Delivery Systems of Antimicrobial Compounds to Food

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ABSTRACT

Background: Food antimicrobial compounds are naturally present or purposefully added in food systems to retard microbial growth or cause microbial death to improve food safety and quality. Directly added antimicrobial compounds to food products may face several major challenges: (1) poor solubility in aqueous systems; (2) limited stability against chemical or physical degradation; (3) uncontrolled release, and (4) possible adverse effects on food sensory qualities. One approach to address these problems is the use of delivery systems.

Scope and approach: In this review, antimicrobial compounds are categorized into synthetic and naturally occurring. They are briefly reviewed with their properties and applications.

Considering their structural and physicochemical aspects, three types of delivery system are discussed: (1) emulsion-based, (2) nanosized carrier-based, and (3) film or coating-based.

Applications of antimicrobial delivery systems in food are discussed.

Key findings and conclusions: Compared with the direct addition of antimicrobial compounds, the use of delivery systems may protect active compounds from degradation, improve their solubility in aqueous phase, decrease their impact on food sensory qualities, and control their release. Although numerous delivery systems have shown efficacies under *in vitro* conditions, their antimicrobial performances need to be verified in real food systems. The availability of low-cost, food-grade carrier materials and the knowledge of interactions between delivery systems and other food components are essential to achieving industrial success for the design and use of delivery systems of antimicrobial compounds.

Keywords: Antimicrobial compounds; Emulsions; Nanosized carriers; Films and coatings; Delivery systems

1. Introduction

Foodborne disease is a major concern in today's world. Pathogens, especially bacteria are responsible for causing a wide variety of infections and intoxications through contaminated foods that result in moderate to severe illness and deaths (Scallan et al., 2011). On the other hand, food deterioration caused by spoilage microorganisms throughout storage and distribution has serious impact on food quality, shelf-life, and food loss. Therefore, protecting food systems against pathogenic and spoilage microbes deserves our special attention in order to improve food safety, quality, and security.

A number of physical processing techniques have been used in food preservation to maintain food safety and quality. Such techniques include thermal processing, high-pressure processing, pulsed electric field processing (PEF), plasma processing, food irradiation, etc. At present, thermal processing remains the most important method of food preservation. It is involved in most types of food products. For example, liquid foods, especially fruit juice and dairy products, are often disinfected or sterilized by heating such as pasteurization and ultra-high temperature treatment. High-pressure processing has been used to treat sliced cooked ham and juices in the US market (Balasubramaniam, Martinez-Monteagudo, & Gupta, 2015). Recently, Sampedro et al. (2013) evaluated a commercial scale PEF system using orange juice as a model. However, PEF processing has not been used in an actual food production line. Food irradiation has been approved in more than 60 countries, with about

500,000 metric tons of food annually processed worldwide. In the USA, irradiation has found applications in raw foods of both animal and plant origins. In EU, due to consumers' negative impression on irradiation, only dried herbs, spices, and seasonings can be processed with irradiation (Arvanitoyannis, Stratakis, & Tsarouhas, 2010).

While showing various levels of success, these processing techniques have several limitations. The first challenge of using these techniques in food preservation is the high installation and maintenance cost. Secondly, processes such as thermal treatment may reduce the nutrition value or trigger non-desirable physicochemical reactions that may affect sensory characteristics of food. Thirdly, there could be a negative public perception for the use of some non-conventional techniques such as radiation. Therefore, there has been a great need to establish alternative preservation strategies to achieve high levels of food safety and security. Among these, one strategy to inhibit the growth of spoilage and pathogenic microorganisms is the application of antimicrobial compounds. Antimicrobials are defined as food additives that inhibit the growth of or inactivate spoilage and pathogenic microorganisms in food systems, thereby enhancing the shelf-life of foods (Tiwari et al., 2009). Successful applications of antimicrobials may overcome, at least partially, the limitations associated with the use of physical processing techniques.

However, the use of antimicrobial compounds in food systems faces considerable challenges. For example, different food components (e.g. enzymes, glutathione, sodium metabisulphite and titanium dioxide) may significantly degrade antimicrobials (such as peptides) by specific or non-specific interactions (Bhatti, Veeramachaneni, & Shelef, 2004).

Similarly, plant-derived antimicrobials such as essential oil components (thymol, eugenol, carvacrol and trans-cinnamaldehyde) also can be depleted in food systems. They may bind with hydrophobic food components, such as lipids and proteins, which reduce their effective concentration against the target organism (Shah, Davidson, & Zhong, 2012). Additionally, essential oil components have limited water solubility, which affects their antimicrobial properties since sufficient concentration cannot be achieved in the aqueous phase (Gaysinsky et al., 2007). Due to poor water solubility, some compounds have to be used at elevated concentrations, leading to sensory defects due to their strong medicinal aroma (Shah, Davidson, & Zhong, 2012). Compounds such as thymol and carvacrol are highly volatile in nature. They can exert their antimicrobial activity in the vapor phase at relatively lower concentrations than the liquid phase, thereby causing minimum effect on organoleptic properties. However, high volatility also means that such compounds tend to lose their antimicrobial activity due to rapid evaporation and chemical interactions including oxidation, which sometimes cannot meet the requirements for controlled release in food preservation (Kamimura, Santos, Hill, & Gomes, 2014). In general, there is a genuine need to address these challenges through the protection of antimicrobials in foods.

One important strategy of protection is through the combination of antimicrobials with a delivery vehicle. A number of delivery systems have been designed for the encapsulation, protection, and sustained release of functional molecules such as antimicrobials, antioxidants, flavors, colors, vitamins, fatty acids, carotenoids, and phytosterols in food systems. Systems including microemulsions, nanoemulsions, liposomes,

nanoparticles, and nanofibers have shown increasing usage in the delivery of antimicrobials in foods (McClements, Decker, Park, & Weiss, 2009). With the development of novel delivery technologies, the uses of antimicrobials in food science would be more effective.

This review will elaborate different types of food antimicrobial compounds according to their sources (e.g. synthetic and naturally occurring) along with various types of delivery systems that may have potential in food applications. Thereafter, reported studies of antimicrobial delivery systems are discussed in detail.

2. Types of food antimicrobials

Food antimicrobials are chemical compounds that are naturally present in or purposefully added to foods, food packaging, food contact surfaces, or food-processing environments to retard microbial growth or cause microbial death (Davidson, Critzer, & Taylor, 2013). The major targets for antimicrobials are food pathogenic and spoilage microorganisms, whose metabolic products induce adverse elements such as toxins, off-flavors, or texture changes (Davidson, 2001). According to the different sources, food antimicrobials can be classified into two groups, i.e. synthetic and naturally occurring antimicrobials (Carocho, Barreiro, Morales, & Ferreira, 2014). Recently, a trend of ‘green’ consumerism appears and consumers desire fewer synthetic additives and more environmentally friendly compounds (Burt, 2004). Hence, there is an increasing demand for naturally occurring antimicrobials (e.g. lactoferrin, lysozyme, eugenol, thymol, chitosan,

natamycin, ϵ -polylysine, and nisin), which would serve as promising alternative food preservatives to control pathogenic and spoilage microorganisms (Tajkarimi, Ibrahim, & Cliver, 2010). In this section, we will first introduce synthetic antimicrobials. Thereafter, naturally occurring antimicrobials are subdivided into animal sourced, plant sourced, and microbial sourced. Those that have been approved for food by the FDA are summarized and discussed briefly.

2.1. Synthetic antimicrobials

Although consumers have become concerned about the safety of synthetic antimicrobials used in food, they are still widely used in the food industry. Among the 36 antimicrobials approved as food preservatives by the European Food Safety Authority (EFSA, 2014), 33 are synthetic antimicrobials, and only 3 (nisin, natamycin and lysozyme) are natural. Common synthetic antimicrobial preservatives and their applications in food are listed in **Table 1**.

In the form of weak organic acids or salts, benzoates, sorbates, and propionates are the most commonly used chemical preservatives in foodstuffs. They are generally used to retard the growth of yeasts and molds and also effective against a wide range of bacteria (Suhr & Nielsen, 2004). The antimicrobial mechanism of these compounds is associated with their pH-dependent equilibrium between the undissociated and dissociated state. Specifically, the compounds are able to enter bacterial cells freely when they are in the uncharged,

undissociated state at low pH. Subsequently, the molecule will dissociate upon encountering the higher pH inside the cell. As a result, there is a release of charged anions and protons inside the bacterial cells, leading to the membrane disruption, accumulation of toxic anions, and inhibition of essential metabolism (Brul & Coote, 1999). This mechanism is consistent with the fact that these antimicrobials are most active in foods of low pH values and essentially ineffective in foods at neutral pH (Ashagrie & Abate, 2012). The applications of these compounds in various foods are summarized in **Table 1**.

Other synthetic antimicrobials such as sodium nitrites and nitrates, and sulfur dioxide and sulfites, are also popularly used in the food industry. For example, sodium nitrite is a preservative widely used in meat products to control the bacteria that cause botulism. Biphenyl/diphenyl-derivatives are particularly used in citrus fruits to avoid spoilage caused by molds. Among the synthetic antimicrobials, some may cause negative side effects. Some studies have shown that the consumption of sodium benzoate may cause hyperactivity amongst children (McCann et al., 2007). The use of nitrite and nitrate in meat products also causes consumer's concern for they are probably carcinogenic to humans, although currently published articles indicated that there was no association between estimated intake of nitrite and nitrate in the diet and stomach cancer (Bryan et al., 2012). Controversial issues and consumers' concerns about the safety of artificial antimicrobials used in food are promoting researchers and food manufacturers to develop natural alternatives to such compounds.

2.2. Naturally occurring antimicrobials

In recent years, an increasing consumers' concern about the potential side effects of synthetic antimicrobials on health has created a demand for the use of naturally occurring alternatives in foods (Tajkarimi, Ibrahim, & Cliver, 2010). Compounds derived from natural sources are preferred due to their “green” nature as well as antimicrobial properties against a broad range of foodborne pathogens. In this review, naturally occurring antimicrobials are discussed briefly in three groups according to their sources: animals, plants, and microbes. Several antimicrobials (such as lactoferrin, thymol, nisin, and natamycin) derived from these sources are summarized in **Table 2**. They have been listed as GRAS (generally regarded as safe) by the FDA and widely studied for their role as antimicrobial agents against food spoilage and pathogenic microbes.

2.2.1. Animal sources

Numerous antimicrobials are derived from animal sources, most of which are in the form of antimicrobial peptides (polypeptides) such as lactoferrin, lactoferricin, lactoperoxidase, lysozyme, and ovotransferrin (Tiwari et al., 2009). In addition, chitosan represents the only antimicrobial of animal origin without the polypeptide structure. Antimicrobials derived from animal sources exhibit differing levels of antimicrobial activity and mode of action against microorganisms (**Table 2**). Currently, lactoferrin and lysozyme were accorded GRAS status by the FDA. Chitosan has also been considered safe as a food additive by countries such as Korea and Japan (Kong, Chen, Xing, & Park, 2010). However, all chitosan GRAS applications are “At notifier’s request, FDA ceased to evaluate the notice”

at this time, except for chitosan from *Aspergillus niger* as a secondary direct food ingredient in alcoholic beverage production at limited levels (FDA, 2013)

2.2.2. *Plant sources*

Plants have been found to be rich sources of antimicrobials, many of which are phenolic compounds found in the essential oils (EOs) of leaves, seeds, flowers, and bulbs and are therefore very aromatic in nature. It is thought that in nature these secondary metabolites allow the plant to resist phytopathogens and insects, but many have demonstrated inhibition or inactivation of bacterial and fungal species of importance in the food-processing arena (Hintz, Matthews, & Di, 2015).

Essential oils (EOs), also known as volatile or ethereal oils, are natural substances categorized as GRAS by the FDA (21 CFR §182.20; Burt, 2004), and most of them are derived from plants. Today, there are about 3000 essential oils that are known globally, and out of them, 300 have been well characterized and are important industrially (Burt, 2004). EO components demonstrate different biological activities including anti-inflammatory, expectorant property, psychoactivity, and antimicrobial activity (Monu, David, Schmidt, & Davidson, 2014). The antibacterial mechanism and cellular targets of EO components vary greatly due to the differences in their molecular structures and physicochemical properties (Lambert, Skandamis, Coote, & Nychas, 2001). Studies have confirmed that EO components with a higher proportion of phenolic compounds generally exhibit stronger antibacterial

effects (Lambert, Skandamis, Coote, & Nychas, 2001). Some examples of phenol-rich compounds include thymol, carvacrol, and eugenol. Essential oil components can penetrate the cell wall, cytoplasmic membrane, and the mitochondrial membrane structures resulting in the leakage of the membrane, disruption of proton motive force, and excessive loss of critical ions and molecules that leads to cell death (Ultee, Bennik, & Moezelaar, 2002). The antimicrobial properties of EO components such as thymol and eugenol can be found in **Table 2**.

2.2.3. Microbial sources

Antimicrobials derived from microbial sources are primarily the bacteriocins of Gram-positive bacteria. Bacteriocins are microbial sourced peptides that can kill bacteria or inhibit bacterial growth (Cleveland, Montville, Nes, & Chikindas, 2001). They are usually hydrophobic or amphiphilic, and able to act on negatively charged bacterial membrane due to their cationic nature. Generally, bacteriocins do not inhibit Gram-negative bacteria since they cannot penetrate the bacterial outer membrane. Therefore, it is often necessary to combine bacteriocins with other preservation methods. Although there are various bacteriocins in nature (e.g. nisin, pediocin, reuterin, plantaricin, helveticin etc.), nisin is the only FDA registered GRAS bacteriocin which is extensively used in the food processing industry (de Arauz, Jozala, Mazzola, & Penna, 2009). In addition to bacteriocins, other antimicrobials of microbial origin registered with the FDA are polylysine (ϵ -poly-L-lysine, EPL) and natamycin. Details about these microbial antimicrobials are summarized in **Table 2**.

3. Types of delivery systems

Many antimicrobials are confronted with various problems when directly added into foods. They can be poorly soluble in aqueous systems, have limited stability against chemical or physical degradation, or show uncontrolled release or adverse effects on food organoleptic properties (Sagalowicz & Leser, 2010). For example, the cationic nature of ethyl lauroyl arginate (LAE, a newly EFSA and FDA-registered synthetic antimicrobial) is problematic when it comes to application since the compound readily forms complexes with negatively charged compounds such as anionic polymers (carrageen, protein, and so on) (Terjung et al., 2014). Nisin may lose its efficacy due to diffusion in the food mass or interactions with other food components such as proteases, glutathione, sodium metabisulphite, and titanium dioxide (Bhatti, Veeramachaneni, & Shelef, 2004). Thymol is chemically stable, however, it may be easily lost by evaporation due to high volatility or interact with hydrophobic food components; it can also cause an unpleasant odor to food products (Shah, Davidson, & Zhong, 2012). To solve these problems, one effective strategy is the use of delivery systems (Weiss, Gaysinsky, Davidson, & McClements, 2009). Delivery systems are carriers that allow for the protection of the active ingredient from oxidation, precipitation, crystallization, denaturation, light, temperature, pH, moisture, ionic strength variations, adsorption, diffusion, and biochemical interactions. It should also allow for the sustained or controlled release of the active molecules at the targeted site triggered by factors such as changes in pH, ionic strength, and enzyme activity (McClements, Decker, Park, & Weiss, 2009), or the presence of

microorganisms. Considering the structural and physicochemical aspects of delivery systems, they can be classified into three major categories: (1) emulsion-based delivery systems, (2) nanosized carrier-based delivery systems, and (3) film/coating-based delivery systems. The properties, advantages, and disadvantages of each type of delivery system for food antimicrobials are summarized in **Table 3**. Below is a brief introduction of these systems in general.

3.1. Emulsion-based delivery systems

Emulsions are of enormous practical interest for their widespread occurrence in processes related to the food, cosmetics, and pharmaceutical industries. Conventional emulsions consist of two immiscible phases, oil and water with one phase dispersed in the other in the form of small droplets. Based on their spatial distribution, they can be classified into oil-in-water (O/W) or water-in-oil (W/O) emulsions (McClements, Decker, Park, & Weiss, 2009). Multiple emulsions such as water-in-oil-in-water (W/O/W) and oil-in-water-in-oil (O/W/O) can also be prepared. Multilayered emulsions can be created by the interaction between different layers of emulsifiers of opposite electrostatic charge. This method of layer-by-layer deposition of emulsifying agents on the droplet surface may provide stability to the active molecules (McClements, Decker, Park, & Weiss, 2009). Conventional emulsion systems have been used for the formulation, protection, and delivery of different functional compounds in food systems. For example, multilayered emulsions were shown to protect ω -3 fatty acids against oxidation in food systems (Sagalowicz & Leser, 2010; Shaw,

McClements, & Decker, 2007). As for antimicrobials, O/W emulsion systems have been used for the encapsulation of essential oil components such as eugenol (Suriyarak & Weiss, 2014). Multiple emulsion (O/W/O) systems have been created for the protection of essential oil component from summer savory (*Satureja hortensis*) against *Staphylococcus aureus* (Hosseini et al., 2013).

Microemulsions are colloidal dispersions of oil and water stabilized by an interfacial layer of surfactant molecules, typically in conjunction with a co-surfactant, with particle sizes ranging from 1-100 nm, usually 10-50 nm (Rao & McClements, 2011; Slomkowski et al., 2011). Unlike conventional colloidal dispersions such as emulsions or suspensions, microemulsions are thermodynamically stable, transparent isotropic solutions, which make them good candidates to deliver hydrophobic compounds in aqueous systems in the food industry (Flanagan & Singh, 2006). Numerous applications of microemulsions have been found in areas such as pharmaceutical, cosmetics, and food. For example, Garti and Yuli-Amar (2008) showed that microemulsion delivery systems could significantly improve the solubilization of lipophilic nutrients, such as β -carotene, lycopene, lutein, or phytosterol. Food-grade microemulsions loaded with natural antioxidants, such as gallic acid, vitamin C, or vitamin E, have been fabricated to enhance their antioxidative activities (Chatzidaki et al., 2015). Dong et al. (2016) developed artocarpinone and ascorbic acid loaded microemulsions and showed their strong anti-browning effects in apple juice at room temperature. In addition, some studies showed that microemulsions containing eugenol, monolaurin, or cinnamon essential oil were effective against pathogenic bacteria or spoilage molds in food models such

as milk, noodles, and pears (Fu, Huang, & Feng, 2008; Gaysinsky et al., 2007; Wang et al., 2014)

Nanoemulsions (also known as submicron emulsions or miniemulsions) are conventional emulsions that contain very small particles (50 nm-1 μ m), formed by dispersing one liquid in another immiscible liquid using suitable emulsifiers (Rao & McClements; Slomkowski et al., 2011). The small size of the particles endows nanoemulsions with potential benefits for certain applications: enhanced long-term stability, high optical clarity, and increased bioavailability (McClements, 2012). Compared to microemulsions, nanoemulsions can be made using a wide range of surface-active agents. Nanoemulsions can be used for the encapsulation and delivery of functional compounds in the food industry. For example, functional compounds that can be encapsulated using nanoemulsions include fatty acids, carotenoids, antioxidants, and phytosterols (Silva, Cerqueira, & Vicente, 2012). Several food antimicrobial agents (mostly essential oils such as basil oil, carvacrol, eugenol, and thymol) have also been encapsulated in nanoemulsions to retard spoilage and pathogenic microorganisms in food systems such as fresh lettuce, zucchini, cooked meat sausages, and fruit juices (Bhargava, Conti, da Rocha, & Zhang, 2015; Donsì, Cuomo, Marchese, & Ferrari, 2014; Ghosh, Mukherjee, & Chandrasekaran, 2013, 2014).

Pickering emulsions are defined as emulsions stabilized by solid particles and not by surfactants (Binks & Lumsdon, 2001; Chevalier & Bolzinger, 2013). Pickering emulsions retain the fundamental properties of conventional emulsions but shows extended stability (Aveyard, Binks, & Clint, 2003; Chevalier & Bolzinger, 2013). Pickering emulsions provide

greater advantages compared to conventional emulsions: (1) they are more resistant to emulsion destabilization mechanisms such as coalescence, flocculation, and Ostwald ripening, and (2) they are prepared without surfactants, which allows their usage in different fields (Aveyard, Binks, & Clint, 2003; Chevalier & Bolzinger, 2013). Pickering emulsions have been used for the protection and release of bioactives including flavonoids such as tiliroside, rutin, and naringin (Luo et al., 2011), bioactive peptides such as lactoferrin (Shimoni, Levi, Tal, & Lesmes, 2013), and antimicrobials such as nisin and thyme oil (Bi, Yang, Bhunia, & Yao, 2011b; Wu, Lin, & Zhong, 2014). In general, there is a growing interest in the use of Pickering emulsions for the delivery of functional molecules in food systems.

3.2. Nanosized carrier-based delivery systems

Nanosized carrier-based delivery systems are nanometric carriers in the form of nanospheres, nanocapsules, micelles, and liposomes, etc., theoretically with dimensions between 1 and 100 nm. Practically, nanosized carriers also comprise submicron particles with size below 1000 nm (Liu et al., 2008). Due to their nanometer range, nanosized carriers usually show unique physicochemical properties such as high surface area to volume ratio. Over the past few decades, nanosized carriers have emerged as a group of novel carriers of active ingredients in pharmaceutical, food, and medical applications (Blanco-Padilla, Soto, Iturriaga, & Mendoza, 2014). Presently, nanosized carriers have been widely used to deliver drugs, polypeptides, proteins, vaccines, and antimicrobials. The highly noticeable nanosized

carriers include liposomes, polymer nanoparticles, solid lipid nanoparticles (SLNs), and nanostructured lipid carriers (NLCs) (Fathi, Mozafari, & Mohebbi, 2012).

Liposomes are lipid vesicles formed from polar lipids that produce bilayer structures (Taylor, Davidson, Bruce, & Weiss, 2005). They are spherical in shape and range from nanometer to micrometer in dimensions. Liposomes contain a single or multilayer of amphiphilic membranes. Liposomes with single layer of membranes are known as single (<30 nm) or large unilamellar vesicles (30-100 nm) (SUV or LUV). Liposomes with multilayer membranes are classified as multilamellar vesicles (MLV) or multivesicular vesicles (MVV) (Taylor, Davidson, Bruce, & Weiss, 2005). Due to the presence of both lipid and aqueous phases in the structure of lipid vesicles, liposome can be utilized in the entrapment, delivery, and release of water-soluble, lipid-soluble, and amphiphilic materials such as antimicrobials, fat soluble vitamins, enzymes and minerals (Malheiros, Daroit, & Brandelli, 2010). For example, the incorporation of docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) into liposomes could protect them from degradation, and these liposomes have potential for functional food applications (Liu et al., 2011). Toniazzi et al. (2014) developed a yogurt with β -carotene encapsulated in liposomes as a functional ingredient and natural colorant. For antimicrobials, research work has been focused on developing nisin-loaded liposomes and testing their antilisterial efficacy in various foods such as cheese and ground beef (Boualem, Subirade, Desjardins, & Saucier, 2013; Malheiros, Daroit, & Brandelli, 2010).

Polymer nanoparticles are frequently defined as solid, colloidal particles in the range of 10–1000 nm. “Polymer nanoparticles” can be a collective term used for any type of polymeric nanosized particles, or specifically for polymer nanospheres and nanocapsules (Rao & Geckeler, 2011; Soppimath, 2001). Polymer nanospheres are matrix particles, that is, particles whose entire mass is solid. Bioactive molecules may be adsorbed either at the sphere surface or encapsulated within the particle. Noticeably, some “nanospheres” with a nonspherical shape are also reported in the literature (Rao & Geckeler, 2011). Unlike polymer nanospheres, polymer nanocapsules are vesicular systems, acting as a reservoir, in which the bioactive agents are confined to an aqueous core and surrounded by the polymeric shell around them (Lu, Wu, Li, & Chen, 2011). Compounds that have been encapsulated into nanoparticles for potential use in food include antimicrobials, antioxidants, aromas, and fatty acids. Giroux and Britten (2011) produced hydrophobic aroma-loaded whey protein nanoparticles which showed good potential to retard the aroma release in foods or beverages. Relkin et al. (2014) incorporated vitamin E into protein- or fat-based nanoparticles to protect α -Tocopherol from pro-oxidant factors. Polymeric materials suitable for preparing food antimicrobial carriers include poly (DL-lactide-co-glycolide) (PLGA), phytoglycogen octenyl succinate (PG-OS), chitosan, zein, zinc oxide (ZnO), etc. Several natural antimicrobial compounds have been encapsulated or stabilized, such as nisin, thymol, carvacrol, and eugenol (Bi, Yang, Bhunia, & Yao, 2011a; Blanco-Padilla, Soto, Iturriaga, & Mendoza, 2014; Gomes, Moreira, & Castell-Perez, 2011; Li et al., 2013).

Solid lipid nanoparticles (SLNs) refer to nanoscale particles prepared using lipids that remain solid at room temperature. SLNs are manufactured by a homogenization process to form an emulsion of liquid lipids and an aqueous solution of surfactants at a temperature greater than the lipids melting temperature. The emulsion is thereafter cooled below the crystallization temperature to obtain SLNs. SLNs have been considered as alternatives to conventional polymer nanoparticles by combining functional advantages of polymer nanomaterials, liposomes, and emulsion systems. For example, the use of solid lipid structures instead of liquids help achieve controlled release of functional components and enhance the stability of such compounds by reducing the rate of chemical reactions such as oxidation. SLNs have found applications in the protection and delivery of bioactives such as nisin (Prombutara et al., 2012), resveratrol (Pandita, Kumar, Poonia, & Lather, 2014), and quercetin (Aditya et al., 2014). SLNs have also been used for the preparation of edible films for the protection of fruits such as guava (Zambrano-Zaragoza et al, 2013).

Nanostructured lipid carriers (NLCs) are novel delivery systems developed at the turn of the millennium (Jenning, Thünemann, & Gohla, 2000). NLCs are regarded as the second-generation of SLNs. SLNs consist of pure solid lipids, while NLCs are made of a solid matrix entrapping variable liquid nanocompartments (Müller, Radtke, & Wissing, 2002). NLCs developed possess many advantages over SLNs, such as good biocompatibility, controlled release, and the possibility of production on large industrial scale (Müller, Radtke, & Wissing, 2002). Problems associated with SLNs such as limited loading capacity, recrystallization risk and possibility of expulsion during storage are avoided by this new

generation of carriers (Patidar, Thakur, Kumar, & Verma, 2010). First designed as drug delivery systems, there are few reports relevant to the application of NLCs in food systems. Associated with their functionalities, NLCs may have the potential to deliver lipophilic substances as alternatives to SLNs for food applications.

3.3. Film/coating-based delivery systems

Antimicrobials can be applied on food solid surfaces to control microbial growth, using different techniques such as spraying, dipping, or brushing. However, some disadvantages may result from the direct application of antimicrobials. For example, the antimicrobials may interact or react with other additives or components present in the food matrix, which reduces their effective concentration (Ture, Eroglu, Ozen, & Soyer, 2011). Incorporation of antimicrobials at food interfaces by the use of films and coatings can diminish such interactions due to its presence being restricted mainly to the surface. It decreases the migration of antimicrobial agents from surface to bulk of the product, and may achieve controlled release of active ingredients at a high concentration (Resa, Jagus, & Gerschenson, 2014). As a consequence, films and coatings with antimicrobial activity constitute a promising form of antimicrobial delivery for food preservation.

Film/coating-based delivery systems have been prepared from biopolymers including carbohydrates, proteins, lipids, and their combinations. The choices of materials for a film or coating largely depend on the desired function (Cha & Chinnan, 2004). Carbohydrate-based

films and coatings from starch, cellulose, pectin, guar gum, chitosan, alginate, galactomannans, and cyclodextrins have found numerous applications in incorporating active ingredients (Fathi, Martín, & McClements, 2014). Proteins have also found applications in film-based delivery of bioactives. Proteins such as collagen, gelatin, fish myofibrillar proteins, keratin, egg white protein (albumen), casein, whey proteins, corn zein, wheat gluten, rice bran protein, soy protein, and peanut protein have been used for the encapsulation and delivery of functional molecules (Cha & Chinnan, 2004; Fernández-Pan, Mendoza, & Mate, 2013). Certain lipid-based materials have also been used as film-forming agents such as waxes, emulsifiers, or surface-active agents (Morillon et al., 2002). Recently, films and coatings have been utilized for the protection and controlled delivery of different active components including flavors, aroma, antimicrobials, probiotics, and antioxidants. For example, whey protein isolate-based edible coating containing natamycin was prepared to improve the shelf life of cheese; the presence of coating decreased water loss, hardness, and color changes, and reduced microbial development of cheeses throughout the 60-day storage (Ramos et al., 2012). The study by Robles-Sánchez et al. (2013) showed that alginate-based edible coating containing ascorbic acid contributed to improved color retention and antioxidant potential of fresh-cut mangoes. The study of de Lacey et al. (2014) showed that the use of agar films with green tea extract and probiotics could extend the shelf life of fish at least for a week.

4. Delivery systems of antimicrobial compounds

As discussed earlier, the direct application of antimicrobial compounds in food systems faces challenges such as water insolubility, physical and chemical degradation, and impact on food organoleptic properties. There is a need to incorporate antimicrobial substances into suitable delivery systems: (1) to retain their biological activity and protect them from interactions with other food ingredients and from environmental stresses experienced during food manufacturing and storage, (2) to minimize their effects on the organoleptic properties of foods, or (3) to improve their solubility in aqueous phase. Various studies have been carried out to design delivery systems of antimicrobial compounds to food. Most of the studies are conducted in culture medium models. Some antimicrobial delivery systems studied using culture medium models are listed in **Table 4**. Meanwhile, there are a few studies on delivery systems used in actual food matrices. Food systems are highly complicated with diverse formats and components. For solid food surface, films and coatings are current choices to deliver antimicrobial agents, using dipping, spraying, or packaging technologies. For liquid food products, antimicrobial agents can be incorporated into dispersible carriers and added directly to food systems. Following are detailed discussions on the delivery systems of antimicrobial compounds in food systems.

4.1. Emulsion-based delivery systems of antimicrobial compounds in food systems

For emulsion-based delivery systems, Gaysinsky et al. (2007) investigated the antimicrobial activity of eugenol microemulsions against *Listeria monocytogenes* and *Escherichia coli* O157:H7 in ultrahigh temperature pasteurized milk containing different

percentages of milk fat. Eugenol in microemulsions showed a significant increase in solubility. Antimicrobial test indicated that eugenol microemulsions completely prevented the growth of *L. monocytogenes* for up to 48 h in skim milk and reduced the strains of *E. coli* O157:H7 to less than detectable levels in less than 1 h. While in 2% and whole milk, eugenol microemulsions could only inhibit sensitive strains of *E. coli* O157:H7. The results indicated that the emulsified eugenol was more applicable in aqueous food systems compared to free eugenol for its increased solubility. However, food compositions, especially the fat level, might affect the efficiency of eugenol microemulsions against targeted pathogens. Fu et al. (2008) studied the effects of food-grade monolaurin microemulsions (MMEs) on the shelf-life of fresh noodles. The microemulsion system was prepared by dispersing monolaurin, Tween 20 and Tween 80 (surfactants) and pentanol and dodecanol (co-surfactants) in distilled water. The shelf-life of fresh noodles prepared without monolaurin and with monolaurin alone was about 4 days at 37 °C, whereas the addition of MMEs extended shelf-life by 10 days at least. Wang et al. (2014) investigated the effect of cinnamon essential oil (CM) microemulsions on controlling gray mold caused by *Botrytis cinerea* in pears and possible influences of microemulsions on quality characteristics. The decay incidence of samples treated with 500 µg/L CM microemulsion was reduced by 18.7% in comparison to that of 500 µg/L CM non-microemulsion after 4-day storage at 20 °C, and the microemulsions showed no significant influence on pear qualities such as firmness and color.

As for nanoemulsions, Ghosh et al. (2014) fabricated eugenol-loaded antimicrobial nanoemulsion using sesame oil, non-ionic surfactant (Tween 20 and Tween 80), and water using ultrasound cavitation. Stable sesame oil nanoemulsion obtained with 1:3 (v/v) of oil-surfactant mixing ratio exhibited antibacterial effect on the native cultivable bacteria populations in orange juice. The nanoemulsion showed enhanced *in situ* antibacterial activity over the same concentration of sodium benzoate, and better results were obtained at storage temperature of 4 °C. Donsì et al. (2014) designed carvacrol nanoemulsions and determined the infusion and antimicrobial activity of the emulsions in zucchini samples and cooked meat sausages. When the droplet size of emulsion was below the characteristic size of inter- and intracellular interstices, the emulsion exhibited a significantly enhanced effective diffusivity that promoted the antimicrobial action of carvacrol. Conceivably, nanoemulsion-based delivery systems for essential oils not only enabled a slow and sustained release of the antimicrobial compounds, but also contributed to their incorporation in complex food systems. Bhargava et al. (2015) applied an oregano oil nanoemulsion to control foodborne pathogens on fresh lettuce. Compared to control (sterile distilled water), 0.05% oregano oil nanoemulsion showed up to 3.44, 2.31, and 3.05 log CFU/g reductions with *L. monocytogenes*, *Salmonella* Typhimurium, and *E. coli* O157:H7, respectively. Up to 3.57, 3.26, and 3.35 log CFU/g reductions were observed on the same bacteria by the 0.1% oregano oil nanoemulsion treatment. A 2.31–3.57 log reduction in foodborne bacteria achieved by oregano oil nanoemulsions on lettuce over a 72 h period in this study is an indication of great promise of plant essential oils in enhancing food safety.

Although the Pickering emulsions have been used to deliver antimicrobial compounds with the results tested in medium models (**Table 4**), studies on their efficiency in food systems were not found from our literature search. The Pickering emulsions have better stability than conventional emulsions and can be used to control the release of incorporated compounds. Undoubtedly, there will be considerable potential in applying the Pickering emulsions for the delivery of antimicrobial materials to real food systems.

4.2. Nanosized carrier-based delivery systems of antimicrobial compounds in food systems

There are several studies on the application of liposome-based carriers for antimicrobials in food systems. Malheiros et al. (2010) summarized some applications of bacteriocin-loaded liposomes in foods and model systems. They later encapsulated nisin and bacteriocin-like substance P34 into partially purified soybean phosphatidylcholine (PC-1) and evaluated the effect of the bacteriocin-loaded liposome on *L. monocytogenes* growth in Minas frescal cheese during 21 days of storage at 7 °C. The encapsulation of nisin and P34 in PC-1 liposomes resulted in better inhibitory effect after 10 days of storage in comparison with free bacteriocins, suggesting that the bacteriocins are being released from the PC-1 liposomes after this time (Malheiros et al., 2012). Boualem et al. (2013) developed an encapsulation system that would optimize nisin activity when used in meat. The dipalmitoylphosphatidylcholine (DPPC) liposomes were formed in phosphate buffer with or without nisin. The liposomes containing nisin were stable for up to 7 days at 4 °C. Stability of the liposome to heat was tested, and at over 37 °C nisin was released from the melted

liposomes to form zones of inhibition. They tested the activity of free and encapsulated nisin in raw and cooked (71 °C) ground beef. Free nisin lost its activity in raw beef, whereas DPPC-encapsulated nisin remained active and was released upon melting of the liposome during heat treatment. Using liposome, Cui et al. (2015) enhanced the chemical stability of clove oil. The liposome-encapsulated clove oil could efficiently inhibit *S. aureus* in tofu. Controlled release of clove oil from liposome was achieved with pore-forming toxins (PFTs) secreted from *S. aureus*, that is, PFTs would insert into the liposome membranes and form pores, through which the encapsulated clove oil was released.

Studies on the application of polymer nanoparticles as carriers for antimicrobials in food systems are also widely reported. The common antimicrobial substance incorporated in polymer nanoparticles is nisin. Zohri et al. (2013) prepared a hybrid of nisin (at concentration of 450 IU/mL) with chitosan-alginate nanospheres and investigated its activity against *L. monocytogenes* and *S. aureus* in ultra-filtered Feta cheese. Antibacterial assay indicated that the nisin-loaded nanoparticles were able to achieve up to 5- and 7-fold reductions on a logarithmic scale of the population of *S. aureus* and *L. monocytogenes* in comparison to free nisin, respectively. The sensory acceptance and physicochemical features of ultra-filtered Feta cheese were not adversely affected by using nisin-loaded nanoparticles as compared with those of free nisin. Overall, greater antibacterial strengths and less undesirable influences of this hybrid than those of free nisin on the original quality of Feta cheese would make this hybrid a promising biopreservative in dairy products. Chopra et al. (2014) loaded nisin into chitosan-carrageenan nanocapsules using an ionic complexation method and

evaluated their antibacterial activity in tomato juice for six months. Bacterial growth in tomato juice occurred after only one day without nisin or without nanocapsules. With pure nisin in tomato juice, there was no growth detected until one month. The growth of bacteria in tomato juice was visible after two months if nanocapsules without nisin were used. When nisin-loaded nanocapsules were used, microbial growth did not take place for six months. The results indicated a synergistic effect between nisin and nanocapsules that led to a long-term antimicrobial effect.

Thus far, there are no reports found for the effect of antimicrobial loaded SLNs or NLCs on food. However, as potential carriers for antimicrobials, they may represent a promising technology for the control of foodborne pathogens.

4.3. Film/coating-based delivery systems of antimicrobial compounds in food systems

Many studies have focused on the incorporation of antimicrobials using edible films and coatings. Edible films and coatings from soy, carboxymethyl cellulose, starch, and pectin have been used to deliver: (1) thyme and oregano essential oils in ground beef patties (Emiroğlu, Yemiş, Coşkun, & Candoğan, 2010), (2) potassium sorbate in fresh pistachios (Sayanjali, Ghanbarzadeh, & Ghiassifar, 2011), (3) natamycin in cheese systems (Resa, Jagus, & Gerschenson, 2014), and (4) oregano essential oil in chicken breast fillets (Fernández-Pan, Carrión-Granda, & Maté, 2013). Higuera et al. (2013) incorporated 1, 5, or 10% ethyl lauroyl arginate (LAE) in chitosan-based films and determined the LAE release and

antimicrobial activity of developed films. Chitosan films incorporated with LAE were continuous, flexible, and transparent, and able to release the antimicrobial agent when exposed to highly humid media. Films of chitosan-5% LAE reduced satisfactorily the microbiota commonly found in fresh chicken, therefore presenting excellent potential for the packaging of fresh poultry products. In the study of Ture et al. (2011), natamycin was incorporated in wheat gluten films and the effect of wheat gluten films containing natamycin on the growth of *Aspergillus niger* and *Penicillium roquefortii* was investigated on fresh kashar cheese. Wheat gluten films containing at or above 2 mg natamycin per 10 g film completely inhibited *A. niger* development on the surface of fresh kashar cheese during 30 days of storage at 10 °C. However, the same films were not very effective in inhibiting the growth of *P. roquefortii*.

Composites combining two film-forming materials for antimicrobial delivery in foods have also been reported. Gómez-Estaca et al. (2010) developed gelatin-chitosan films containing clove essential oils and tested the effect of films applied to fish at 2 ± 1 °C during 11 days against six selected microorganisms. The growth of microorganisms was drastically reduced for Gram-negative bacteria, especially for Enterobacteria, whereas lactic acid bacteria remained practically constant for much of the storage period. Some recent applications of antimicrobial compound-loaded edible films and coatings in food systems are listed in **Table 5**.

Conclusions

Compared with directly adding antimicrobial compounds to food, using delivery systems may protect the compounds from degradation, improve their solubility, and control their release. Appropriate antimicrobial delivery systems should be designed based on fundamental understanding of the structures and functions of carriers selected, as well as their interactions with the compounds to be protected and delivered. Thus far, most studies on the delivery systems have been employing culture medium models, and these studies usually did not involve or focus on the interactions between the delivery systems and food matrices, the impact on the sensory qualities, and the assessment on the safety of carriers. While a number of delivery systems have shown efficacies under the model conditions, their performances still need to be demonstrated in real foods. In general, the delivery systems can be designed using culture medium models, and the food models with industrial implications are essential to the development of products.

Thus far, there have been only a few studies conducted using food systems, and our patent and literature search revealed very limited cases for the industrial applications of antimicrobial delivery systems. Indeed, most patented delivery systems were not developed for food. For the research and development of food applications, there are several challenges that need to be properly addressed: (1) to identify additional food grade materials for preparing carriers, (2) to better understand the interactions of the delivery systems with other food components in the complicated food matrices, and (3) to substantially reduce the cost for manufacturing the delivery systems of antimicrobial compounds. Considering the current trends in food consumption and development of food ingredient technology, we believe that

the following technologies are desirable and feasible for delivering antimicrobial compounds:

(1) the broad use of clean-label carriers, such as native proteins, fibers, and starches for the complexation and delivery purposes, (2) release of multiple, synergistic antimicrobial compounds for optimized efficacy at reduced materials cost, and (3) controlled or programmed release of antimicrobials triggered by adverse environmental conditions, such as abnormal temperature increase in the cold chain and humidity increase during low-moisture-content storage.

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Table 1: Commonly used synthetic antimicrobial compounds for Food

E number	Chemical compound	Applications
E200, E202-203	Sorbic acid and sorbates	Cheese, wine, yogurt, dried meats, apple cider, baked goods
E210-213	Benzoic acid and benzoates	Salad dressings, carbonated drinks, jams, margarine, pickles, condiments
E214-215, E218-219	Hydroxybenzoates/parabens	Bakery products, soft drinks, pickles, salad dressings
E220-224, E226-228	Sulfur dioxide and sulfites	Dried fruits, wine making, lemon juice
E230	Biphenyl/diphenyl-derivatives	Particularly used in citrus fruits
E249-252	Nitrites and nitrates	Particularly used in meats
E280-283	Propionic acid and propionates	Baked goods such as bread, cakes
E243	Ethyl lauroyl arginate	Meat, poultry

Table 2: Naturally occurring antimicrobials listed as GRAS by the FDA

Antimicrobial	Source	Target microorganism	Mode of action	Reference
Animal origin				
Lactoferrin	Bovine milk	<i>Bacillus cereus</i> , <i>Candida albicans</i> , <i>Listeria innocua</i> , <i>Listeria monocytogenes</i> , <i>Staphylococcus aureus</i>	Chelation of iron from bacterial pathogens; Iron-independent interaction with the lipid A part of Gram-negative bacterial lipopolysaccharide (LPS), leading to increased membrane permeability	Jenssen & Hancock, 2009 Olmo et al., 2010
Lysozyme	Hen's egg, mammalian milk	<i>Aspergillus</i> , <i>Bacillus</i> spp., <i>Candida</i> , <i>Clostridium</i> spp., <i>Lactobacillus</i> spp., <i>L. monocytogenes</i> , <i>Pseudomonas</i> spp., <i>Saccharomyces</i>	Hydrolysis of the β -1, 4 linkage between N-acetylmuramic acid and N-acetylglucosamine in the peptidoglycan of the microbial cell wall; Gram-negative bacteria being resistant to lysozyme for the out membrane can act as a barrier	Branen & Davidson, 2004 Gyawali & Ibrahim, 2014 Tiwari et al., 2009
Chitosan ^a	Exoskeletons of crustaceans and arthropods	Lactic acid bacteria, <i>L. monocytogenes</i> , <i>Salmonella</i> Typhimurium, <i>Zygosaccharomyces bailii</i>	Electrostatic interaction with negatively charged bacterial cell wall; Adsorption to DNA molecules and thus blocking the transcription of RNA from DNA; Chelation of essential minerals	Jayakumar et al., 2007 van den Broek et al., 2015
Plant origin^b				
Thymol	Thyme oil	<i>C. albicans</i> , <i>Escherichia coli</i> O157:H7, Lactic acid bacteria, <i>L. monocytogenes</i> , <i>Salmonella</i> spp., <i>S. aureus</i>	For Gram-negative bacteria, acting on the outer membrane containing lipopolysaccharides and increasing the depletion of intracellular ATP; For Gram-positive bacteria, binding to the fatty acids of the phospholipid membrane, thus increasing the cellular permeability	Topuz et al., 2016 Ultee et al., 2002
Eugenol	Clove oil	<i>Aspergillus</i> , <i>Candida</i> , <i>E. coli</i> , <i>E. coli</i> O157:H7, <i>L. monocytogenes</i> , <i>L. innocua</i> , <i>Salmonella</i> spp., <i>S. aureus</i>	Disruption of the bacterial cytoplasmic membrane, thus increasing its non-specific permeability, ultimately leading to the loss of cellular contents and cell death	Devi et al., 2010
Carvacrol	Oregano oil	<i>C. albicans</i> , <i>Bacillus subtilis</i> , <i>E. coli</i> , <i>L. monocytogenes</i> , <i>Saccharomyces cerevisiae</i> , <i>Salmonella</i> spp., <i>S. aureus</i>	Being similar to thymol	Lambert et al., 2001
Microbial origin				
Nisin	<i>Lactococcus lactis</i>	<i>Clostridium</i> spp., <i>L. monocytogenes</i>	Electrostatic interaction with the negatively charged bacterial cell membrane; Interaction specifically to lipid II molecules of the Gram-positive bacterial cell membrane, leading to pore formation	Breukink & de Kruijff, 2006 de Arauz et al., 2009
Polylysine	<i>Streptomyces albulus</i>	<i>Candida</i> , <i>B. subtilis</i> , <i>E. coli</i> , Lactic acid bacteria, <i>L. monocytogenes</i> , <i>S. cerevisiae</i> , <i>S. Typhimurium</i> , <i>S. aureus</i>	Electrostatic interaction with the negatively charged bacterial cell surface, leading to distortion of the outer membrane and producing abnormal distribution of the cytoplasm	Hylgaard et al., 2014 Miya et al., 2014
Natamycin	<i>Streptomyces natalensis</i>	Fungi and yeasts, ineffective against bacteria	Binding to the fungal membrane sterols; Inhibition of the vacuolar fusion	Welscher et al., 2008, 2010

a Chitosan from exoskeletons of crustaceans and arthropods is not registered as GRAS by the FDA. But it is regarded as safe additive in many other countries.

b Essential oils such as thyme oil, clove oil, and oregano oil are all approved as GRAS by the FDA. Thymol, eugenol, and carvacrol are the main antimicrobial components of thyme, clove, and oregano oils. The GRAS status was not accorded to the pure substances, but the essential oils.

Table 3: Advantages and disadvantages of each type of delivery system for antimicrobial compounds

Delivery system	Advantage	Disadvantage	Reference
Emulsion			
Conventional emulsion	Containing lipophilic oil phase, hydrophilic aqueous phase and amphiphilic interfacial layer; Being prepared entirely from food-grade ingredients with fairly simple processing operations	Physical instability under various environmental stresses; Rapid release	McClements et al., 2007
Microemulsion	Thermodynamically stable and transparent isotropic emulsion formed spontaneously; Carrier of lipophilic and crystallizing compounds; Increase in bioavailability	A lack of suitable food-grade surfactants and co-surfactants; Large amounts of surfactant needed	Flanagan & Singh, 2006
Nanoemulsion	High optical clarity and long-term stability; Rapid absorption and increase in bioavailability; A wide range of surface-active agents being available	Thermodynamic instability and low stability in acidic condition; Rapid release; High-energy method for preparation	McClements, 2012
Pickering emulsion	Extended stability compared to conventional emulsion; Being stabilized by solid particles, not surfactants; Controlled release	Usually opaque emulsion; Suitable food-grade solid particles limited in the food industry	Aveyard et al., 2003 Chevalier & Bolzinger, 2013
Nanosized carrier			
Liposome	Feasibility to deliver both lipophilic and hydrophilic molecules; High stability of compounds in foodstuff with high water content	Relatively low encapsulation efficiency; Short shelf life; High cost of phospholipids	Malheiros et al., 2010
Polymer nanoparticles	Large surface-to-volume ratio; Increase in bioavailability and biodegradability; Controlled release of insoluble molecules	Lack of stability of some active substances; Large dispersion of encapsulated compounds; High production costs	Blanco-Padilla et al., 2014
SLN	Good chemical protection of sensitive antimicrobials such as essential oils; Controlled release	Limited loading capacity; Lack of physical stability for possible recrystallization and expulsion	Fathi et al., 2012
NLC	Good biocompatibility and excellent stability; High loading capacity; Controlled release; Possibility of production on large industrial scale	A lack of suitable food-grade ingredients limiting the applications in the food industry	Müller et al., 2002 Patidar et al., 2010
Films and coatings	Decreasing the migration of antimicrobials from surface to bulk of the food product, thus protecting the antimicrobials from interaction or reaction with other additives or components present in the food matrix; Controlled release; Being suitable for large scale production	A lack of evenness and uniformity of films/coatings, thus leading to irregular distribution of the active constituents; Development being limited due to availability of new biopolymer materials and antimicrobials, regulatory concerns	Carocho et al., 2014 Ollé Resa et al., 2014

Table 4: Examples of delivery systems for antimicrobial compounds in medium models

Delivery system	Antimicrobial	Target microorganism	Main finding	Reference
Emulsions				
Conventional emulsion	Carvacrol	<i>Bacillus cereus</i> , <i>Escherichia coli</i> , <i>Salmonella Typhimurium</i> , <i>Staphylococcus aureus</i>	<i>Satureja hortensis</i> essential oil exhibiting a notable inhibitory effect against selected bacteria with controlled release when incorporated in microparticles via multiple emulsion/ionic gelation method	Hosseini et al., 2013
	Eugenol	<i>Staphylococcus carnosus</i>	Maximum amount of eugenol encapsulated to maintain both the antimicrobial activity and physical stability of model O/W emulsions	Suriyarak et al., 2014
Microemulsion	Glycerol monolaurate	<i>Escherichia coli</i> , <i>Staphylococcus aureus</i>	A food-grade fully dilutable microemulsion system demonstrated to kill pathogenic bacteria by interaction with the bacterial cell membrane, resulting in DNA leakage	Zhang et al., 2009
	Eugenol	<i>Bacillus cereus</i> , <i>Staphylococcus aureus</i>	Microemulsion improving the antimicrobial and antioxidant activities of eugenol compared with the free eugenol	Hamed et al., 2012
Nanoemulsion	Eucalyptus oil	<i>Bacillus cereus</i> , <i>Escherichia coli</i> , <i>Staphylococcus aureus</i>	Eucalyptus oil-loaded nanoemulsion fabricated and demonstrated to inactivate <i>B. cereus</i> at 0th min, <i>S. aureus</i> at 15 min, and <i>E. coli</i> at 1h	Sugumar et al., 2012
	Lactoferrin	<i>Bacillus cereus</i> , <i>Candida albicans</i> , <i>Listeria innocua</i> , <i>Staphylococcus aureus</i>	Lactoferrin encapsulated in the multiple nanoemulsions showing full stability after long-term storage, proving the effectiveness of encapsulation	Balcão et al., 2013
	Thyme oil, lauric arginate	<i>Zygosaccharomyces bailii</i>	The addition of a cationic surfactant (lauric arginate) substantially increasing the antimicrobial efficacy of the thyme oil nanoemulsions	Chang et al., 2015
	Essential oils	<i>Escherichia coli</i>	The antimicrobial activity of nanoemulsions determined by the essential oil type used in the formulation rather than by their droplet size	Salvia-Trujillo et al., 2015
Pickering emulsion	Nisin	<i>Listeria monocytogenes</i>	Pickering emulsion significantly prolonging nisin efficacy during storage	Bi et al., 2011b
	Thyme oil	<i>Escherichia coli</i> , <i>Listeria monocytogenes</i> , <i>Salmonella Enteritidis</i>	Soybean polysaccharide suitable to prepare essential oil emulsions, showing no impact on the antimicrobial activities	Wu et al., 2014
Nanosized carriers				
Liposome	Nisin	<i>Listeria monocytogenes</i> , <i>Staphylococcus aureus</i>	Nisin-loaded liposome significantly prolonging and enhancing the antimicrobial activity when compared with free nisin	Zou et al., 2012
	Cinnamaldehyde	<i>Bacillus cereus</i> , <i>Escherichia coli</i>	The antimicrobial effect of cinnamaldehyde enhanced by encapsulation in nanoliposomes	Makwana et al., 2014
	Nisin	<i>Listeria monocytogenes</i>	Lecithin-encapsulated nisin stable for 6 weeks, showing better antibacterial activity compared to free nisin	Imran et al., 2014

Polymer nanoparticles	Cinnamaldehyde, eugenol	<i>Listeria</i> spp., <i>Salmonella</i> spp.,	The entrapment efficiency for eugenol and trans-cinnamaldehyde being approximately 98% and 92%, respectively; Controlled release being observed	Gomes et al., 2011
	Nisin	<i>Listeria monocytogenes</i>	Nisin activity against pathogens being prolonged when loaded in phytoglycogen based nanoparticles	Bi et al., 2011a
	Thymol, carvacrol	<i>Escherichia coli</i>	Encapsulation of essential oils in zein nanoparticles enhancing their solubility up to 14 fold without affecting the antimicrobial activity	Wu et al., 2012
	Thymol	<i>Staphylococcus aureus</i>	The zein nanoparticles possessing strong loading capacity for thymol, and being stable over 8-month storage. Sustained release being observed	Li et al., 2013
SLN	Nisin	<i>Lactobacillus plantarum</i> , <i>Listeria monocytogenes</i>	Compared to free nisin, the antibacterial activity of nisin loaded in SLNs being prolonged evidently	Prombutara et al., 2012
NLC	Green tea extract	<i>Escherichia coli</i>	0.1 wt% green tea extract being needed to obtain excellent physical stability and smallest particle size	Manea et al., 2014
Films and coatings				
	Cinnamon oil	<i>Escherichia coli</i> , <i>Listeria monocytogenes</i> , <i>Pseudomonas fluorescens</i>	Incorporation of cinnamon oil into chitosan-based films at higher than 0.4% (v/v) exhibiting a clear inhibitory zone, whereas chitosan control films showing no inhibitory zone	Ojagh et al., 2010
	Chitosan	<i>Escherichia coli</i> , <i>Listeria monocytogenes</i>	Bi-layer edible and biodegradable films based on chitosan and gelatin developed	Pereda et al., 2011
	Lysozyme	<i>Micrococcus lysodeikticus</i>	Incorporation of lysozyme into the sol-gel modified poly (ethylene terephthalate) showing no impact on the activity of the antimicrobial agent	Corradini et al., 2013
	Natamycin	<i>Aspergillus niger</i> , <i>Penicillium chrysogenum</i> <i>Penicillium commune</i> <i>Penicillium roqueforti</i>	The release rates of natamycin from the alginate-based films strongly depending on the cross-linking agent used, and all active films being able to inhibit the growth of the four microorganisms	Bierhalz et al., 2014
Others				
Molecular Complex	Essential oils	<i>Listeria innocua</i> , <i>Salmonella Typhimurium</i>	Essential oil complexes inhibiting both bacterial strains at lower active compound concentrations than free oils, likely due to their increased water solubility	Hill et al., 2013
Nanofiber	ϵ -Polylysine	<i>Escherichia coli</i> , <i>Staphylococcus aureus</i>	ϵ -Polylysine successfully introduced onto the bacterial cellulose nanofibers via crosslinking method, the antimicrobial activity enhanced	Gao et al., 2014
Niosome	Nisin, EDTA	<i>Escherichia coli</i> , <i>Staphylococcus aureus</i>	Nisin/EDTA-loaded niosomes having a higher and longer-lasting inhibition effect against <i>S. aureus</i> as compared to their free forms	Kopermsub et al., 2011

Table 5: Antimicrobial compound-loaded films and coatings in food systems

Material used	Antimicrobial	Food matrix	Target microorganism	Main finding	Reference
Carbohydrates					
Alginate	Nisin	Smoked salmon	<i>Listeria monocytogenes</i>	Salmon covered with nisin-incorporated films having a bacteriostatic effect on <i>Listeria</i> for 28 days, exceeding the industrial standard shelf life for smoked salmon	Concha-Meyer et al., 2011
Carrageenan	Ovotransferrin	Chicken breast	<i>Candida albicans</i> , <i>Escherichia coli</i> , <i>Salmonella</i> Typhimurium	The carrageenan-based films containing ovotransferrin combined with EDTA showing the strongest inhibition effect against tested bacteria of chicken breast	Seol et al., 2009
Cellulose	Lysozyme, lactoferrin	Meat slices	<i>Escherichia coli</i> , <i>Listeria innocua</i>	Lysozyme and lactoferrin incorporated into carboxymethyl cellulose and a synergistic antimicrobial activity observed	Barbiroli et al., 2012
Chitosan	Ethyl lauroyl arginate	Chicken breast fillets	<i>Aspergillus niger</i> , <i>Escherichia coli</i> , <i>Saccharomyces cerevisiae</i> , <i>Staphylococcus aureus</i>	Chitosan films alone showing antimicrobial activity in the range 0.47-2.96 log reductions, while 5% lauric arginate-incorporated chitosan film producing 1.78-5.81 log reduction	Higueras et al., 2013
Pectin	Oregano oil	Shrimp, cucumber	<i>Escherichia coli</i> , <i>Listeria monocytogenes</i> , <i>Staphylococcus aureus</i>	Application of pectin-oregano oil films effective in reducing total coliforms, yeast, and molds of shrimp and cucumber slices stored at 4 °C during 15 d	Alvarez et al., 2014
Starch	Natamycin	Cheese	<i>Saccharomyces cerevisiae</i> , <i>Yarrowia lipolytica</i> , <i>Zygosaccharomyces rouxii</i>	Natamycin bioavailability not compromised by the polymeric supporting matrix, and film action showing greater efficiency than spraying	Resa et al., 2014
Proteins					
Corn zein	Lysozyme, EDTA	Ground beef patties	<i>Listeria monocytogenes</i> , <i>Salmonella</i> Typhimurium	Lysozyme/EDTA incorporated zein films on beef patties significantly decreasing tested pathogens after 5 days of storage compared to those of control patties	Unalan et al., 2011
Gelatin	Nisin	Rainbow trout	<i>Listeria monocytogenes</i>	Films with nisin reducing <i>L. monocytogenes</i> counts on the trout fillets below the detection limit (0.3 log CFU/g) after 30 d storage at 4 °C	Han et al., 2013
Soy protein isolate	Essential oil monomers	Chilled pork	<i>Escherichia coli</i> , <i>Pseudomonas</i> , <i>Staphylococcus aureus</i>	Essential oil monomers-loaded films significantly inhibiting bacterial growth, among which cinnamaldehyde-loaded films showing the highest antimicrobial activity	Zhang et al., 2013
Wheat gluten	Natamycin	Kashar cheese	<i>Aspergillus niger</i>	Wheat gluten film incorporating 2 mg natamycin/10 g sufficient to eliminate <i>A. niger</i> on cheese surface	Ture et al., 2011
Whey protein isolate	Clove oil, oregano oil	Chicken breast	<i>Enterobacteriaceae</i> , <i>Pseudomonas</i> spp.	The antimicrobial edible films showing high effectiveness against the main spoilage microbes developed on the surface of skinless chicken breasts cold-stored for 8 days	Fernandez-Pan et al., 2013
Lipids^a	-	-	-	-	-
Composites					
Pectin/Polylactic acid (PLA)	Nisin	Liquid egg, orange juice	<i>Listeria monocytogenes</i>	Pectin/PLA films incorporating nisin showing promise for the inhibition of pathogenic <i>L. monocytogenes</i>	Jin et al., 2009
Gelatin/Chitosan	Essential oils	Fish	Enterobacteriaceae	Gelatin/chitosan film incorporating clove essential oil drastically reducing the natural microbiota of fish during chilled storage with a slow release of the antimicrobial	Gómez-Estaca et al., 2010

a Reports not found