Comprehensive REVIEWS in Food Science and Food Safety

Natural Antimicrobial Edible Coatings for Microbial Safety and Food Quality Enhancement

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Abstract: Natural antimicrobial agents have been investigated as alternatives to synthetic ones for ensuring food safety and quality. However, the practical use of these preservatives in the food industry is limited due to their negative impact on the odor and taste of food products, as well as the early loss of functionality due to their rapid diffusion and interaction with food components. The incorporation of natural antimicrobial agents into edible coatings has been investigated to control diffusion of active compounds and maintain their concentrations at a critical level on a food surface. Recently, nanoencapsulating and multilayered/nanolaminate delivery systems have emerged as promising tools to enhance the functionality of edible coatings. This review highlights the potential use of polymeric edible coatings for the incorporation of natural antimicrobial agents and the improvement of their controlled release in food systems. The methods used to assess the antimicrobial activity of encapsulated natural antimicrobial agents and the most recent findings regarding the application of nanoencapsulating and multilayered/nanolaminate delivery systems in food products are also discussed.

Keywords: edible coatings, food quality, food safety, nanodelivery systems, natural antimicrobial agents

Introduction

Food products are highly susceptible to microbial contamination that may affect their quality attributes and reduce their nutritional value. Moreover, the possible presence of microbial toxins or pathogenic microorganisms such as *Salmonella*, *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus, Campylobacter, Clostridium perfringens*, *and Aspergillus niger* may even endanger consumer safety and contribute to foodborne illness (Nummer and others 2012). Although synthetic fungicides, mainly nitrite and sulfites, proved to be highly effective against a wide variety of pathogenic microorganisms in foods, their potential negative impact on human health has prompted research on the use of naturally occurring antimicrobial agents to inhibit the growth of foodborne pathogens and prevent food spoilage. A wide variety of natural antimicrobial agents, including essential oils (EOs) derived from plants, animal-based enzymes (such as lysozyme, lactoferrin), bacteriocins from microbial sources (such as nisin, pediocin), and biopolymers (chitosan [CH]), have been tested for their antimicrobial potential against pathogens and spoilage bacteria in various food products (Pellegrino and Tirelli 2000; Abdollahzadeh and others 2014; Ahmed and others 2014; de Oliveira and others 2015). Although successful examples of the use of natural antimicrobial agents as food preservatives have been reported, some of them may have an impaired effect *in situ* due to their rapid diffusion within the bulk of foods and/or their possible interaction with food components, which may reduce their antimicrobial activity against target cells. In fact, the presence of high concentrations of fat and/or protein in food products may provide a protective layer around the microorganism or absorb the antimicrobial substance, thus reducing its concentration and effectiveness in aqueous media (Perricone and others 2015). The development of bioactive packaging systems through the incorporation of antimicrobial agents into biopolymer-based coatings could make a significant contribution toward shelf-life extension and food safety preservation. In addition to their effectiveness as selective barriers to gas, moisture, and solute migration, edible coatings can effectively reduce microbial growth in solid and semisolid food products by decreasing the diffusion rate of antimicrobial agents from coating materials into the food, and consequently sustaining a relatively high concentration of antimicrobial agents on the surface of the food product for a long time (Appendini and Hotchkiss 2002). Edible coatings can be produced from different natural components, including polysaccharides, proteins, and lipids, obtained from renewable agricultural resources and/or food processing wastes. The effectiveness of an edible coating in preserving food quality and extending shelf life is closely related to its barrier property to moisture, oxygen, and carbon dioxide, which mainly depends on the chemical composition and structure of the coating materials, the characteristics of the coated product, and the storage conditions (Lin and Zhao 2007).Polysaccharides such as sodium alginate (NaAlg), CH, and hydroxypropylmethylcellulose (HPMC) have been used in edible coating formulations for fresh products owing to their excellent coating-forming properties and selective permeability to O_2 and CO_2. In fact, these coatings are able to reduce gas exchange

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between food products and the environment, which may decrease respiration rates and thereby delay ripening and senescence of fresh produce in a similar way to storage under modified/controlled atmosphere (Janjarasskul and Krochta 2010). Several studies have demonstrated the ability of polysaccharide-based coatings carrying different natural antimicrobial agents to preserve quality and increase safety of many fresh and minimally processed fruits such as orange (Aloui and others 2015), mango (Cissé and others 2015), strawberry (Duran and others 2016), grape (Aloui and others 2014a), blueberry (Vieira and others 2016), minimally processed papaya (Narsaiah and others 2014), fresh-cut pineapple (Azarakhsh and others 2014), and fresh-cut apple (Chiabrando and Giacalone 2016). Likewise, different natural antimicrobial agents mainly EOs have shown potential, when added to edible coating formulations, for prolonging the shelf life of chicken meat (Fernández-Pan and others 2014; Bazargani-Gilani and others 2015) and seafood products (Aşı k and Candoğan 2014) by reducing oxygen and moisture transmission, limiting microbial contamination and discoloration, and preserving texture, color, and flavor (Gennadios and others 1997). Despite their effectiveness in maintaining quality and extending shelf life of many perishable foods, the practical application of edible coatings as effective antimicrobial carriers in the food industry is limited due to the weak adhesion of coating materials to the hydrophilic surface of the food, the degradation of the antimicrobial agent, or its quick desorption through coating materials (Campos and others 2011). In the last few years, nanotechnology has been investigated as a promising strategy to enhance the performance of natural antimicrobial agents and improve their effectiveness in preserving food quality, through the development of nanoencapsulating and multilayered/nanolaminate delivery systems. In fact, these nanocarriers have the potential to modulate the release of antimicrobial agents, which may reduce the amount required to achieve the desired antimicrobial effect. Moreover, these nanodelivery systems may potentially protect antimicrobial compounds against unfavorable environmental conditions and chemical reactions (such as oxidation or hydrolysis), limit their possible interaction with food components, improve their solubility, and preserve their stability during food processing and storage (McClements and others 2009). Among the nanoencapsulating systems currently used for the delivery of bioactive compounds, nanoemulsions have received particular attention because they can be formulated with natural food-grade ingredients and their production process is easily scalable in the industry by high-pressure homogenization process (Donsì and others 2011). Moreover, being kinetically stable and transparent, these nanoemulsions are assumed to be suitable for incorporation into food products without affecting their optical properties. Despite their efficacy in reducing pathogenic and food spoilage microorganisms and preserving quality attributes of various food and beverage products (Jo and others 2015; Maté and others 2016), using these systems in solid foods such as fruits and meat products is often limited because of the difficulty in immobilizing nanodroplets on the surface of foods. Recently, the development of *in situ* nanoemulsions from biopolymer-based edible coating formulations has been investigated as an effective strategy to place antimicrobial agents on the surface of solid products. Interesting results of improving quality and safety of meat-based products (Wu and others 2016), fresh fruits (Kim and others 2014; Salvia-Trujillo and others 2015), and vegetables (Severino and others 2015) have been obtained when incorporating antimicrobial nanoemulsions into bio-based edible coatings. Recently, a layer-by-layer (LbL) assembly technique has also received great interest as a new film preparation strategy to coat perishable foods, mainly fresh-cut fruits such as fresh-cut pineapples (Mantilla and others 2013), fresh-cut watermelon, and fresh-cut melon (Sipahi and others 2013; Moreira and others 2014). In addition to its added advantages of experimental simplicity and controlled release of antimicrobial agents, this technique leads to the development of multilayer coatings with specific thicknesses, properties, and performance, able to improve both quality and stability of coated food products. The main objectives of this review are to discuss the recent applications of natural antimicrobial agents from different sources as food preservatives and to highlight the potential use of bio-based edible coatings as polymeric matrices for the incorporation and/or the controlled release of natural antimicrobial agents in food systems. Moreover, this study reviews the different approaches used to quantify and/or screen the antimicrobial effect of the entrapped antimicrobial agents and focuses on the most recent findings regarding the application of nanotechnology in food packaging mainly on the use of nanoencapsulating and multilayered/nanolaminate delivery systems as promising tools to enhance the functionality of edible coatings and improve their effectiveness in preserving food quality.

Natural Antimicrobial Agents for Food Preservation

Natural antimicrobial compounds have been investigated as alternatives to synthetic ones for preserving food quality, owing to their effectiveness against food spoilage and foodborne pathogens. Based on their source of production, natural antimicrobial agents can be classified into different groups as shown in Figure 1. They include mainly plant-derived compounds (EOs and plant extracts), antimicrobial agents from animal sources, antimicrobial substances derived from bacterial cell metabolism (Lucera and others 2012), and antagonistic microorganisms.

Plant-derived compounds

Natural plant-derived compounds can be obtained from fruits and vegetables (garlic, pepper, onion, cabbage, xoconostle, and guava), seeds and leaves (olive leaves, parsley, caraway, nutmeg, fennel, and grape seeds), and herbs and spices (marjoram, basil, oregano, rosemary, thyme, sage, clove, and cardamom) (Tajkarimi and others 2010). Plant-derived EOs and extracts have long been used as food additives, not only to enhance taste and impart characteristic flavors, but also to prolong food shelf life by preventing rancidity and controlling microbial contamination. In fact, due to their high content of secondary metabolites, mainly phenolic compounds, iso-flavonoids, terpenes, ketones, aliphatic alcohols, acids, and aldehydes, these compounds are able to reduce or inhibit the growth of pathogenic microorganisms (Tiwari and others 2009). The antimicrobial activity of plant-derived compounds mainly depends on microorganism type, inoculum size, culture medium, extraction method, and method for antimicrobial activity determination (Tajkarimi and others 2010). Based on their chemical composition, EOs and plant extracts can exert their antimicrobial activity through different mechanisms of action. These mechanisms include changes in the cell membrane permeability, disintegration of the cytoplasmic membrane, release of cellular constituents, changes in the fatty acid and phospholipid composition, changes in the synthesis of DNA and RNA synthesis, and destruction of protein translocation (Amensour and others 2010). In the last decades, several studies have examined the potential use of EOs and other plant extracts as natural antimicrobial agents in different food products (Table 1).

The application of plant EOs and extracts for reducing the growth of food spoilage bacteria and foodborne pathogens in

Table 1–Antimicrobial effects of plant-derived extracts in food systems.

Product	Storage conditions	Target microorganism	Antimicrobial compound	Concentration	Antimicrobial effects	Reference
Pork meat	4 °C/25 °C for 72 h	*Listeria monocytogenes*	*Satureja horvatii* EO	10 and 20 mg/mL	Total inhibition	Bukvički and others (2014)
Minced fish meat	4 °C for 12 d	*L. monocytogenes*	Thyme EO	0.8% and 1.2%	Reduction of viable count below 2 log CFU/g from day 6 until the end of storage	Pellegrino and Tirelli (2000)
Ham	4 °C for 35 d under aerobic conditions	*L. monocytogenes*	Oregano and cinnamon cassia EOs	500 ppm	Reduction of the growth rate by 19% and 10% with oregano and cinnamon cassia EOs, respectively	Dussault and others (2014)
Fresh Tuscan sausage	7 °C for 14 d	Coliforms	Bay leaf EO	0.1 g/100 g	A 2.8 log reduction in total coliforms count at day 12	da Silveira and others (2014)
Peaches	4 d at room temperature	*Monilinia laxa, Monilinia fructigena Monilinia fructicola*	Vervain EOs	1000 ppm	Reduction of brown rot lesions diameter	Elshafie and others (2015)
			Thyme EOs	500 ppm		
Apple juice		*E. coli O157:H7*	Lemon EO	75 μL/L + Thermal treatment at 54 °C for 10 min	A 5 log reduction in the initial population	Espina and others (2012)
Lemon	20 °C in darkness for 3 d	*Penicillium digitatum Penicillium italicum*	Thymol Carvacrol	250 μL/L 500 μL/L	No incidence of fungal mycelium was observed on lemon surface	Pérez-Alfonso and others (2012)
Chocolate	20 °C for 9 d	*E. coli O157:H7*	Lemon EO	0.1 mL/100 g	A 1.7 log reduction in *E. coli O157:H7* population	Kotzekidou and others (2008)
Raw peeled undeveined shrimp	3 h at 4 °C	Total viable count	Olive leaves extract	2% (w/v)	A 2 log reduction in the initial population	Ahmed and others (2014)
Chicken meat	4 d at 4 °C	*Campylobacter jejuni*	Chestnut inner shell extract	2 mg/g	Total inhibition of *C. jejuni* at an inoculum level of 3 log CFU/g	Lee and others (2016)

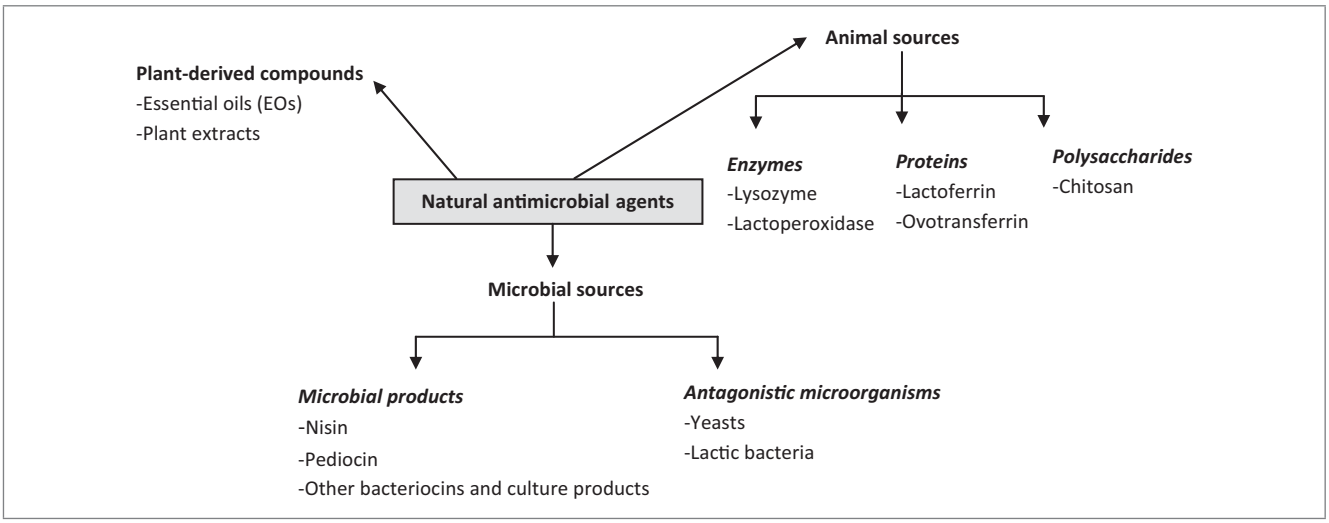

Figure 1–Classification of natural antimicrobial agents according to their source of production.

food systems requires detailed knowledge of their antimicrobial properties (mode of action, minimum inhibitory concentration (MIC), target microorganisms), and their interactions with food components and with other antimicrobial compounds (Hyldgaard and others 2012). Although some EOs have shown potential for food preservation, their practical use in the food industry is often restricted because of their application costs, their strong aroma and taste as well as their potential toxicity. In fact, due to their possible interaction with lipids, proteins, and other food components, higher concentrations of EOs and extracts are usually required to achieve a similar effect as *in vitro.* The use of high levels of EOs may endanger human health as they may induce several disorders, including intoxications, mutation events in somatic and germinal tissues, and development of somatic diseases, teratogenic effects, and inherited genetic damages (Sousa and others 2010). Likewise, the direct addition of high concentration of EOs may affect the organoleptic properties of food, and thus its acceptance by the consumer (Jouki and others 2014). In this sense, a green color combined with a grassy aroma was detected when crude ethanolic extracts from *Eremophila duttonii* and *Eremophila alternifolia* were added to milk and food homogenates (Owen and Palombo 2007). In another study, da Silveira and others (2014) claimed that consumers appreciated control sausage samples more than samples enriched with 0.05 and 0.1 g/100 g of *Laurus nobilis* EO, for which a strong flavor was detected. Likewise, Abdollahzadeh and others (2014) and García-Díez and others (2016) reported a significant decrease in the taste and overall acceptability scores of minced fish enriched with 0.8% of thyme EO and dry cured sausage treated with 0.05% of oregano and garlic EOs, respectively.

Antimicrobial agents from animal sources

Natural antimicrobial compounds from animal sources include enzymes and proteins present in milk and eggs, such as lysozyme, lactoferrin, and lactoperoxidase, as well as some polysaccharides, mainly CH extracted from crustaceans and shrimp shells.

Lysozyme, generally recognized as safe (GRAS) for direct incorporation into food systems (FDA 1998), has received particular attention due to its stability over a wide range of temperature (4 to 95 °C) and pH (pH 2 to 10) conditions and its high antimicrobial activity against several pathogenic Gram-positive bacteria such as *Bacillus stearothermophilus*, *Micrococcus* spp, *Clostridium tyrobutyricum*, and *Listeria monocytogenes* (Davidson and others 2013). Commercially, lysozyme was added to semihard cheeses to inhibit late blowing, caused by *C. tyrobutyricum* (Pellegrino and Tirelli 2000). In this context, a conspicuous bacteriostatic effect against *C. tyrobutyricum* was observed by Danyluk and Kijowski (2001) when using lysozyme with an activity of 250 to 500 U/mL bacteria suspension. Recently, lysozyme was added to white and red wines instead of sulfur dioxide to control the proliferation of lactic acid bacteria (LAB) (Liburdi and others 2014). The antimicrobial activity of lysozyme has been mainly ascribed to its ability to break down the peptidoglycan of bacterial cell walls by catalyzing the hydrolysis of the β-1, 4-bond between *N*-acetyl-muramic acid and *N*-acetyl-D-glucosamine residues (Masschalck and Michiels 2003). Lysozyme has a limited effect on Gram-negative bacteria due to the presence of the lipopolysaccharidic layer that may prevent the access of lysozyme to the target peptidoglycan. Different techniques have been proposed to enhance the antimicrobial activity of lysozyme against Gram-negative bacteria, and consequently increase its practical use in the food industry. These approaches include mainly thermal denaturation, covalent attachment of saturated fatty acids at lysine residues, glycosylation, reduction of disulfide bonds, and use of chelating compounds (Masschalck and Michiels 2003). In this context, Liang and others (2006) reported the efficacy of the combined application of nisin (27.5 U/mL) and lysozyme (690 U/mL) with a pulsed electric field at a field strength of 27 kV/cm and a pulse rate of 200 pulses/s, in reducing spoilage microorganisms in apple cider. The lactoperoxidase system (LPS) is another antimicrobial enzyme that is very abundant in bovine milk (30 mg/L). This system has a high antimicrobial activity against several foodborne pathogens, including *Salmonella* Typhimurium, *S. aureus*, *L. monocytogens*, and *Campylobacter jejuni* (Kennedy and others 2000; Armenteros and others 2007). The activation of LPS has been widely used as a means for preventing deterioration of raw milk by the action of undesirable microorganisms during collection, transport, and processing, especially in countries with inadequate refrigeration (Armenteros and others 2007). However, only a few studies have been attempted to apply LPS on poultry and meat products (Kennedy and others 2000; Jooyandeh and others 2011).

Lactoferrin, an antimicrobial glycoprotein, is known to be effective against several foodborne microorganisms, including *E.*

coli, *Carnobacterium*, *Klebsiella*, and *L. monocytogenes* (Gyawali and Ibrahim 2014). Al-Nabulsi and others (2009) reported the ability of lactoferrin in 0.2% peptone to reduce initial counts of *Cronobacter* spp by 4 log colony forming units (CFU)/mL of within a 4-h incubation at 37 °C. Likewise, a 4 log cycle reduction of *L. monocytogenes* was achieved by at lactoferrin concentration of 1 mg/mL within 8 h of incubation at 37 °C (Ripolles and others 2015). The antimicrobial activity of this peptide is attributed either to its ability to limit microbial access to nutrients via iron chelation and/or to disturb the outer membrane of Gram-negative bacteria (Gyawali and Ibrahim 2014). In another work, Wang and others (2013) reported the efficacy of lactoferrin at a concentration of 100 mg/L in inhibiting tube elongation and spore germination of *Botrytis cinerea* after incubation for 8 h at 23 °C. According to these authors, the mechanisms by which lactoferrin exerts its antifungal activity are mainly related to the disruption of the conidia plasma membrane and the leakage of cytoplasmic materials from the hyphae. Lactoferrin is authorized for beef preservation in the United States (USDA-FSIS 2010). Recently, a 0.6 log CFU/g reduction of *E. coli* O157:H7 was achieved when chicken breast fillets were mixed by hands with lactoferrin at 0.5 mg/g in a stomacher bag, before being stored at 5 °C for 9 d.

In addition to peptides, CH extracted from the exoskeletons of arthropods and crustaceans has shown antimicrobial potential against several Gram-positive and Gram-negative bacteria including *Salmonella* Typhimurium, *S. aureus*, *B. cereus, L. monocytogenes*, *Shigella dysenteriae*, and *E. coli* (Gyawali and Ibrahim 2014), as well as pathogenic fungi such as *Aspergillus flavus*, *Alternaria* spp, *Penicillium* spp, and *Cladosporium* spp (Hafdani and Sadeghinia 2011). The antimicrobial effect of CH can vary depending on molecular weight, molecular structure, degree of deacetylation, pH, and microorganism species (Xu and others 2007). Several hypotheses have been proposed to explain the mechanisms by which CH exerts its antimicrobial activity. According to Yadav and Bhise (2004), this cationic polysaccharide may interact with the negatively charged microbial cell membrane, disturbing its permeability and leading to loss of intracellular constituents. Another explanation could be the interaction of CH oligomers with the microbial DNA. In fact, owing to their low molecular weight, these CH hydrolysis products are assumed to diffuse through the bacterial cell wall after interaction of their cationic charges with the negatively charged lipopolysaccharides of the bacterial outer membrane. The diffused cationic oligomers may interact with the negatively charged DNA molecule, which may inhibit DNA transcription and consequently affect RNA and protein synthesis, and thus the activity of enzymes responsible for the growth of the target microorganism (Rabea and others 2003). Although CH has been shown effective against several foodborne microorganisms, its practical application as a food preservative has been limited due to its low solubility at neutral and higher pH (Hugo and Hugo 2015). However, studies on the antimicrobial activity of CH incorporated in food systems have been reported against some foodborne pathogens. In this context, Rhoades and Roller (2000) reported that the addition of CH at a concentration of 0.3 mg/mL completely inhibited the growth of yeasts in pasteurized apple-elderflower juice during storage at 7 °C for 13 d. In another study, Soultos and others (2008) reported a 1.1 log CFU/g reduction in the initial counts of *Enterobacteriaceae* when pork sausage was treated with 1% (w/w) CH, and stored at 4 °C for 7 d. Likewise, Chantarasataporn and others (2014) showed that oligochitosan concentrations of 0.4% reduced the growth of *Enterobacteriaceae* and *Staphylococcus* in minced pork meat, respectively, by 1 and 2 log CFU/g.

Antimicrobial agents from microbial sources

Antagonistic microorganisms and/or their derived antimicrobial metabolites have been proposed as natural preservatives to inhibit or prevent the growth of spoilage and pathogenic microorganisms in food systems and, consequently, to enhance their safety and prolong their shelf life. Among microbial antagonists, LAB have been widely used as potential protective cultures, not only because most of them are GRAS, but also because they are able to act as potential competitors against food spoilage microorganisms, through competition for nutrients or the production of primary and secondary antimicrobial metabolites (Ghanbari and others 2013). The survival of LAB during storage in food systems is mainly related to the composition of the food matrix and the environmental conditions such as pH, temperature, and ionic strength (da Cruz and others 2009). LAB have been reported to show low survival ability in low-pH food products such as condiments, fruit juices, and salads, as well as in foods prepared or stored at high temperatures (70 to 80 °C) (Rodgers 2007).

Some researchers have reported the promising potential of LAB as protective cultures for inhibiting pathogenic and spoilage bacteria in many food systems. In this context, a total inhibition of yeast contamination has been observed by Delavenne and others (2015) when *Lactobacillus harbinensis* K.V9.3.1Np was added as a bioprotective agent in yogurt. Likewise, Cheong and others (2014) reported the effectiveness of *Lactobacillus plantarum* isolates in controlling the growth of *Penicillium commune* on cottage cheese for up to 18 d at room temperature. Recently, Gao and others (2015) reported a decrease in *L. monocytogenes* CMCC 54002 to an undetectable level (<10 CFU/g) at day 30 when *Lactobacillus sakei* C2 and its bacteriocin sakacin C2 were applied to vacuum-packed sliced cooked ham, stored at 4 °C.

Among the various bacteriocins from LAB, nisin and pediocin, GRAS, have received much attention as food preservatives (Cotter and others 2005; Papagianni and Anastasiadou 2009).

Nisin synthesized by some strains of *Lactococcus lactis* is a heat-stable bacteriocin peptide with a high antimicrobial activity toward a wide range of Gram-positive bacteria including *Staphylococcus*, *B. cereus*, *Lactobacillus*, *Enterococcus*, *L. monocytogenes*, *Leuconostoc*, *Clostridium sporogenes*, *Clostridium botulinum*, *Pediococcus*, and *Micrococcus*, while showing limited activity against yeasts, molds, and Gram-negative bacteria (Tiwari and others 2009). Nisin inhibits target cells via specific binding to the cell wall precursor lipid II, followed by formation of pores in the bacterial cell membrane and subsequent loss of intracellular constituents (Bauer and Dicks 2005). The reduced antimicrobial effect of nisin against Gram-negative bacteria is mainly attributed to the presence of the outer membrane, which may protect the cytoplasmic membrane and peptidoglycan layer of Gram-negative cells (Helander and Mattila-Sandholm (2000). Recently, the combination of nisin and chelating agents, such as sodium salts of ethylenediamine tetraacetate (disodium ethylenediaminetetraacetic acid), enhanced the antimicrobial activity against Gram-negative bacteria such as *Salmonella* Typhimurium and *E. Coli* O157:H7 (Fang and Tsai 2003; Prudêncio and others 2016). In fact, it has been reported that chelating agents are able to disintegrate the outer membrane of Gram-negative bacteria by removing divalent cations (notably Ca^{2+} and Mg^{2+}) that are responsible for its stability, allowing bacteriocin to reach the cytoplasmic membrane (Martin-Visscher and others 2011).Commercially, nisin has been widely used as a

food preservative in acidic foods owing to its high solubility and stability at low pH values. (Jeevaratnam and others 2005). Primarily, nisin has been added as a food preservative to inhibit growth and sporulation of *C. sporogenes* and *C. botulinum* and in cheese (Mattick and Hirsch 1956; Delves-Broughton and others 1996). Recently, many researchers have demonstrated the great potential of nisin for the control of pathogenic and spoilage bacteria in dairy and nondairy food products. Mitra and others (2011) showed that the addition of nisin at a concentration of 1000 AU/mL to both skim milk and whole milk was effective in reducing the initial count of *Pseudomonas fluorescens*, *Enterococcus italicus*, *Lactobacillus paracasei*, *Enterococcus mundtii*, *B. cereus*, *Enterococcus faecalis*, *Bacillus thuringiensis*, and *Acinetobacter* spp, to an undetectable level during incubation at 8 °C for 8 to 20 h. In another study, a reduction of 1.2 and 2.0 log cycles in the *S. aureus* count has been noticed, respectively, after addition of nisin at 100 and 500 IU/mL to Minas traditional Serro cheese (Pinto and others 2011). Likewise, Felicio and others (2015) reported the effectiveness of nisin in reducing the growth of *S. aureus* in Minas frescal cheese. Recently, de Oliveira and others (2015) evaluated the antimicrobial potential of nisin against *L. monocytogenes*, *B. cereus*, *Alicyclobacillus acidoterrestris*, and *S. aureus* in mango, cashew, peach, and soursop juices. A 4 log reduction in viable cells of *A. acidoterrestris* was observed in mango, soursop, and peach juices enriched with nisin at 5000 IU/mL after 8 h of incubation at 4 °C, while no viable cells were observed in cashew juice. On the other hand, a 4 log decrease in the initial count of *S. aureus* was observed in mango juice after 24 h of incubation at 4 °C. Likewise, at least a 4 log reduction in the initial count of *B. cereus* was observed after 24 h of incubation at 4 °C in the presence of nisin.

Pediocin is another heat-stable bacteriocin produced by *Pediococcus* species such as *Pediococcus acidilactici* and *Pediococcus pentosaceus*. Most of these peptides are thermostable and active over a wide range of pH (pH 2 to 8). In contrast to nisin, pediocin has a relatively narrow spectrum of activity. Overall, pediocins are active against some species of *Enterococcus*, *Clostridium*, *Lactobacillus*, *Carnobacterium*, *Pediococcus*, and occasionally, *Leuconostoc and Streptococcus*; however, they exhibit a high antimicrobial activity against *L. monocytogenes* (Zhu and others 2005). Pediocins have been used as food preservatives in various food products, including cheese and meat-based products. In this context, Rodríguez and others (2005) have reported the efficacy of pediocin preparations from *L. lactis* CL1 and *L. lactis* CL2 in reducing *E. coli* O157:H7 counts by 0.84 and 1.69 log units, *S. aureus* by 0.98 and 0.40 log units, and *L. monocytogenes* by 2.97 and 1.64 log units, compared with the control cheese at day 30. In another study, Nieto-Lozano and others (2010) reported a reduction of 2 and 0.6 log cycles in the initial counts of *L. monocytogenes* after storage at 4 °C for 60 d and at 15 °C for 30 d, respectively, when pediocin PA-1 was added at 5000 bacteriocin units/mL (BU/mL) in frankfurters.

Although most studies on the practical use of protective cultures have focused on LAB and their bacteriocins, some researchers have reported the promising potential of yeasts as biocontrol agents, for preserving postharvest quality and reducing microbial contamination in fresh fruits and vegetables, owing to their high inhibitory capacity and rapid colonization of fruit wounds (El-Tarabily and Sivasithamparam 2006). Several mechanisms of action were suggested to be involved in the protective effect of antagonistic yeasts, including competition for space and nutrients, production of high levels of ethanol, pH changes as a result of organic acid production or growth-coupled ion exchange, and production of killer toxins or "mycocins" (Muccilli and Restuccia 2015). Numerous yeast antagonists have been reported to successfully control postharvest infections in many fruit commodities. Luo and others (2013) reported the efficacy of *Pichia membranaefaciens* in reducing the incidence of both green and blue mold infections by 66% and 83%, respectively, in inoculated citrus fruits after 4 d of storage at 20 °C. In another study, Platania and others (2012) highlighted the strong inhibitory effect of *Wickerhamomyes anomalus* strain (BS91) against *P. digitatum* in Tarocco oranges (*Citrus sinensis*), where less than 15% of fruits were decayed up to the 10th d of storage at 20 °C. Recently, Parafati and others (2015) reported the efficacy of *W. anomalus* strain BS91, *Aureobasidium pullulans* strain PI1, and *Metschnikowia pulcherrima* strain MPR3 in reducing the size of disease lesions caused by *B. cinerea* in table grapes.

In addition to their effectiveness in controlling postharvest diseases of fruits, some yeasts have great potential to prevent microbial contamination during the vinification process. In this context, de Ullivarri and others (2014) reported the ability of *W. anomalus* Cf20 and *Saccharomyces cerevisiae* Cf8 to inhibit the growth of some wine spoilage yeasts, including *Dekkera anomala* BDa15 and *P. membranaefaciens* BPm481, in the range of 7% to 48% and 61% to 91%, respectively.

Natural Antimicrobial Agents in Edible Coatings

Although some researchers have reported the efficacy of natural antimicrobial agents, when directly added to food systems, in reducing microbial contamination, the rapid diffusion of these agents within the bulk of food, as well as their possible interaction with food components, may decrease their antimicrobial activity during storage and thus limit their practical application in the food industry. Recently the use of edible coatings as polymeric matrices for the entrapment of natural antimicrobial agents has been investigated as a promising alternative to overcome these limitations by lowering the diffusion of active compounds onto food surfaces and hence maintaining their concentrations at a critical level for microbial growth inhibition over long periods of storage (Gyawali and Ibrahim 2014) (Figure 2). Furthermore, compared to direct application, this approach may impart a highly localized functional effect without affecting its organoleptic properties (Campos and others 2011). Moreover, edible coatings may act as a semipermeable barrier providing an additional protection for foods against moisture loss, solute migration, gas exchange, respiration, and oxidative reactions (Quirós-Sauceda and others 2014) (Figure 2).

Edible coatings are thin layers prepared from naturally occurring polymers and applied on food surfaces by different mechanical procedures, such as spraying, brushing, and dipping (Dhall 2013), or by electrostatic deposition (Poverenov and others 2014). Overall, the functional properties of edible coatings depend on different factors including coating characteristics (composition, chemical structure, viscosity of the coating solutions, coating thickness, degree of crosslinking), coating processing conditions (temperature, pH, type of solvent), and type and concentration of additives (emulsifiers, plasticizers, or cross-linking agents). Recently, the performance of edible coatings was improved by the incorporation of different bioactive compounds, mainly antimicrobial agents that may not only increase antimicrobial properties but also reduce biochemical deteriorations caused by processing, such as texture breakdown, enzymatic browning, and off-flavors development (Valdés and others 2015). In this context, several studies have investigated the application of edible coatings as natural antimicrobial delivery systems to extend shelf life of highly perishable foodstuff, mainly fresh and minimally processed fruits and meat products.

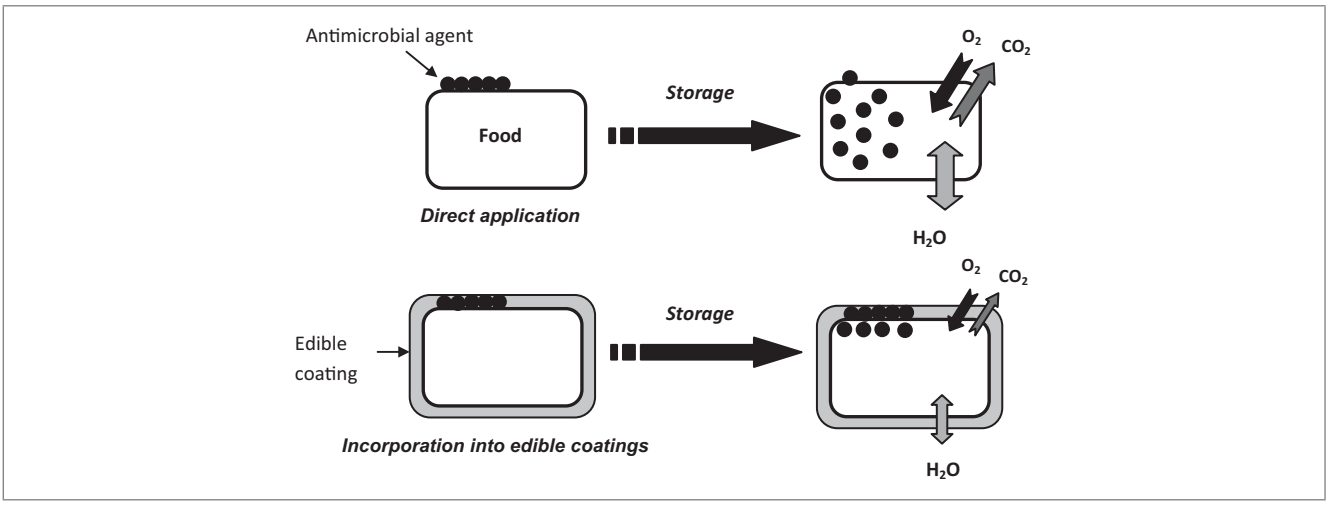

Figure 2–Functional properties of an edible coating.

Antimicrobial edible coatings for fresh and minimally processed fruits

Polysaccharides such as NaAlg, CH, and HPMC have been widely used as coating materials for fresh and minimally processed fruits and vegetables owing to their excellent film-forming properties and selective permeability to O_2 and CO_2. In fact, these coatings may reduce respiration rates and thereby delay ripening and senescence of fresh produce in a similar way to storage under modified/controlled atmosphere (Janjarasskul and Krochta 2010). However, accumulation of CO_2 and depletion of O_2 in a fruit's internal atmosphere may lead to anaerobic fermentation and production of fermentative metabolites that contribute to the development of off-odors and off-tastes (Lin and Zhao 2007). The effectiveness of edible coatings in preserving the quality of fresh fruits and vegetables is strongly related to the selection of a suitable coating material that is able to provide a desirable internal gas composition depending on both respiration and transpiration rates of the commodity. Moreover, the control of the wettability of coating formulations is also of great importance as it may affect coating thickness and thereby its permeability (Casariego and others 2008). In addition, controlling environmental conditions of the storage area, mainly temperature and relative humidity, also have an important influence on the internal atmosphere of fresh fruits, as they may strongly affect coating permeability and respiration rates (Lin and Zhao 2007).

Several studies have demonstrated the ability of polysaccharide-based coatings as carriers of natural antimicrobial substances, mainly plant-derived compounds, to preserve postharvest quality and to reduce microbial contamination of fresh and minimally processed fruits by decreasing respiration rates, reducing weight loss, preserving texture and flavor, and reducing microorganism proliferation and metabolic activity (Table 2).

Antimicrobial edible coatings for meat products

Edible coatings incorporating natural antimicrobial agents have been investigated in the meat industry for their ability to increase microbial safety and extend shelf life of meat products by reducing oxygen and moisture transmission, limiting microbial contamination and discoloration, and preserving texture, color, and flavor (Gennadios and others 1997). Several studies have demonstrated the high potential of edible coatings carrying different natural antimicrobial agents to preserve poultry, meat, and seafood products. Fernández-Pan and others (2014) reported the ability of whey protein isolate-based coatings enriched with oregano EO at a concentration of 20 g/kg to extend the refrigerated shelf life of chicken breast from 6 to 13 d, while at the same time, maintaining total mesophilic aerobic, LAB, and *Pseudomonas* spp counts below the microbiological critical limits established for distribution and consumption. In agreement with this study, Bazargani-Gilani and others (2015) reported the efficacy of CH-based coatings enriched with 2% *Zataria multiflora* EO in controlling microbial growth, delaying chemical changes, improving sensory attributes, and extending shelf life of chicken breast previously dipped in pomegranate juice by 15 d during refrigerated storage. Similarly, CH-based coatings supplemented with antimicrobial agents have shown great potential for preserving seafood products and extending their shelf life. Jasour and others (2015) reported the ability of the lactoperoxidase system (LPS) incorporated into CH coatings to increase shelf life of trout fillets and maintaining their sensory attributes at high acceptability until the 16th d of storage at 4 °C. In agreement with these findings, Aşi k and Candoğan (2014) reported the effectiveness of CH coatings incorporating garlic oil in reducing aerobic bacteria counts and extending the refrigerated shelf life of shrimp meat by 2 d. Apart from CH, other biopolymers, including polysaccharides and proteins, have been investigated as effective antimicrobial delivery systems to curb undesirable effects in seafood products. In this context, Ariaii and others (2015) studied the effect of methylcellulose-based coatings incorporating 1.5% of *Pimpinella affinis* EO on the quality and shelf life of fresh silver carp (*Hypophthalmicthys molitrix*) fillets during storage at 4 °C for 20 d. They highlighted the ability of these coatings to reduce microbial growth and to extend shelf life of silver fillets up to 12 d without affecting their color, odor, texture, or overall acceptability. Likewise, Heydari and others (2015) reported the effectiveness of NaAlg coatings incorporating 1% horsemint EO in controlling microbial deterioration and limiting lipid oxidation of bighead carp (*Aristichthys nobilis*) fillets. Recently, Hosseini and others (2016) found that fish gelatin-based coatings enriched with 1.2% oregano EO significantly reduced volatile base formations in rainbow trout (*Oncorhynchus mykiss*) stored at 4 °C for 16 d, and resulted in a 1.05 CFU/g reduction of psychrotrophic bacteria counts.

Table 2–Antimicrobial coatings for fresh and minimally processed fruits and vegetables

Antimicrobial compound	Coating formulation	Food	Storage conditions	Main effects	Reference
Grapefruit EO (1%, v/v) Grapefruit seed extract (1%, v/v)	1 and 2% (w/v) NaAlg +15% (w/v) glycerol	Table grapes	4 °C for 15 d	• Reducing weight loss in the range of 14% to 24% • Reducing firmness loss by 25% • Preserving antioxidant activity • Reducing fungal decay caused by *P. digitatum* in inoculated berries	Aloui and others (2014a)
Aloe vera (0.5%, v/v)	0.5% (w/v) CH + 0.5% (w/v)glycerol + 0.1% (w/v) Tween 80	Blueberry	5 °C for 25 d	• Reducing microbial growth and weight loss by 50% and 42%, respectively • Extending shelf life of blueberries up to 5 d	Vieira and others (2016)
Natamycin (1%, w/v) Nisin (1%, w/v) Pomegranate extract (1%, w/v)	1.5% (w/v) CH + 0.5% (v/v) polyethylene glycol	Strawberry	4 °C for 40 d	• Improving pH stability and total soluble content • Maintaining fruit firmness • Reducing respiration rate and microbial counts	Duran and others (2016)
CH (1%, w/v)	1% (w/v) Carboxymethylcellulose + 0.75% glycol monostearate 1% (w/v) Hydroxypropylmethylcelulose + 0.75% glycol monostearate	Strawberry	11 °C for 12 d	• Reducing weight loss in the range of 64% to 72% at day 8 • Reducing decay percentage by more than 75% at the end of storage • Maintaining high total soluble solids content • Maintaining high contents of total phenols and anthocyanins up to 12 d of storage • Maintaining ascorbic acid content at higher levels during the entire storage period • Inhibiting cell wall-degrading enzymes • Maintaining fruit firmness	Gol and others (2013)

(*Continued*)

Table 2–Continued.

Antimicrobial compound	Coating formulation	Food	Storage conditions	Main effects	Reference
LPS	CH (1%, w/v) + 25% glycerol (p/p CH)	Mango		• Reducing weight loss by more than 46% • Maintaining fruit color • Inhibiting fungal proliferation • Delaying fruit ripening	Cissé and others (2015)
Cinnamon bark EO (0.3%, v/v) Rosemary EO (0.3%, v/v)	2% (w/v) NaAlg + 1.5% (v/v) glycerol + 1% (w/v) calcium ascorbate	Fresh cut-apple	0 °C in darkness for 10 d	• Reducing respiration rate • Reducing weight loss • Preserving the original color and lightness • Reducing polyphenoloxidase and peroxidase activities	Chiabrando and Giacalone (2016)
Wickerhamomyces anomalus BS91 killer yeast (10^7 CFU/mL)	Alginate (2%, w/v) + glycerol (20%, w/w based on biopolymer content) Locust bean gum (1%, w/v) + glycerol (20%, w/w based on biopolymer content)	Valencia orange	25 °C and 75% RH for 15 d	• Reducing firmness loss by more than 21% at day 15 • Reducing green mold in inoculated fruits by more than 73% after 13 d • Reducing weight loss of coated oranges in the range of 28% to 33%	Aloui and others (2015)
Lemon EO (3%, w/w)	CH (1%, w/w)	Strawberry	4 °C and 90% RH for 10 d	• Reducing respiration rate • Enhancing the antifungal activity of CH against *B. cinerea* in inoculated strawberries	Perdones and others (2012)

(*Continued*)

Table 2–Continued.

Antimicrobial compound	Coating formulation	Food	Storage conditions	Main effects	Reference
Pediocin (20%, v/v)	Alginate (2%, w/w)	Minimally processed papaya	4 °C for 21 d	• Reducing O_2 levels (1.8-fold) and acidity percentage (2.7-fold) • Reducing firmness and weight losses (8.7- and 7.4-fold, respectively, higher compared with uncoated samples) • A 4 log reduction in microbial count	Narsaiah and others (2014)
Lemongrass EOs (0.3%, w/v)	NaAlg (1.29%, w/v) + glycerol (1.16%, w/v) + sunflower oil 0.025% (w/v)	Fresh-cut pineapple	10 °C and 65% RH for 16 d	• Reducing respiration rate • Reducing weight loss • Maintaining firmness • Reducing total plate count, yeast, and mold counts • Maintaining sensory characteristics of the fruit	Azarakhsh and others (2014)
Cinnamon bark EO (0.3%, v/v) -Fennel EO (0.3%, v/v)	Cassava starch (2% and 3% w/v) + glycerol (1:0.25)	Apple slices	5 °C for 5 d	• Inhibiting the growth of *S. aureus* and *Salmonella choleraesuis* • Increasing water vapor resistance and reducing respiration rates of coated apple slices • Protecting fruit surface against enzymatic browning	Oriani and others (2014)
Propolis extract (5%, v/v) and cinnamon EO (0.1, % v/v)	Gum arabic (5%, w/v) + Tween 80 (0.2%, w/v)	Chilli	13 °C and 80% to 90% RH for 28 d	• Reducing weight loss by more than 60% • Reducing firmness loss by more than 89% • Reducing disease incidence by more than 85%	Ali and others (2014)

NaAlg, sodium alginate; CH, chitosan; LPS, lactoperoxidase system.

Nanoscale Antimicrobial Delivery Systems for Food Preservation

Although several studies have reported the effectiveness of natural antimicrobial compounds when incorporated into edible coatings, compared to their direct incorporation into food products, some papers on the desorption phenomenon of bioactive agents from single component based films demonstrated a quick diffusion of these compounds with a rapid loss of their activity (Barba and others 2015). Nonetheless, the effectiveness of antimicrobial films and coatings can be also affected by the connectivity of the macromolecular network and the intermolecular forces between coating material and antimicrobial substance (Calderón-Aguirre and others 2015). Moreover, the weak adhesion of coating materials to the hydrophilic surface of the food may also limit the practical application of conventional edible coatings as efficient antimicrobial delivery systems in the food industry (Campos and others 2011). Consequently, microencapsulation technology based on the entrapment of antimicrobial agents inside a tiny microsphere/microcapsule, with an average diameter of 1 μm to several hundred micrometers, has been investigated as a promising tool to delay and control desorption of antimicrobial compounds, while at the same time ensuring their protection against chemical reactions and undesirable interactions with other food components. Although some researchers have reported the efficiency of these microencapsulation delivery systems in controlling microbial growth and extending shelf life of perishable foods (Huq and others 2015; Wu and others 2015), their practical application in the food industry is often limited due to their negative impact on the sensory properties of food products, especially when their size exceeds 100 μm (Champagne and Fustier 2007). Nowadays, nanoencapsulation systems (nanometer-scale systems), and multilayered delivery systems (nanolaminates), have emerged as a new generation of antimicrobial delivery systems for enhancing microbial safety and preserving food quality. In fact, thanks to their high surface area-to-volume ratio, these nanostructures may increase the antimicrobial concentration in food areas with high microbial load and improve passive cellular absorption mechanisms leading to higher antimicrobial activity.

Nanoencapsulation-based antimicrobial systems for food preservation

Among the nanoencapsulating systems currently used as food antimicrobial delivery systems, nanoemulsions have received particular attention because they can be formulated with natural food-grade ingredients and their production process is easily scalable in the industry by high-pressure homogenization process (Donsì and others 2011). Nanoemulsions are defined as heterogeneous systems within nanometric size (≤100 nm) composed of 2 immiscible liquids (oil and water) that are mixed to make 1 homogeneous and/or stable phase through the use of an appropriate emulsifier. In particular, oil-in-water nanoemulsions, which have attracted widespread attention as food delivery systems, are consisting of nanometric oil droplets dispersed in an aqueous continuous phase, with each nanodroplet being surrounded by a thin interfacial layer of a food-grade emulsifier or biopolymer (McClements and others 2007) (Figure 3).

Emulsifiers play a crucial role in the elaboration of nanoscale oil droplets by reducing the interfacial tension and preventing nanodroplets aggregation through the creation of repulsive interacting forces. The selection of emulsifier type is a key factor controlling the interfacial properties (thickness, charge, droplet size, and rheology), as well as the response of nanodroplets to the different environmental stimuli such as ionic strength, temperature, pH, and enzyme activity. The ability of nanoemulsions to act as matrices for the entrapment and the controlled release of functional compounds depends not only on the molecular characteristics of the entrapped molecules, but also on the composition, microstructure, and the properties of the nanoemulsions themselves (McClements and Rao 2011). Bioactive compounds can be encapsulated either in the inner oil phase (bioactive-enriched core) or into the outer emulsifier layer (bioactive-enriched shell), as shown in Figure 3. Encapsulation of functional components within oil droplets is assumed to protect the entrapped molecules from chemical degradation by controlling the properties of the emulsifier layer surrounding them (McClements and Decker 2000). On the other hand, the encapsulation of functional compounds into the emulsifier layer is assumed to reduce the possibility of a controlled-release mechanism. Nanoemulsions have been frequently investigated as carriers of lipophilic and amphiphilic antimicrobial agents to solve problems related to their poor solubility in food systems, while at the same time improving their bioavailability and antimicrobial effect. In fact, these nanoemulsions are believed to act as "nano-tanks" for antimicrobial agents, improving their dispersion in aqueous media and providing a sustained concentration of the entrapped compounds over an extended period by ensuring a continuous diffusion of new active molecules from the emulsion droplets (Donsì and others 2014). Additionally, owing to their subcellular size, nanoemulsions may facilitate the diffusion of active molecules through biological membranes, thus increasing the bioavailability of bioactive compounds and enhancing their antimicrobial effect (Blanco-Padilla and others 2014). Moreover, being kinetically stable and transparent, these nanoemulsions are assumed to be suitable for incorporation into food products without affecting their optical properties. In the last few years, antimicrobial nanoemulsions have been investigated by the food industry for their potential, as antimicrobial delivery systems, to control foodborne disease and food spoilage microorganisms and preserving quality attributes of various food and beverage products. In this sense, Bhargava and others (2015) evaluated the antibacterial potential of oregano oil nanoemulsions against foodborne bacteria in fresh lettuce stored at 4 °C for 72 h. They reported the ability of these nanoemulsions, at a concentration of 0.1%, to reduce initial counts of *Salmonella* Typhimurium, *L. monocytogenes*, *and E. coli* O157:H7 by more than 3.26, 3.57, and 3.35 log CFU/g, respectively. Similarly, Jo and others (2015) reported the effectiveness of 0.8% trans-cinnamaldehyd nanoemulsions in inhibiting the growth of *S. aureus* and *Salmonella* Typhimurium in water melon juice after incubation at 37 °C for 72 h. Recently, Maté and others (2016) reported a 3 log cycle reduction in the initial count of *L. monocytogenes* after application of a D-limonene and nisin-based nanoemulsion in tryptic soy broth growth medium, chicken broth, and vegetable cream incubated at 37 °C for 90 min. In another study, Shadman and others (2016) reported the ability of sunflower oil-based nanoemulsions, containing 1% of *Zataria multiflora* Boiss. EO to reduce lipid oxidation and extend the refrigerated shelf life of trout (*O. mykiss*) fillets, by at least 15 d. Despite their efficacy in reducing pathogenic and food spoilage microorganisms and preserving quality attributes of various food and beverage products (Jo and others 2015; Maté and others 2016), the use of nanoemulsions in solid foods such as fruits and meat products is often limited because of the difficulty in immobilizing nanodroplets on the surface of foods. Recently, the development of *in situ* nanoemulsions from biopolymer-based edible coating

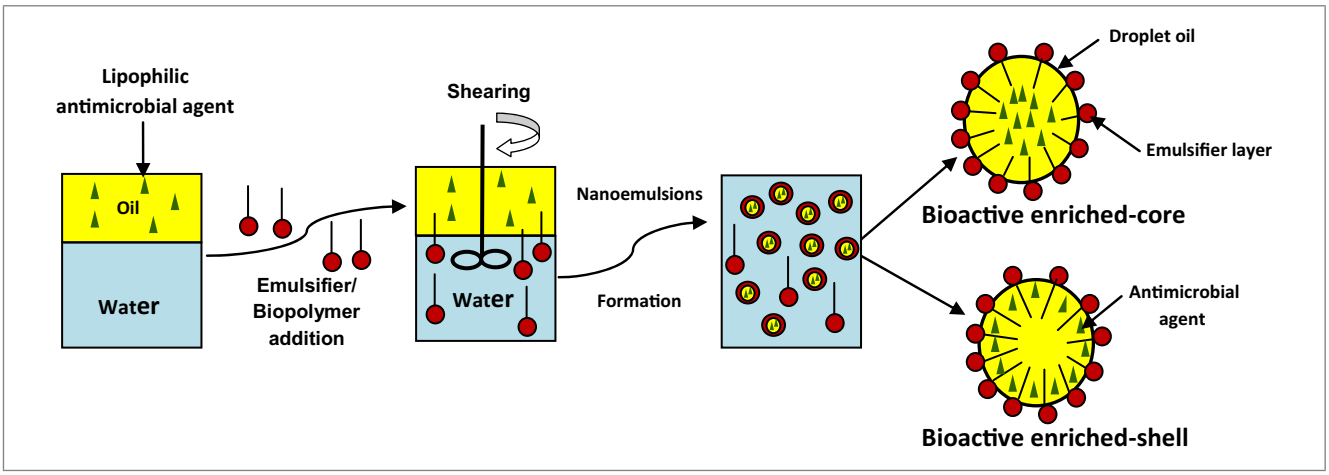

Figure 3–Oil-in-water nanoemulsion as nanoencapsulating system for lipophilic antimicrobial agents: formation and possible localizations of the entrapped antimicrobial compound.

formulations has been investigated as an effective strategy to place antimicrobial agents on the surface of solid products (Wu and others 2016). In fact, the incorporation of nanoemulsions into edible-coating solutions may improve their distribution and enhance their adhesion to the surface of solid products, which may increase potential synergies between the incorporated nanoemulsions and the coated product, and thus improve the functionality of antimicrobial nanoemulsions in retarding food deterioration. Recently, Severino and others (2015) reported the efficacy of modified CH-based coatings incorporating 0.05% of mandarin nanoemulsions in combination with ultraviolet-C treatment in reducing *Listeria innocua* populations by 3 log CFU/g, while at the same time maintaining firmness and preserving color of green beans after 15 d of storage at 4 °C. Likewise, Kim and others (2014) showed that the incorporation of 3% (w/w) of lemongrass nanoemulsions into carnauba wax-based coatings reduced weight loss, maintained firmness, antioxidant activity, and phenolic compounds, delayed the increase in total anthocyanin content in grape berries, and completely inhibited the growth of *Salmonella* Typhimurium and *E. coli* O157:H7 after 15 and 28 d of cold storage, respectively. In another study, Salvia-Trujillo and others (2015) compared the efficacy of NaAlg-based coatings containing lemongrass EO either in the form of nanoemulsions or conventional emulsions in enhancing microbial safety and preserving quality attributes of fresh-cut Fuji apples during 15 d of cold storage. Nanoemulsion-based coatings containing lemongrass EO at a concentration of 0.1% (v/v) were shown effective in reducing *E.coli* counts up to undetectable levels after 11 d of cold storage, while more than 10^3 CFU/mL of viable *E. coli* cells were detected in fresh-cut apples treated with conventional emulsions at the same lemongrass EO concentration after 14 d of cold storage. According to these authors, a reduction in oil droplet size may accelerate the penetration of antimicrobial compounds in bacterial cells, which may explain the enhancement in functionality of lemongrass oil when incorporated in the form of nanoemulsions, compared to conventional emulsions. Similarly, Wu and others (2016) highlighted the great potential of CH-based coatings incorporating citrus EO nanoemulsions for controlling microbial growth, inhibiting lipid oxidation, and prolonging the shelf life of silver pomfret (*Pampus argenteus*) fish from 12 to 16 d during refrigerated storage, when compared to conventional emulsions.

Antimicrobial multilayer coatings for food preservation

Antimicrobial multilayer coatings are another promising approach for providing better antimicrobial agent retention with a controlled release mechanism. This innovative approach lies in incorporating antimicrobial compounds into multilayered/nanolaminate systems (control layer/matrix layer/barrier layer) formed by the LbL electrostatic deposition technique, which consists in immersing solid substrates into film-forming solutions of oppositely charged polyelectrolytes, followed by a drying step to remove the excess solution attached to the surface after each dipping step (Figure 4).

These multilayered structures are able to act as effective antimicrobial delivery systems, thanks to their inner and barrier layers that may, respectively, control the diffusion rate of antimicrobial compounds embedded in the matrix layer and prevent their migration toward the outside of the package (Karam and others 2013). Overall, the diffusion process of antimicrobial agents through multilayer architecture depends on the assembly thickness, the tortuosity of the diffusion pathway, and the interactions between the polymer and the antimicrobial agent. Recently, the LbL assembly technique has gained great interest as a new strategy for coating perishable foods such fresh fruits and meat products. In addition to its added advantages of experimental simplicity and controlled release of antimicrobial agents, this technique leads to the development of multilayer coatings with specific thicknesses, properties, and performance, and is able to improve both quality and stability of coated food products. In this context, Mantilla and others (2013) reported the high potential of multilayered edible coatings, made from NaAlg, pectin, and calcium chloride, and incorporated with an antimicrobial complex (beta-cyclodextrin and trans-cinnamaldehyde) in the form of nanoemulsions for controlling microbial growth, preserving color and texture of fresh-cut pineapples, and extending their shelf life to 15 d at 4 °C. In another study, Sipahi and others (2013) showed that the application of a multilayered edible coating based on 1% (w/w) alginate, 2% (w/w) of a natural antimicrobial complex (beta-cyclodextrin and trans-cinnamaldehyde), and 2% (w/w) pectin on fresh-cut watermelon reduced their weight loss by more than 60%, decreased coliforms and yeast and mold counts, respectively, by 1.5 log CFU/g and 4 log CFU/mL, and prolonged their shelf life to 15 d during refrigerated storage, without affecting their firmness and sensory

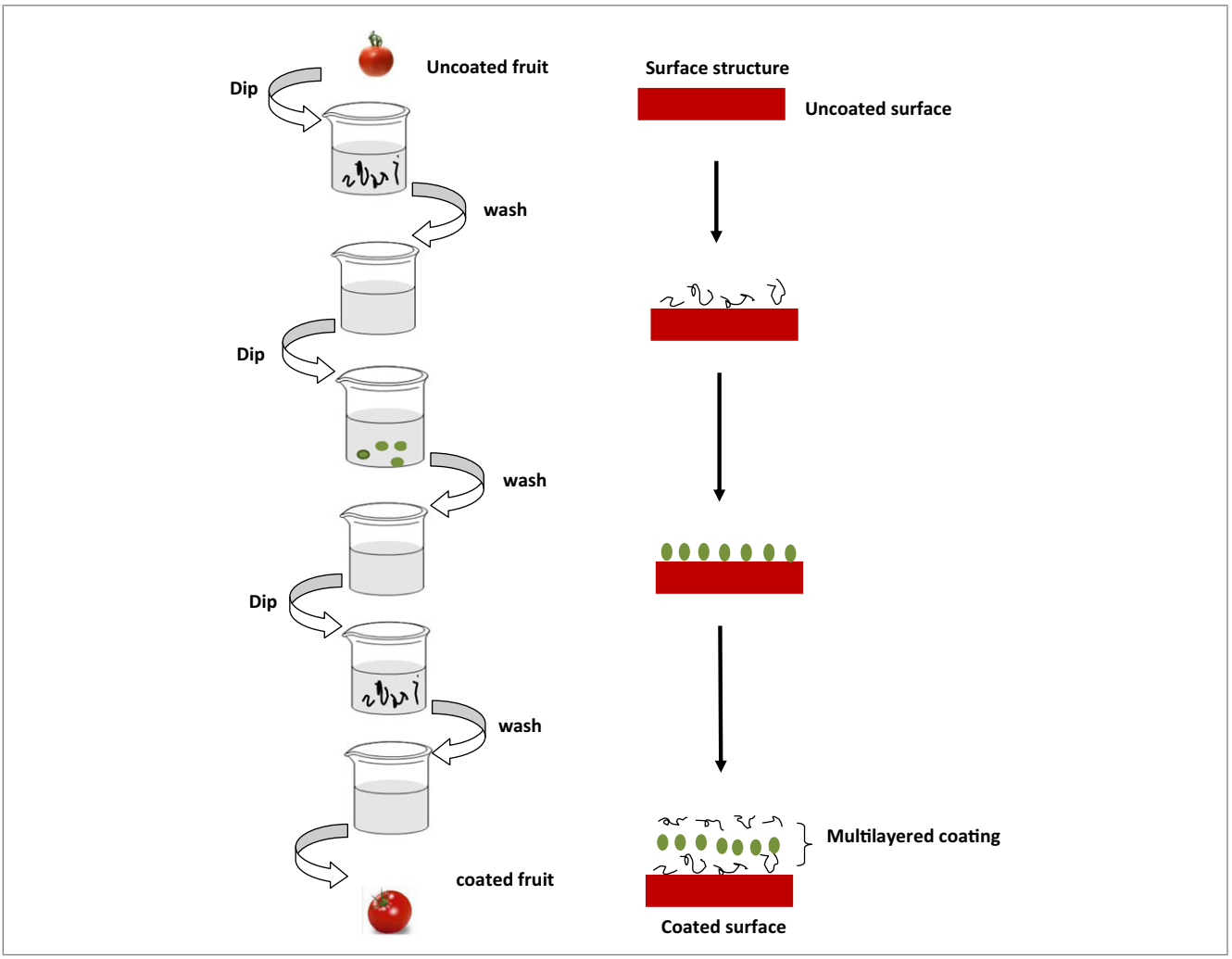

Figure 4–Schematic representation of a multilayered coating formation on fruit surface.

attributes. Likewise, Moreira and others (2014) highlighted the high potential of multilayered antimicrobial edible coatings based on CH and pectin incorporating a beta-cyclodextrin and trans-cinnamaldehyde antimicrobial complex, in the form of nanoemulsions, for reducing weight loss of fresh-cut melon, maintaining their texture, preserving their color and total carotenoids content, and extending their shelf life up to 15 d during refrigerated storage.

Methods for the Antimicrobial Evaluation of Edible Films and Coatings

The most common antimicrobial agents used are organic acids, CH, nisin, the LPS, and some plant extracts and their EOs (Campos and others 2011; Khwaldia 2011; Atarés and Chiralt 2016). Although several standardized assays have been established for antimicrobial susceptibility testing of conventional drugs, there is no standard methodology to evaluate the inhibitory activity of potential preservatives from natural resources. Modifications have been made on these standard and approved methods to evaluate the antimicrobial activity of natural compounds and extracts and to determine their MICs (Burt 2004). Many published papers have described the *in vitro* tests of antimicrobial activity assessment of naturally occurring antimicrobial agents (Burt 2004; Das and others 2010; Nasir and others 2015). It is not easy to perform a comparison of the results obtained from different published papers because the antimicrobial efficacy of a natural extract may be affected by many factors, such as the type, origin, chemical structure, solubility and extraction method of antimicrobial compounds, assay choice, test microorganisms, the volume of inoculum, culture medium, and growth and incubation conditions (McHugh and others 2009). In fact, the selection of an antimicrobial susceptibility testing methodology may be based on several criteria: reproducibility, reliability, ease of performance, purpose of the assay, antimicrobial nature, characteristics of target microorganisms, cost, accuracy, flexibility, and availability of skilled personnel (Campos and others 2011; OIE 2012).

In vitro methods

Many researchers have been interested in evaluating the efficacy of these antimicrobial agents in edible films and coatings. In the literature, several *in vitro* studies have been performed to assess the antimicrobial activity of film-forming solutions incorporated with natural or GRAS antimicrobial agents and their resulting films. Table 3 summarizes relevant *in vitro* methods for the antimicrobial evaluation of coating solutions and films. These *in vitro* methods include agar disk diffusion, well diffusion, dilution methods, poisoned food technique, spore germination assay, enumeration by plate count method, and film surface inoculation test.

Table 3–*In vitro* studies of coating formulations and films incorporating natural or GRAS antimicrobial agents

Polymer formulation	Antimicrobial agents	Loading	Microorganisms	Assay performed	Results	Reference
NaAlg	Methyl cinnamate Carvacrol	0.25% to 1.25% (w/w)	*E. coli* and *B. cinerea*.	Overlay diffusion test (for coating solutions)	Growth inhibition of *E. coli* and *B. cinerea*	Peretto and others (2014)
NaAlg Locust bean gum (LBG)	*Wickerhamomyces anomalus* killer yeast	10^7 CFU/mL	*P. digitatum*	Count of *P. digitatum* in potato dextrose agar (PDA) medium	Growth inhibition of *P. digitatum* in PDA medium High viability of killer yeasts after 15 d of storage	Aloui and others (2015)
Sodium caseinate (NaCas)	CH	CH/protein = 0.8/1 (w/w)	Native microfloras of carrot, cheese, and salami	Well agar diffusion method (for film-forming solutions) Tube-assay method (for film-forming solutions) Diffusion-type assay (for films): measurement of inhibition area	Inhibition of the microfloras of carrot and cheese CH and NaCas/CH films exerted significant antimicrobial effects on the microflora of cheese and salami	Moreira and others (2011)
Whey protein isolate	LPS	11 to 29 mg LPOS/g coating		Plate counting test Disc-covering and disc-surface-spreading tests	Inoculation before or after application of coatings had no effect on inhibition of *L. monocytogenes* Inhibition of the growth of *L. monocytogenes* by 4.2 log CFU/cm^2 for coatings incorporating LPOS (29 mg/g film) and placed either above or below the inoculum	Min and others (2005)
CH	Lime and thyme	0.1%	*Rhizopus stolonifer*	Measurement of Mycelial growth Determination of sporulation (number of spores/mL)	Coating formulations had high activity against *E. coli* Coating formulations containing CH, beeswax, and lime EO completely inhibited growth of both microorganisms	Ramos-García and others (2012)
	EOs Besswax		*E. coli* DH5α	Count of *E. coli* colonies on agar plates incubated for 48 h		
CH	*Mentha piperita* L.(MPEO) or *Mentha* × *villosa* Huds (MVEO) EOs	1.25 or 2.5 μL/mL	*Aspergillus niger, Botrytiscinerea, Penicillium expansum*, and *Rhizopus stolonifer*	Determination of the percent inhibition rates of the radial mycelial growth using the "poison food" technique Determination of the percent inhibition of spore germination using the "cavity slide" method	The different coating formulations based on CH /MPEO and CH/MVEO strongly inhibited the growth of all tested fungi CH/EOs formulations inhibited conidial germination of all tested fungal strains by more than 75%	Guerra and others (2015)

(*Continued*)

Table 3–Continued

Polymer formulation	Antimicrobial agents	Loading	Microorganisms	Assay performed	Results	Reference
CH	Clove oil	0.05% (v/v)	*E. coli* and *S. aureus*	For coating solutions: Paper disc-agar diffusion assay Growth inhibition assay	The highest inhibition rates of *E. coli* and *S. aureus* (99.17 and 96.42%, respectively) were achieved by CH-based coating solutions incorporating clove oil and ethylenediaminetetraacetate	He and others (2014)
CH	Allyl isothiocyanate (AIT)	5% (v/v)	*Listeria innocua*	Broth macrodilution assay (for films)	Composite films made from microemulsions significantly inactivated *Listeria innocua*	Guo and others (2015)
CH	Bergamot or bitter orange EOs	2% (v/v)	*A. flavus*	Mycelium growth assay using the "poison food" technique	Inhibition of both mycelial growth and conidial germination of *A. flavus* (by 55% and 85%, respectively)	Aloui and others (2014b)
Locust bean gum (LBG)				Conidial germination inhibition assay using the "cavity slide" method		
CH Carboxymethylcellulose NaCas	Rosemary, oreganum, olive, capsicum, garlic, onion, and cranberry oleoresins	1% (w/w)	Native microflora of butternut squash, *Listeria monocytogenes*	Agar diffusion assay (for film-forming solutions)	Low antimicrobial effects against target microorganisms	Ponce and others (2008)
Gelatin	Lime juice and garlic extract	30% to 50% (v/v)	Fishborne pathogens and spoilage bacteria (*E. coli, Salmonella typhi, Y. enterocolitica, S. aureus*, *Bacillus subtilis*, *Micrococcus* sp., *B. cereus,* and *Bacillus pumilus)*	For coating solutions: Agar well diffusion method Microbroth dilution method	Coating formulations based on gelatin/garlic extract were more effective against target microorganisms than gelatin/lime juice coating solutions	Thaker and others (2015)

NaAlg, sodium alginate; CH, chitosan; LPS, lactoperoxidase system; NaCas, sodium caseinate.

Disk agar diffusion assay. This is a simple qualitative test, which has been widely used to measure the antimicrobial activity of antimicrobial agents alone or incorporated in coating solutions and films on a seeded solid medium (Ponce and others 2008; Moreira and others 2011; He and others 2014). This preliminary screening test is based on the determination of the diameter of an inhibition zone around paper disks impregnated with film-forming solutions or film disks containing the antimicrobial substance. Although its low cost, reproducibility, and ease of performance are acceptable, this diffusion method is not suitable for the MIC determination and the distinction between bacteriostatic and bactericidal effects (Burt 2004). Moreover, many factors, including size, polarity and shape of the diffusing molecule, and the chemical structures of the agar and the film matrix, can affect the diffusion of tested antimicrobial agents (Campos and others 2011). This assay in not suitable for mixtures containing substances with different diffusion rates (McHugh and others 2009).

Agar well diffusion assay. This is based on the same principle as the disk diffusion assay and consists of introducing antimicrobial samples into *punched wells* of *inoculated agar* plates (Moreira and others 2011; Thaker and others 2015). After incubation, the diameters of clear zones are measured. This screening test is inexpensive, simple, and easy to interpret and to reproduce (Nasir and others 2015).

Dilution methods. These are reproducible and quantitative tests used to determine the MIC of antimicrobial substances. They can be used for many microorganisms and are able to *distinguish between* bactericidal and bacteriostatic effects (OIE 2012).

The *agar dilution assay* involves the incorporation of coating solutions containing the antimicrobial at known concentrations into

an agar medium followed by the application of a defined inoculum to the agar surface (OIE 2012). This simple method is inexpensive, does not necessitate specialized laboratory facilities, and offers the possibility to screen a large number of samples. However, this method has some limitations which include the subjectivity and time used in manual interpretation of results, the difficulty of obtaining a stable dispersion of antimicrobial substances in agar, the dependence of inhibition zone on inoculum size, incubation temperature, the nature and amount of surfactants, and the presence of volatile compounds (Panda 2012).

Broth dilution methods are based on testing microorganisms of standard concentrations against different concentrations of an antimicrobial agent (alone or incorporated in coating solutions and films) in a liquid medium of predetermined formulation. In contrast to disk diffusion and agar dilution methods, the broth dilution assay can be used to monitor antibacterial activity over time. The macrodilution assay is performed in tubes containing a minimum volume of 2 mL, while the microdilution method utilizes smaller volumes of tested antimicrobial substances and is performed using microtitration plates (OIE 2012). In several studies assessing the antimicrobial activity of coating formulations and films by broth dilution tests, the most widely used methods for end point determination include the optical density measurements (Min and others 2005; Moreira and others 2011; Bustos and others 2016), the enumeration of viable cells (He and others 2014; Guo and others 2015), and the absorbance evaluation (Severino and others 2015).

The broth microdilution is a fast, simple, and cost-effective method which allows the simultaneous screening of a large number of samples. However, this assay may be inconvenient for highly colored extracts because of their possible interference with the end point colorimetric method. Moreover, unreliable results can be obtained when testing anaerobic microorganisms, which exhibited little growth in the presence of oxygen (CLSI 2009).

Poisoned food technique. This is frequently employed for evaluating the antifungal activity of antifungal agents and plant extracts alone or incorporated in coating solutions and films. This technique consists of poisoning a solid agar or a liquid medium with different concentrations of antifungal substances to be tested and then allowing a test fungus to grow on this medium (Ali-Shtayeh and Abu Ghdeib 1999). This assay is based on the determination of mycelial growth inhibition percentage by measuring the mycelial growth diameter in the treated plate (with antifungal substances) and the untreated plate (Marandi and others 2011). The failure to distinguish between effects on sporulation and those on growth is a deficiency of the poisoned food technique. The antifungal response is dependent on many factors such as presence of surfactants, fungal inoculum size, and pH of the medium.

Spore germination assay or "cavity slide" technique. This is a specific test for fungal pathogens (Cronin and others 1996). It consists in placing different concentrations of the antifungal substance to be tested on cavity slides containing a standard concentration of the conidial suspension. This assay is based on microscopic observation of spore germination at different time intervals (Dubey 1991). The major limitations of this method include the large amount of labor needed for microscopic evaluation of germination and its inconvenience for many antifungal compounds that do not affect spore germination. Both spore germination and mycelial growth assays are highly useful as they provide information on the possible mechanism of action of antifungal compounds (Slawecki and others 2002).

Plate counting method. This is used to evaluate the antimicrobial efficacy of films and coatings. It consists in counting microbial populations over time, on previously inoculated surface agar plates in contact with the film disk incorporated with antimicrobial agents (Campos and others 2011). The results of this assay can provide information on the behavior of a film or coating in contact with a contaminated surface (Min and others 2005). This assay is time consuming and space consuming and requires specialized equipment that must be prepared correctly.

Film surface inoculation test. This is based on counting the microbial population previously inoculated on the surface of a film disk placed on a semisolid medium (Campos and others 2011). The results of this assay can provide information on the ability of a film or coating to act as a barrier to external contamination, and to simulate microbial contamination on coated products (Vásconez and others 2009).

Methods for edible coatings applied on food systems

Although many *in vitro* assays have been developed for the antimicrobial evaluation of coating solutions and films, the interpretation of results is onerous because of the influence of biological and technical factors. Moreover, more pronounced antimicrobial effects of edible films and coatings have been reported in culture media than in real food systems (Campos and others 2011).

The assays performed to assess the antimicrobial activity of films and coatings applied on foods are based on counting the native or inoculated microorganisms over storage time (Table 4). In this context, Fernández-Pan and others (2014) developed whey protein isolate coatings containing oregano or clove EOs and studied their effects on the microbial quality and shelf life of chicken breast fillets. They reported high effectiveness of these antimicrobial coatings compared to the direct application of tested EOs on chicken breast fillets. The most effective formulations against mesophilic and psychrotrophic bacteria were those incorporating 20 g/kg oregano EO. These coating formulations achieved the highest microbial count reductions on chicken fillets and extended their shelf life up to 13 d at 4 °C. He and others (2014) reported that CH/clove oil coatings on pork slices reduced total viable counts by 3.09 log CFU/g compared with uncoated samples. Similar studies based on counting the native microorganisms over storage time in shrimp meat (Aşı k and Candoğan 2014), fish fillet (Jasour and others 2015), rainbow trout (Hosseini and others 2016), and pork meat (Bonilla and others 2013) have achieved the same results. Likewise, Moreira and others (2011) evaluated the antimicrobial efficiency of coatings and wrappers based on CH or sodium caseinate/CH and applied on cheese, salami, and carrots. The tested coatings and wrappers reduced mesophilic, psychrotrophic, and yeast and mold counts in the range of 2 to 4.5 log CFU/g.

In another study, Neetoo and Mahomoodally (2014) found that cellulose-based coatings containing nisin (25000 IU/mL), 0.3% potassium sorbate (PS), and 0.1% sodium benzoate (SB) significantly reduced the population of *L. monocytogenes* in surface-inoculated cold-smoked salmon slices stored at 4 °C for 4 wk. These coating treatments based on ternary combinations of nisin, PS, and SB reduced *L. monocytogenes* by 2.9 log CFU/cm^2 at the end of the storage period at 4 °C, exhibiting the best antilisterial activity. Likewise, Guo and others (2015) proved the antimicrobial activity of CH-allyl isothiocyanate coatings on ready-to-eat meat samples. The periodical enumeration of *L. innocua* in coated and

Table 4–Methods used to evaluate the antimicrobial performance of edible coatings applied on food systems

Polymer formulation	Antimicrobial agents	Loading	Microorganisms	Assay performed	Results	Reference
PLA	CH	5% and 10% (w/w)	Aerobic mesophilic and coliform microorganisms	Microbial counts of coated minced pork meat samples	A 2 log reduction in the coliform counts	Bonilla and others (2013)
PLA	PLA EDTA, SB	0.25% (w/w)	*E. coli* O157:H7 and *Salmonella stanley*	Enumeration of inoculated microbial population on the apple surface	A 4.7 log reduction in *E. coli* and *S. Stanley* populations in apples coated with PLA/SB + LA or PLA/SB + LA + EDTA and stored for 14 d	Jin and Niemira (2011)
NaCas	CH	Weight ratio CH/Protein = 0.8/1	Mesophilic and psychrotrophic aerobic bacteria and yeasts and molds	Microbial counts of coated cheese, salami, and carrots	Reduction in mesophilic, psychrotrophic, and yeasts and molds counts (2 to 4.5 log CFU/g)	Moreira and others (2011)
NaAlg	Malic acid, cinnamon, palmarosa, and lemongrass EOs	0.3% and 0.7%	Mesophilic and psychrophilic bacteria, yeasts and molds	Count of native flora in coated melon	Reduction in mesophilic and psychrophilic counts in fresh-cut melon coated with NaAlg/malic acid+EOs	Raybaudi-Massilia and others (2008)
	Eugenol, geraniol, and citral	0.5%	*Salmonella enteritidis*	Enumeration of *S. enteritidis* in coated inoculated fresh-cut melon	The highest reductions in *S. enteritidis* population were achieved by NaAlg-based coatings incorporating 0.3% of lemongrass and 0.7% of cinnamon	
NaAlg	Grapefruit seed	1%	*P. digitatum*	Determination of decay percentage of inoculated	Reduction in mold counts (36% to 42%)	Aloui and others (2014a)

(*Continued*)

Table 4–Continued.

Polymer formulation	Antimicrobial agents	Loading	Microorganisms	Assay performed	Results	Reference
	extract (GSE) and grapefruit essential oil (GEO)	(w/w)		grape berries during storage at 20 °C		
NaAlg pectin	Citral and eugenol	Eugenol at 0.1% and 0.2% Citral at 0.15% and 0.3%	Aerobic mesophilic and psychrophilic bacteria, molds and yeasts.	Count of native flora in coated strawberries	Reduction of microbial spoilage during a 13-d storage	Guerreiro and others (2015)
NaAlg LBG	*W. anomalus* killer yeast	10^7 CFU/mL	*Penicillium digitatum*	Determination of green mold incidence on "Valencia" oranges artificially inoculated with *P. digitatum* during storage at 25 °C	Reduction of green mold by 73% at the end of the storage period	Aloui and others (2015)
Methylcellulose/HPMC	Nisin, SL, SD, PS, SB	(25000 IU/mL) Binary or ternary combinations of nisin and chemical antimicrobial agents were tested	*L. monocytogenes* Spoilage aerobic and anaerobic bacteria	Enumeration of *L. monocytogenes* in coated inoculated cold smoked salmon Count of spoilage microflora in coated cold smoked salmon	Reduction in *L. monocytogenes*, and anaerobic and aerobic spoilage populations by more than 4.2 log CFU/cm^2 at the end of a 4-wk storage	Neetoo and Mahomoodally (2014)
Whey protein isolate (WPI)	Oregano and clove EOs	10 or 20 g/kg	Total aerobic mesophilic bacteria, *Enterobacteriaceae*, total aerobic psychrotrophic bacteria, lactic acid bacteria, and *Pseudomonas* spp	Microbial counts of coated chicken breast fillets	The most effective coatings in reducing microbial counts in chicken breast fillets and increasing their shelf life up to 13 d were those containing 20 g/kg oregano EO	Fernández-Pan and others (2014)
CH	Clove oil	0.05% (v/v)	Total viable count	The spread-plate method	A 3.09 log reduction in total viable count	Poverenov and others (2014)
CH	Natamycin Nisin Pomegranate extract	1% (w/v)	Aerobic mesophilic bacteria, coliform bacteria, and yeasts and molds	Microbial counts of coated strawberries	The highest microbial count reductions were achieved by CH coatings incorporating pomegranate extract or natamycin	Duran and others (2016)

(*Continued*)

Table 4–Continued.

Polymer formulation	Antimicrobial agents	Loading	Microorganisms	Assay performed	Results	Reference
CH	*Mentha piperita* L (MPEO) or *Mentha* × *villosa* Huds (MVEO) EOs.	1.25 or 2.5 μL/mL	*A. niger*, *Botrytis cinerea*, *Penicillium expansum*, and *Rhizopus stolonifer*	Determination of disease incidence in inoculated cherry tomato fruits	Inhibition of the growth of all tested fungi during storage at room temperature or low temperature.	Guerra and others (2015)
CH	Lysozyme	60% (dry weight of lysozyme/dry weight of CH)	*L. monocytogenes*, or *Salmonella enterica Ser. Enteritidis*	Enumeration of *L. monocytogenes* and *S. Enteritidis* in coated and inoculated peeled eggs	A 4 log reduction in *S. Enteritidis* population	Kim and others (2008)
			Total plate counts, coliforms, yeasts, molds	Microbial counts of coated peeled eggs	Total inhibition of TPC, coliforms, yeasts, and molds during the 6-wk storage	
CH	*Aloe vera* extract	0.5% (v/v)	Total yeasts and molds	Total yeasts and molds counts of coated blueberries	Inhibition of *B. cinerea* growth on noninoculated blueberries during storage for 6 d Microbial growth delay in artificially inoculated blueberries	Vieira and others (2016)
CH	AIT	5% (v/v)	*Listeria innocua*	Enumeration of *Listeria innocua* in coated and inoculated ready-to-eat meat samples	A 3 log reduction of *L. innocua* after 35 d of storage	Guo and others (2015)
Modified CH (3% *N*-palmitoylCH)	Carvacrol nanoemulsion	0.05% (w/v)	*E. coli* O157:H7 and *Salmonella* Typhimurium *E. coli* O157:H7 and *Salmonella* Typhimurium	Count of *E. coli* O157:H7 and *Salmonella* Typhimurium populations in coated and inoculated green beans	Reduction of bacterial growth on green beans stored at 4 °C for 13 d	Severino and others (2015)
CH LBG	Bergamot or bitter orange EOs	2% (v/v)	*A. flavus*	Determination of decay percentage of inoculated dates during storage at 25 °C	Reduction of fungal decay in dates in the range of 52% to 62% at day 12.	Aloui and others (2014b)
CH Carboxymethyl cellulose NaCas	Rosemary, oreganum, olive, capsicum, garlic, onion, and cranberry oleoresins	1% (w /w)	Mesophilic aerobic bacteria	Count of mesophilic bacterial in coated butternut slices	No significant antimicrobial effect of CH coatings containing rosemary and olive oleoresins	Ponce and others (2008)

PLA, polylactic acid; CH, chitosan; EDTA, ethylenediaminetetraacetic acid; SB, sodium benzoate; NaCas, sodium caseinate; NaAlg, sodium alginate; LBG, locust bean gum; HPMC, hydroxypropylmethylcellulose; SL, sodium lactate; SD, sodium diacetate; PS, potassium sorbate; AIT, allyl isothiocyanate.

inoculated ready-to-eat meat samples revealed significant reductions after 35 d of storage at 10 °C. Kim and others (2008) reported that *S. enteritidis* growth was effectively controlled on hard-boiled eggs by CH-lysozyme coatings, which reduced *S. enteritidis* by 4 log after 4 wk of storage at 10 °C compared with untreated eggs. Microbial enumeration was conducted on previously inoculated eggs at 10^4 CFU/g and coated by immersion and then stored for 4 wk at 10 °C. Another coating formulation containing alginate and lemongrass EO was developed by Raybaudi-Massilia and others (2008) who found that it was most effective in reducing *S. enteritidis* counts in fresh-cut melon stored at 5 °C for 21 d. CH-based coatings incorporated with beeswax or oleic acid and lime or thyme EO were applied on tomatoes at a small scale and at the semicommercial level by Ramos-García and others (2012). Tomatoes artificially wounded were dipped in antimicrobial coatings. After drying (2 h), 20 mL of the *Rhizopus* spore suspension (10^5 spores/mL) and 35 mL bacterial solution with 10^5 CFU/μL were distributed over the injured fruit surface. At the semicommercial level, it was demonstrated that the lowest disease incidence (44.6%) was observed in fruit coated with CH + oleic acid and stored at 12 °C. A complete control of *E. coli* DH5α at different maturity stages (breaker, pink, and red) and at 2 storage temperatures (12 and 25 °C) was provided by CH + beeswax + lime EO, and CH + beeswax coatings. The effect of MPEO or MVEO incorporated in CH-based coatings on the control of pathogenic fungi causing postharvest mold infections in cherry tomato fruit was assessed by Guerra and others (2015) who determined disease incidence in tomatoes inoculated with *A. niger, B. cinerea, Penicillium expansum*, or *Rhizopus stolonifer* and coated with CH/MPEO or CH/MVEO solutions. The results indicated that these active treatments completely inhibited fungal growth at 12 or 25 °C.

On the other hand, Vieira and others (2016) tested the antimicrobial efficiency of CH coatings incorporated with *Aloe vera* extract on blueberries, and they found microbial count reductions over 25 d. Guerreiro and others (2015) reported the effectiveness of coatings based on NaAlg and pectin containing 2 main EO constituents, citral and eugenol, in enhancing the shelf life of strawberries. Similar studies based on the standard plate count method for microbiological analysis of coated and uncoated strawberries (Duran and others 2016), minimally processed papayas (Narsaiah and others 2014), and fresh-cut pineapples (Azarakhsh and others 2014) had similar results.

Antimicrobial agents can be transported from the edible coating to the food matrix by diffusion release. Solubility and permeability of the antimicrobial agent are among the main factors affecting the diffusion release through the coating matrix (Quirós-Sauceda and others 2014). The quantification of the release rate of an antimicrobial is highly useful as it has a great impact on antimicrobial effectiveness. Depending on application, antimicrobial coatings are formulated to provide either a quick release or a slow release of the antimicrobial over storage time. Many recent studies have been published on the evaluation of both the antimicrobial properties of edible films and coatings and the release mechanism of the incorporated antimicrobial agents (Boyaci and others 2016; Bustos and others 2016; Chen and Liu 2016).

Conclusions

A wide variety of naturally occurring antimicrobial compounds derived from animal, plant, and microbial sources have been investigated as alternatives to synthetic ones for ensuring food safety and quality, owing to their antimicrobial properties against a broad range of foodborne microorganisms. Although successful examples of the use of natural antimicrobial agents as food preservatives have been reported in the literature, their practical application in the food industry still faces limitations due to their high impact on organoleptic characteristics of food products, their rapid diffusion in the food, and their possible interaction with food components. Recently, the incorporation of natural antimicrobial agents into bio-based polymeric matrices has been investigated as a promising alternative to overcome these limitations. In this context, different approaches have been used to evaluate the antimicrobial performance of the resulting antimicrobial coatings and films either *in vitro* or *in situ*, when directly applied as coatings on food systems. In the last few years, nanoencapsulating and multilayered/nanolaminate antimicrobial delivery systems have been developed to enhance the functionality of edible coatings and improve their effectiveness in preserving food quality. Nanoemulsions and nanolaminated-based coatings have shown great potential for controlling spoilage and growth of pathogenic microorganisms and extending shelf life of a variety of perishable foods, mainly meat products and fresh and minimally processed fruits and vegetables. A new generation of bionanocomposite coatings is being currently developed through the incorporation of bio-based nanofillers such as CH and nanocellulose nanoparticles into coating formulations. These bionanocomposite coatings may be suitable as carriers for antimicrobial agents, since they are able to confer greater retention efficiency and slower active compound release due to the formation of tortuous pathways into the polymeric matrix that may reduce the diffusivity of the entrapped antimicrobial compound.

Future trends should focus more on investigations pertaining to physicochemical properties of nanodelivery systems as well as on their interactions with food matrices. On the other hand, mathematical models for prediction of bioactive release kinetics from nanocarriers should be studied to enhance understanding of nanodelivery systems' functionality and optimizing their performance.

Author Contributions

All authors contributed to the writing and the final approval of the manuscript.

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