lipids
- The generation of a multilayer structure

might contribute to minimal degradation and/or low diffusion and/or gradual release and/or adequate bioavailability of the antimicrobial.

Information nowadays about all these topics is abundant but not systematic because researchers have a great variety of objectives when designing their experiments and not all of them are working for the production of edible films and coatings as supporters of antimicrobials. A deeper insight concerning this subject is needed to elucidate the magnitude of the ratio benefit/cost and not only from a financial point of view.

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