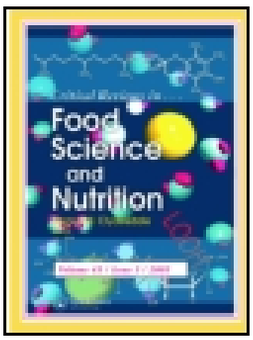

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Critical Reviews in Food Science and Nutrition

ISSN: 1040-8398 (Print) 1549-7852 (Online) Journal homepage: http://www.tandfonline.com/loi/bfsn20

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To cite this article: Nathalie Almeida Lopes & Adriano Brandelli (2017): Nanostructures for delivery of natural antimicrobials in food, Critical Reviews in Food Science and Nutrition, DOI: 10.1080/10408398.2017.1308915

To link to this article: http://dx.doi.org/10.1080/10408398.2017.1308915

Accepted author version posted online: 10 Apr 2017.

Nanostructures for delivery of natural antimicrobials in food

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Abstract

Natural antimicrobial compounds are a topic of utmost interest in food science due to the increased demand for safe and high-quality foods with minimal processing. The use of nanostructures is an interesting alternative to protect and delivery antimicrobials in food, also providing controlled release of natural compounds such as bacteriocins and antimicrobial proteins, and also for delivery of plant derived antimicrobials. A diversity of nanostructures are capable of trapping natural antimicrobials maintaining the stability of substances that are frequently sensitive to food processing and storage conditions. This article provides an overview on natural antimicrobials incorporated in nanostructures, showing an effective antimicrobial activity on a diversity of food spoilage and pathogenic microorganisms.

Key words

nanobiotechnology; bacteriocin; nisin; nanoparticles; phytochemicals

1. Introduction

Biomolecules presenting antimicrobial activity are very useful to control the growth of undesirable and post-processing contaminant microorganisms, extending the shelf life and maintaining the quality and safety of foods. The use of natural substances for food preservation meets the actual tendency of consumers for healthier, safe and convenient food products, including the demand for minimally processed and additive-free foods.

A variety of natural antimicrobial agents such as antimicrobial peptides (bacteriocins, lactoferrin), plant-derived substances (essential oils, polyphenols, isothiocyanates) and enzymes (lysozyme) have been used to control spoilage and pathogenic microorganisms by incorporation into the food matrix or in films and coatings (Rhim et al., 2013; Meira et al., 2014). However, many of the natural antimicrobial substances used are highly sensitive to the production process and/or storage conditions. Under high processing temperature and pressure, degradation or evaporation of volatile antimicrobial agents can occur. In addition, undesirable interaction with components of food matrix (usually protein and fat) or degradation by endogenous food enzymes has been reported (Aasen et al., 2003; Sant'Anna et al., 2011).

Nanotechnology arises as an alternative for delivery of natural preservatives in food, generally showing advantages in comparison to the use of free antimicrobials. Nanostructures are attractive carriers to protect bioactive compounds against adverse environmental conditions that can be noticed during food production and storage, thus improving stability and efficacy for controlling pathogenic bacteria (Brandelli, 2015). The effectiveness of nanostructured antimicrobials is determined in part by the release rate of the antimicrobial compound, allowing the migration of adequate amounts from the nanostructure to the food matrix or onto the food

surface, where microbial growth often predominates. An excessively slow release could result that microbial growth is not sufficiently inhibited, whereas a considerably fast release may not sustain the inhibition. The release rate depends on the type of nanostructure, preparation method, interactions between nanocomposite material and antimicrobial agents, and environmental conditions (Meira et al., 2014; Salmieri et al., 2014). Thus, the controlled release that can be achieved using nanostructures is highly advantageous, ensuring that a constant level of the antimicrobial agent reaches the food.

Applications of nanotechnology have been proposed to agriculture and food sectors, and some comprehensive reviews have been published on this topic (Taylor et al., 2005; Ranjan et al., 2014; Dasgupta et al., 2015). In this article, the use of different nanostructures as carriers for delivery of natural antimicrobials in foods is discussed and particular focus is devoted on recent work of nanostructured systems with application in food systems that present antimicrobial activity on a diversity of food spoilage and pathogenic microorganisms.

2. Nanostructures

A diversity of nanostructures are currently available to delivery antimicrobial compounds. Natural antimicrobial substances, including peptides, essential oils, or plant extracts, can be incorporated into different nanostructures including liposomes, polymer nanoparticles (nanospheres and nanocapsules), nanofibers, nanotubes, among other (Brandelli and Taylor 2015). A schematic representation of the variety of nanostructure morphologies is presented in Figure 1. The aptness of the nanostructured system is often associated with the characteristics of the antimicrobial substance and the nanomaterial, and the nature of the food to which the application is intended for. Polymeric nanocapsules, frequently formed by an oily liquid core

surrounded by a polymer layer, are suitable to entrap and deliver hydrophobic substances. Nanoliposomes, formed as concentric phospholipid bilayered vesicles with an aqueous core, can entrap and deliver both hydrophobic and hydrophilic molecules (Brandelli 2012). A summary of the major properties of nanostructures available for delivery of natural antimicrobials is presented in Table 1.

3. Nanostructured bacteriocins and antimicrobial proteins

The direct introduction of antimicrobials into food products, such as using of nisin in its unencapsulated free form is associated with loss of activity due to degradation and a possible interaction between different food components. Currently, some novel encapsulation methods have been introduced to overcome these limitations, which may improve the antimicrobial activity and stability of biopreservatives in complex systems (Ibarguren et al., 2014; Imran et al., 2015). Some examples of nanostructures for delivery of antimicrobial peptides and proteins are presented in Table 2.

3.1 Nisin

Nisin is a bacteriocin produced by strains of *Lactococus lactis* and belongs to the lantibiotic family, which contains the modified amino acids lanthionine and methyllanthionine. It is a cationic and amphiphilic antimicrobial peptide composed of 34 amino acid residues, having an isoelectric point above 8.5 (Arauz et al., 2009). Nisin exists as two major variants (A and Z), which differ by a single amino acid at position 27, being histidine in nisin A and asparagine in nisin Z (Breukink and de Kruijff, 1999).

Nisin has received particular attention because of its antibacterial activity against a wide variety of Gram-positive bacteria, such as the foodborne pathogens *Listeria monocytogenes*, *Staphylococcus aureus*, as well as the effective inhibition of spores of *Bacillus* and *Clostridium*. It is a food additive (E234) approved by the Food and Agriculture Organization/World Health Organization (FAO/WHO), mainly used as a preservative in heat processed and low pH foods. Nisin is also part of the FDA list of Generally Recognized As Safe (GRAS) additives (Ibarguren et al., 2014; Salmieri et al., 2014).

Prombutara et al. (2012) studied the slow release of nisin-loaded solid lipid nanoparticles (SLN) produced by high-pressure homogenization to provide protection from the food environment and prolong the biological activity. Nisin showed a sustained release rate from nanoparticles and could inhibit the growth of *Listeria monocytogenes* DMST 2871 and *Lactobacillus plantarum* TISTR 850 over at least 15-20 days, compared to less than three days for free nisin in solution.

Several authors have described the antimicrobial activity of nisin encapsulated into phosphatidylcholine liposomes (Were et al., 2004; Taylor et al., 2008; Malheiros et al., 2010). More recently, phosphatidylcholine liposomes containing chitosan or chondroitin sulfate were developed by the thin-film hydration method, and their antimicrobial activity was tested against *Listeria* spp. (Silva et al., 2014). The liposome containing chitosan was more stable and more efficient in inhibiting *L. monocytogenes* when compared to the other liposome tested, can be interesting structures for encapsulate nisin. Imran et al. (2015) evaluated purified nisin encapsulated into liposomes made with marine lecithin or soy lecithin using a continuous cell disruption system method of microfluidic format. Liposomes prepared from soy lecithin (5 g/100

mL) provided best encapsulation efficiency (47%) and high physical stability. The antimicrobial assay revealed that the combination of free and encapsulated nisin (1:1) exhibited a better control of *L. monocytogenes* as compared to free or encapsulated nisin alone.

Nisin loaded chitosan/carrageenan nanocapsules were prepared by Chopra et al. (2014) using an ionic complexation method, for controlled and sustained release food preservative formulations. The optimal parameters for synthesis of particles with highest encapsulation efficiency and smallest size were 10.0 mg/mL chitosan, 0.75 mg/mL carrageenan and 1.0 mg/mL surfactant. The antimicrobial activity of the nanoparticulate formulation was evaluated against various microorganisms (*M. luteus*, *P. aeruginosa*, *S. enterica* and *E. aerogenes*) and while incorporated in fresh tomato juice demonstrated long lasting efficiency in controlling microbial contaminations. The combination of chitosan and alginate was successfully used to encapsulate nisin, with 95% encapsulation efficiency (Zohri et al., 2010). These nanoparticles showed increased antimicrobial activity against *S. aureus* than that observed to free nisin, and showed extended antimicrobial activity when tested in milk samples. Another type of polysaccharide-based nanoparticles were tested as carriers for nisin. Phytoglycogen obtained from corn was subjected to β-amylolysis and chemical modifications, resulting in nisin-coated derivatives that were capable to maintain the antimicrobial activity against *L. monocytogenes* for 21 days (Bi et al., 2011).

Nanocomposite films composed of poly (lactic acid) containing cellulose nanocrystals were prepared by an adsorption coating method, using nisin as antimicrobial agent. These films were then used as packages for cooked ham as a food model, showing to be stable after 14 days of storage at 4°C and efficient to inhibit *L. monocytogenes* (Salmieri et al., 2014). Nisin was also

adsorbed on the surface of different nanoclays, namely bentonite, octadecylamine-modified montmorillonite and halloysite. These bacteriocin-nanoclay systems were analyzed using skimmed milk agar as food simulant and the greater antimicrobial activity was observed for halloysite samples (Meira et al., 2015). Starch/halloysite/nisin nanocomposite films were developed as active antimicrobial packaging, which was effective to control *L. monocytogenes* on Minas Frescal cheese (Meira et al., 2016).

Some interesting and innovative strategies have been proposed to develop antimicrobial films containing nanostructured nisin (Figure 2). Nisin encapsulated into phosphatidylcholine liposomes was incorporated to biopolymer-based films of either hydroxyethyl cellulose, gelatin or casein, resulting in active packaging materials that exhibit effective inhibition of *L. monocytogenes* (Imran et al., 2012, Boelter and Brandelli 2016). In addition, nanofibers containing nisin have been described as well. Nisin incorporation into gelatin nanofibers resulted in antimicrobial mats showing effect against *Lactobacillus plantarum*, *S. aureus* and *L. monocytogenes*. The antimicrobial nanofiber maintained its antimicrobial activity after storage for 5 months at 25^{o}C (Dheraprasart et al., 2009). In another approach, nisin was immobilized on carboxylated cellulose nanofibers using a coupling agent. The grafting of nisin on nanocellulose was effective and the nanostructure showed excellent antimicrobial activity against *S. aureus* and *Bacillus subtilis* (Saini et al., 2016).

3.2 Pediocin

Another bacteriocin that attracts research interest is pediocin. This bioactive peptide produced by *Pediococcus acidilactici* has the ability to kill *Listeria* and closely related bacteria. It belongs to the Class II of unmodified bacteriocins, subdivided in groups of the pediocin-like bacteriocins

and two-peptide bacteriocins. Pediocin is a small (<5 kDa), heat stable membrane-active peptide, and its activity is retained at a wide pH range but is sensitive to most proteases. This bacteriocin presents a great interest to the food-processing industry, such as applications in food preservation and potential substitutes for chemical preservatives due to its activity in controlling *Listeria monocytogenes*, a pathogen of special concern in the food industry (Bemena et al., 2014; Espitia et al., 2013).

The use of pediocin associated to nanotechnology allowed the development of new antimicrobial packaging for food preservation. Espitia et al. (2013) developed nanocomposite films of methylcellulose incorporated with pediocin and ZnO nanoparticles. Optimal concentrations were 20% (w/w) ZnO nanoparticles and 15% (w/w) pediocin. Developed nanocomposite films presented antimicrobial activity against *L. monocytogenes* and *S. aureus*, showing the potential use of developed nanocomposite films for the control of these food borne pathogens.

Encapsulation of pediocin into liposomes prepared from partially purified soybean phosphatidylcholine by the film hydration method was successfully achieved by Mello et al. (2013). The efficiency of encapsulation was 80% and the stability of liposomes, maintaining the antimicrobial activity, showed potential for application in food systems. The controlled release behavior of pediocin encapsulated in different materials such as phospholipids, alginate and guar gum, was investigated by Narsaiah et al. (2013). Encapsulated pediocin was more effective inhibiting *Listeria innocua* than free pediocin, and hybrid capsules of alginate plus guar gum incorporating pediocin-loaded phospholipid nanoliposomes was the best delivery system.

As described for nisin, pediocin was also able to adsorb on nanoclays (Meira et al., 2015). Despite the higher adsorption of pediocin was obtained on bentonite, the antimicrobial activity detected using BHI and skimmed milk agar plates as food simulant was better when halloysite nanoclay was used as a support agent. Largest inhibition zones were observed against Gram-positive bacteria, indicating that nanoclays, especially halloysite, are suitable nanocarriers for pediocin adsorption.

3.3 Lactoferrin

Lactoferrin is an 80 kDa iron-binding glycoprotein of the transferrin family, acting to control iron levels in body fluids by sequestering and solubilizing ferric iron. This globular protein has multiple biological functions, and it is not only involved in iron transport, but also has immune response, antioxidant, anticarcinogenity, antiviral and anti-inflammatory properties. Besides, it has considerable potential as a functional ingredient in food, cosmetic and pharmaceutical applications (Guan et al., 2012; Wu et al., 2013).

The use of lactoferrin has the interest of many researchers as a natural compound, which displays a wide array of modes of action to perform its primary antimicrobial function. It contains various antimicrobial peptides encrypted in its sequence, which can be released upon hydrolysis by proteases (Sinha et al., 2013).

Lactoferrin nanoliposomes with high encapsulation efficiency were prepared by reverse-phase evaporation method and tested in simulated gastrointestinal juice in vitro, showing an acceptable stability at 37°C for 4 h. Lactoferrin nanoliposomes and free lactoferrin exhibited a dose-dependant effect on the survival of Caco-2 cells, and lactoferrin nanoliposomes had a more obvious activity on the cells. This approach suggests that lactoferrin nanoliposomes with

different concentration could modulate the growth of tumor cells and may be suitable for use in oral administration (Guan et al., 2012). The possibility of lactoferrin nanoliposomes to administer a drug to specific cells, and facilitating their uptake makes them suitable for potential applications in medicine.

Production of lactoferrin derivatives, encompassing full stabilization of its three-dimensional structure, was obtained by nanoencapsulation with in lipid nanovesicles (Balcão et al., 2013). The use of an electrolyte with a low ionic strength and low lactoferrin concentrations with a homogenization time of 10 min were found as critical variables for producing stable nanovesicle dispersions, which were successfully employed at lab-scale antimicrobial trials.

3.4 Lysozyme and other antimicrobial enzymes

Lysozyme is alkaline molecule with isoelectric point 11.35, which can catalyze the hydrolysis of peptidoglycan and chitodextrins. Furthermore, it has antifungal, antiviral, antitumor, and immune modulatory activities (Sava et al., 1989; Cartel et al., 1992; Gorbenko et al., 2007). It is a safe food preservative and has antibacterial activity against Gram-positive bacteria and Gram-negative bacteria (Davidson et al., 2013). Lysozyme has been used as a model protein for incorporation in nanostructures, and its antibacterial applications can be enhanced through protective coatings and pretreatment for controlled release (Liu et al., 2013).

Lysozyme activity was totally preserved when it was used as a positively charged model protein to study the viability of negatively charged nanoparticles, consisting of polymer blends of poly lactic-co-glycolic acid (PLGA) and poly(styrene-co-4-styrene-sulfonate), to improve the loading capacity and release properties (Cai et al., 2008). Methods of coating with poly-γ-glutamic acid and chitosan have been established to improve the applications of lysozyme. When

the lysozyme was coated with poly-γ-glutamic acid and chitosan, the loading efficiency and loading content reached 76% and 40%, respectively, with outstanding antibacterial activity, providing a reference for coating and controlled release of alkaline proteins (Liu et al., 2013).

The antimicrobial enzyme lysostaphin produced by *Staphylococcus staphylolyticus* belongs to the class of endopeptidases. This enzyme hydrolyzes the cell wall the bacteria through cleavage of cross-linked pentaglycine bridges in the peptidoglycan of *S. aureus* (Bastos et al., 2010). A study on antimicrobial activity of lysostaphin-antibody-nanoparticle and lysostaphin-nanoparticle conjugates against *S. aureus* at different enzyme and antibody ratios was developed. The increased antimicrobial activity was observed for both enzyme-coated and enzyme-antibody-coated nanoparticles for lysostaphin coatings (Satishkumar and Vertegel, 2011). Thus, these nanoparticles have the potential for becoming novel therapeutic agents for treating antibiotic-resistant *S. aureus* infections.

The papain enzyme belongs to the papain superfamily, which shows extensive proteolytic activity towards proteins, short-chain peptides, and amide links and amino acid esters, and is extensively applied in the fields of medicine and food (Amri and Mamboya, 2012). The covalent immobilization of papain to four different types of polymers (low density polyethylene, high density polyethylene, linear low density polyethylene and polycaprolactam) with curcumin as the photocrosslinker was studied by Manohar et al. (2015). After 30 days these papain crosslinked polymers remained stable and active, moreover exhibited antibiofilm activity against both a Gram positive (*S. aureus*), as well as a Gram negative (*Acinetobacter sp.*) strain, showing a promising platform for fabricating nontoxic and renewable antimicrobial films, which may offer a wide range of applications in food and pharmaceutical packaging industries.

3.5 Other antimicrobial peptides

Bificin C6165, a bacteriocin produced by *Bifidobacterium animalis* subsp. *animalis* CICC 6165, is employed to control *Alicyclobacillus acidoterrestris* in fruit juices. The inclusion of bificin C6165 in diluted apple juices prior to heat treatment increased the bacterial heat sensitivity. The inhibitory effect was better at lower pH (pH 3.5) and at a higher temperature of 45°C, as bificin C6165 is relatively heat-stable at acidic pH value. Furthermore, the encapsulation of bificin C6165 with Ca-alginate nanocomposite was investigated, demonstrating a promising method to control *A. acidoterrestris* in food juice industry (Pei et al., 2014).

Piras et al. (2015) developed the encapsulation of the frog-skin derived antimicrobial peptide temporin B, into chitosan nanoparticles. The encapsulation efficiency was 75%, reducing significantly the peptide cytotoxicity against mammalian cells, and showing antibacterial action against strains of *Staphylococcus epidermidis* for up to 4 days. Piras et al. (2015b) also developed a model drug delivery system based on chitosan nanoparticles using Renin substrate I, displaying almost 100% of encapsulation efficacy and a linear release of the Renin substrate I. Despite to chitosan be commonly formulated with negatively charged active agents (such as anionic proteins), the use of a small cationic active agent promoted the formation of "core-shell" nanoparticles, thus the waste of expensive and precious materials such as peptides is highly limited with the described model.

Bacillus sp. P34 produces the antimicrobial peptide P34, with a molecular mass of 1,456 Da and antibacterial activity against pathogenic and food spoilage bacteria, besides stable within a broad range of pH and temperature (Motta et al., 2007). Malheiros et al. (2011) developed liposomes containing the antimicrobial peptide P34 prepared from partially purified soybean

phosphatidylcholine, showing high encapsulation efficiency and stability during storage, and demonstrating potential for use as a biopreservative in food. These liposomes were tested to control the growth of *L. monocytogenes* in Minas frescal cheese resulting in lower values of viable counts as compared with the treatment with free peptide or controls without antimicrobial addition (Malheiros et al., 2012).

The encapsulation of bacteriocins produced *Lactobacillus sakei* subsp. *sakei* 2a into phosphatidylcholine liposomes resulted in antilisterial activity by 5 days in UHT goat milk stored at 7°C. The nanovesicles presented high encapsulation efficiency, low polydispersity index and stability for 28 days, evidencing their potential technological applicability (Malheiros et al., 2016). A similar liposomal system was previously used to encapsulate the antimicrobial peptide P40, resulting in extension of antimicrobial activity against *L. monocytogenes* as compared with the free peptide (Teixeira et al., 2008).

The bacteriocins plantaricin 423 and ST4SA were incorporated into nanofibers prepared with poly(lactic acid)/polyethylene oxide blends, providing high concentration of antimicrobial peptides into nanofibers. The mechanical properties and degradation rate of poly(lactic acid) can be improved by blending with a hydrophilic polymer such as polyethylene oxide, and the release rate could be controlled by selecting the adequate combination of polymers (Heunis et al., 2011). Subtilosin is a cyclic antimicrobial peptide produced by *Bacillus amyloliquefaciens* with antimicrobial spectrum against diverse bacterial pathogens. This antimicrobial also showed antiviral activity on Herpes simplex virus 1 and maintained its antimicrobial activity after incorporation into poly(vinyl alcohol) nanofibers (Torres et al., 2013). These studies indicate that

nanofibers are a promising platform for effective delivery of natural antimicrobial peptides in food.

Antimicrobial peptides carried on silver and gold nanoparticles have been used for the control and treatment of infectious diseases, demonstrating better antibacterial, antifungal and antiviral activities when conjugated with biopolymers (Rai et al., 2015). Silver is considered the foremost metal because of its well-known antimicrobial properties, showing potential activity against antibiotic-resistant organisms as well. Gold nanoparticles exhibit important properties, including antibacterial activity against *S. aureus* and *P. aeruginosa* (Bindhu and Umadevi, 2014; Rai et al., 2015). Thus, the combination of biocidal properties of metallic nanoparticles with the antimicrobial activity of some peptides and proteins has been effectively demonstrated (Brandelli, 2012, Thirumurugan et al., 2013). Silver nanoparticles were functionalized with an antibacterial peptide produced by a food-grade lactic acid bacterium. The resulting in enterocin-coated silver nanoparticles exhibited a broad-spectrum antimicrobial activity against food-borne pathogenic bacteria without any detectable toxicity to red blood cells (Sharma et al., 2012).

4. Encapsulation of plant derived antimicrobials

4.1 Plant extracts and essential oils

Essential oils derived from plants are widely used in medicine and the food industry because of their antibacterial, antifungal, antiviral and antioxidant properties. Essential oils are complex mixtures of volatile constituents biosynthesized by plants, which mainly include terpenes and terpenoids and aromatic and aliphatic constituents, all characterized by owning low molecular weight (Davidson et al., 2013). The antimicrobial activity in essential oils is generally associated with oxygenated terpenoids, such as alcohols and phenolic terpenes, but some hydrocarbons also

exhibit antimicrobial effects. Essential oils show promising activities against many food-borne pathogens in vitro, but in food systems, higher concentrations of essential oils are needed to obtain similar antibacterial effects (Bassolé and Juliani, 2012).

PLGA nanoparticles containing cinnamon bark extract, a natural antimicrobial, were produced using an emulsion-solvent evaporation method (Hill et al., 2013). This nanostructured antimicrobial formulation proved to be inhibitor of *S. enterica* and *L. monocytogenes* after 24 and 72 h at concentrations ranging from 224.4 to 549.2 μg/mL, respectively.

To prevent the long-term instability, the microwave extract of *P. madagascariensis* was encapsulated into alginate beads with high efficiency, showing antimicrobial activity against *Staphylococcus epidermidis*, along with antioxidant capacity and low toxicity in the models tested. This extract is a potential antibacterial skin active ingredient (Rijo et al., 2014). Propolis-chitosan nanobeads permits the encapsulation of natural polyphenols, inhibiting microbial growth of *S. aureus* in liquid culture. These nanoparticles were able to release the bioactive compounds from propolis. Beads proved to be effective against the most common spoilage and pathogenic microorganisms that might be present in fresh food products (Mascheroni et al., 2014).

Noudoost et al. (2015) assessed the modulation of prebiotic properties of biological substances by nanoencapsulation. The nanoliposomes of green tea extract improved its beneficial properties including antibacterial and antioxidant activities. Furthermore, addition of 1% nanoliposomes green tea extract increased the growth rate of *Lactobacillus casei* and *Bifidobacterium lactis* to a significant extent.

The encapsulation of garlic extract into nanometric liposomes has been recently described. The incorporation of garlic extract caused an effective inhibition of different *Listeria* strains when tested in milk as a food model (Pinilla et al., 2017). The co-encapsulation of garlic extract with nisin was also investigated, resulting an extended antimicrobial effect against several foodborne pathogenic bacteria, including *Listeria monocytogenes*, *Staphylococcus aureus*, *Escherichia coli* and *Salmonela enterica* serotype Enteritidis (Pinilla and Brandelli, 2016).

Tragacanth gum is a natural biocompatible and biodegradable polysaccharide used to produce nanocapsules containing plant extracts through a microemulsion method based on ultrasonic homogenization and magnetic stirring in the presence of aluminum chloride and calcium chloride (Ghayempour et al., 2015). The antimicrobial activity of nanocapsules against *E. coli*, *S. aureus* and *C. albicans* showed 100% microbial reduction after 12 h by shake flask method, thus Tragacanth gum is a suitable for using as the wall material during of encapsulation plant extracts.

Nanoemulsions containing essential oils from Amazonian plants were prepared to inhibit *Paenibacillus larvae*, the etiologic agent of American foulbrood disease, which causes severe economic losses in honey production. The nanoemulsions of both Andiroba and Copaiba oils were prepared by high-pressure homogenization and resulted nanoparticles with droplet size around 200 nm. These nanostructures caused a solid inhibition of several *Paenibacillus* species, suggesting a potential strategy for the treatment or prevention of American foulbrood (Vaucher et al., 2015). Nanoemulsions containing lemongrass, clove, thyme, or palmarosa essential oils showed the strongest antimicrobial activity against *E. coli* after 30 min contact (Salvia-Trujillo et

al., 2015). Nanoemulsions of clove bud and oregano essential oils were incorporated into water-soluble methylcellulose films and the antimicrobial activity was tested in bread slices. These antimicrobial films caused a reduction in yeasts and molds counts in the sliced bread during 15 days (Otoni et al., 2014).

4.2 Defined phytochemicals

Phytochemicals are important natural bioactive compounds of fruit and vegetables and are classified into several groups, such as polyphenols (phenolic acids and flavonoids), gluconisolates and polyacetylenes, being widely recognized for their nutraceutical effects and health benefits (Tiwari and Cummins, 2015). The use of bioactive molecules from plants has gained a substantial interest for food, cosmetic and pharmaceutical applications. Polyphenols are among the most powerful active compounds synthesized by plants, in view of their antioxidant activity and bactericidal and fungicidal actions. However, their poor bioavailability, combined with sensitive to light and heat, limits their use in food or in oral medications (Munin and Edwards-Lévy, 2011; Pinho et al., 2014). The chemical structures of some plant-derived substances that have been incorporated into nanoparticles are depicted in Figure 3.

Nanoencapsulation technology has been recently applied to improve the physicochemical properties of some phytochemicals. The optimization of the production of chitosan nanoparticles with polyphenols such as rosmarinic acid, protocatechuic acid and 2,5-dihydroxybenzoic acid was evaluated by Madureira et al. (2015), showing that both free and encapsulated compounds have inhibitory activity against *Bacillus cereus*, *Escherichia coli* O157, *Listeria innocua*, *Staphylococcus aureus*, *Salmonella enterica* serotype Typhimurium and *Yersinia enterocolitica*.

The best encapsulation performance was obtained for nanoparticles produced with the polyphenol rosmarinic acid.

The encapsulation of eugenol and carvacrol, hydrophobic components of plant essential oils, into surfactant micelles composed of polysorbate 20 (Tween 20), Surfynol® 485W, sodium dodecyl sulfate and CytoGuard® LA 20, can assist the decontamination of fresh produce surfaces from bacterial pathogens during postharvest washing (Ruengvisesh et al., 2015). Spinach samples were treated with eugenol-containing micelles applied via spraying or immersion methods, against *Escherichia coli* O157:H7 and *Salmonella enterica* serotype Saintpaul, showing the lowest minimum inhibitory and bacterial concentration for pathogens with sodium dodecyl sulfate and CytoGuard® LA 20 micelles containing essential oil components.

Thymol, a natural antimicrobial agent from thyme and thyme oil, was encapsulated in nanoparticles prepared with sodium caseinate and chitosan. The nanoparticles were more effective in suppressing Gram-positive bacterium than non-encapsulated thymol for a longer period (Zhang et al., 2014). Eugenol is a major phenolic component with diverse biological activities, but its application is limited due to poor water solubility. Thus, eugenol nanoliposomes were prepared by combining the ethanol injection method with dynamic high-pressure microfluidization, exhibiting good storage stability, relatively good sustained release of eugenol and antibacterial activities (Peng et al., 2015).

Banerjee et al. (2015) evaluated the antibacterial activity of free and liposomal formulation of apigenin, a bioflavonoid present in fruits and vegetables, finding high amount of apigenin loading within liposomal suspension. The liposomes were stable over a period of six

weeks, also significantly decreased minimum inhibitory concentrations against both Gram-positive and Gram-negative bacteria. Esfandyari-Manesh et al. (2013) prepared PLGA nanoparticles for prolonging the efficacy of antimicrobial activity of anethole and carvone by nanoprecipitation methods. Studies conducted in vitro at 37°C indicated that release of carvone continued for 4 days and 9 days for anethole, also showed antimicrobial activity of these nanoparticles against Gram-positive and Gram-negative bacteria. Gomes et al. (2011) synthesized PLGA nanoparticles with *trans*-cinnamaldehyde and eugenol with poly(vinyl alcohol) as surfactant by emulsion evaporation method. The entrapment efficiency for eugenol and *trans*-cinnamaldehyde was 98% and 92%, respectively, and all loaded nanoparticles formulations proved to be efficient in inhibiting *Salmonella* spp. and *Listeria* spp. with continuous releasing during 72 h. Cinnamaldehyde was also incorporated to chitosan/polyethylene oxide nanofibers with about 50 nm diameter. These nanofibers caused high inactivation rates against *E. coli* and *P. aeruginosa*, attributed to the rapid release of cinnamaldehyde combined with the intrinsic antimicrobial activity of chitosan (Rieger and Schiffman, 2014).

Curcumin is a major polyphenolic phytochemical of *Curcuma longa* L. The rhizome has been traditionally used as antimicrobial agent and curcumin has receiving significant attention due to a variety of biological activities, including broad-spectrum antibacterial, antiviral and antifungal activities (Moghadamtousi et al., 2014). Curcumin is highly hydrophobic and difficult to incorporate in aqueous food systems. For this reason, it has been used as a model molecule for encapsulation studies in different nanostructures (Figure 4). Curcumin liposomes were prepared by ethanol injection method and then coated with chitosan. The cumulative release rate of

curcumin was faster with temperature increase, but it could be controlled by chitosan covering (Liu et al., 2015). Curcumin nanoemulsion prepared with medium chain triglyceride oil, whey protein concentrate and Tween 80 through ultrasound processing, resulted in stable nanoparticles for 27 days at room temperature, with an encapsulation efficiency of 90%. The nanoemulsion was resistant to pepsin digestion, but pancreatin causes the release of curcumin (Sari et al., 2015). The encapsulation of curcumin was studied by spray-drying dispersion with casein, resulting a powder containing about 16% curcumin and an encapsulation efficiency of 83% (Pan et al., 2013). The encapsulation caused the loss of curcumin crystallinity because it was entrapped in the nanoparticle core through hydrophobic interactions. In addition, the encapsulation of curcumin in k-carrageenan and lysozyme resulted in spherical shaped nanoparticles spontaneously formed by one-step incubation, with an encapsulation efficiency of 71% (Xu et al., 2014). These studies demonstrated that nanoencapsulation has great potential to increase the apparent solubility and delivery of sensitive hydrophobic compounds, protecting them against harsh environment.

Some antimicrobial compounds are generated by the action of plant enzymes on inactive precursors to enhance the plant defence system under adverse conditions. In garlic, mustard and horseradish, the enzyme myrosinase converts glucosinolates into diverse isothiocyanates, some of them showing important antimicrobial activity (Bridges et al., 2002). Allicin, the major antimicrobial compound of garlic, was encapsulated into liposomes prepared with lecithin/cholesterol. These liposomes showed a mean diameter of 145 nm, zeta-potential of -40.1 mV and encapsulation efficiency of 75%, suggesting that this nanostructure was stable and could protect allicin from unfavorable conditions, such as heat, light and alkaline conditions (Lu et al.,

2014). The antimicrobial compound allyl isothiocyanate was encapsulated into nanofibers composed by poly(lactic acid) and soy protein/polyethylene oxide blends (Vega-Lugo and Lim, 2009). The controlled release of the antimicrobial was triggered by increase in the relative humidity, suggesting that the antimicrobial nanofibers may be promising in active food packaging applications.

5. Conclusions and perspectives

Nanostructures are fascinating tools with great potential for delivery and controlled release of natural antimicrobials in food. Natural food antimicrobials, particularly nisin and some essential oils, have been successfully encapsulated into liposomes or polymer nanovesicles. Other natural antimicrobials have been incorporated in nanostructures as well, showing an effective antimicrobial activity on a diversity of food spoilage and pathogenic microorganisms. Nevertheless, studies have been essentially performed *in vitro* and therefore, additional work for incorporation of antimicrobial nanostructures in real food products should be warranted. In this regard, the incorporation of nanostructured natural antimicrobials in food packaging seems to be closer to large-scale applications in the food sector. Diverse nanocomposite active packaging have been developed, and the controlled release to the food matrix appears to comply with the safety aspect.

Toxicology of nanostructured food additives should be also a major point of investigation. The toxicological aspects of formulated nanostructures that could be incorporated to food systems are yet poorly explored. Despite many foods contain nanoscale components that have been safely eaten for a long time, there is a scientific consent that nanomaterials are essentially different substances, which may represent new and unique risks to human health and

environment. Thus, specific forms of safety monitoring are required to warrant food safety and quality. Nanotechnology presents many valuable applications in food science and technology, allowing to create innovative food products and technologies. The correct evaluation and management of food components at the nanometric scale is essential to the effective inclusion of this technology by the food industry.

Acknowledgments

A Brandelli is research awardee from CNPq (Brasilia, Brazil).

Conflicts of interest

Authors declare no conflicts of interest.

References

Aasen, I.M., Markussen, S., Moretro, T., Katla, T., Axelsson, L., and Naterstad, K. (2003). Interactions of the bacteriocins sakacin P and nisin with food constituents. *Int. J. Food Microbiol*. 87: 35-43.

Amri, E., and Mamboya, F. (2012). Papain, a plant enzyme of biological importance: A review. *Am. J. Biochem. Biotechnol*. 8: 99-104.

Arauz, L.J., Jozala, A.F., Mazzola, P.G., and Vessoni-Penna, T.C. (2009). Nisin biotechnological production and application: a review. *Trends Food Sci. Technol*. 20: 146-154.

Balcão, V.M., Costa, C.I., Matos, C.M., Moutinho, C.G., Amorim, M., Pintado, M.E., Gomes, A.P., Vila, M.M., and Teixeira, J.A. (2013). Nanoencapsulation of bovine lactoferrin for food and biopharmaceutical applications. *Food Hydrocolloids*. 32: 425-431.

Banerjee, K., Banerjee, S., Das, S., and Mandal, M. (2015). Probing the potential of apigenin liposomes in enhancing bacterial membrane perturbation and integrity loss. *J. Colloid Interf. Sci*. 453: 48-59.

Bassolé, I.H.N., and Juliani, H.R. (2012). Essential oils in combination and their antimicrobial properties. *Molecules*. 17: 3989-4006.

Bastos, M.C.F., Coutinho, B.G., and Coelho, M.L.V. (2010). Lysostaphin: a staphylococcal bacteriolysin with potential clinical applications. *Pharmaceuticals*. 3: 1139-1161.

Bemena, L.D., Mohamed, L.A., Fernandes, A.M., and Lee1, B.H. (2014). Applications of bacteriocins in food, livestock health and medicine. *Int. J. Curr. Microbiol. Appl. Sci*. 3: 924-949.

Bi, L., Yang, L., Narsimhan, G., Bhunia, A.K., and Yao, Y. (2011). Designing carbohydrate nanoparticles for prolonged efficacy of antimicrobial peptide. *J. Control. Release*. 150: 150-156.

Bindhu, M.R., and Umadevi, M. (2014). Antibacterial activities of green synthesized gold nanoparticles. *Mater. Lett.* 120: 122-125.

Boelter, J.F., and Brandelli A. (2016). Innovative bionanocomposite films of edible proteins containing liposome-encapsulated nisin and halloysite nanoclay. *Colloid. Surf. B.* 145: 740-747.

Brandelli, A. (2012). Nanostructures as promising tools for delivery of antimicrobial peptides. *Mini-Rev. Med. Chem.* 12: 731-741.

Brandelli, A. (2015). Nanobiotechnology strategies for delivery of antimicrobials in food and agriculture. In: Nanotechnologies in Food and Agriculture, pp. 119-139. Rai, M., Ribeiro, C., Duran, N., Eds., Springer, Cham.

Brandelli, A., and Taylor, T.M. (2015). Nanostructured and nanoencapsulated natural antimicrobials for use in food products. In: Handbook of Natural Antimicrobials for Food Safety and Quality, pp. 229-257. Taylor, T.M., Ed., Elsevier, London.

Breukink, E., and de Kruijff, B. (1999). The lantibiotic nisin, a special case or not? *Biochim. Biophys. Acta*. 1462: 223-234.

Bridges, M., Jones, A.M., Bones, A.M., Hodgson, C., Cole, R., Bartlet, E., Wallsgrove, R., Watts, N., and Rossiter, J.T. (2002). Spatial organization of the glucosinolate-myrosinase system in brassica specialist aphids is similar to that of the host plant. *Proc. Biol. Sci.* 269: 187-191.

Cai, C., Bakowsky, U., Rytting, E., Schaper, A.K., and Kissel, T. (2008). Charged nanoparticles as protein delivery systems: a feasibility study using lysozyme as model protein. *Eur. J. Pharmaceut. Biopharm*. 69: 31-42.

Cartel, F., Cartei, G., Ceschia, V., Pacor, S., and Sava, G. (1992). Recovery of lymphocyte $CD4^+$:$CD8^+$ ratio in patients treated with lysozyme. *Drug Invest*. 4: 51-57.

Chopra, M., Kaur, P., Bernela, M., and Thakur, R. (2014). Surfactant assisted nisin loaded chitosan-carageenan nanocapsule synthesis for controlling food pathogens. *Food Control*. 37: 158-164.

Dasgupta, N., Ranjan, S., Mundekkad, D., Ramalingam, C., Shanker, R., and Kumar, A. (2015). Nanotechnology in agro-food: From field to plate. *Food Res. Int.* 69: 381-400.

Davidson, P.M., Critzer, F.J., and Taylor, T.M. (2013). Naturally occurring antimicrobials for minimally processed foods. *Annu. Rev. Food Sci. Technol*. 4: 163-190.

Dheraprasart, C., Rengpipat, S., Supaphol, P., and Tattiyakul, J. (2009). Morphology, release characteristics, and antimicrobial effect of nisin-loaded electrospun gelatin fiber mat. *J. Food Protec.* 72: 2293-2300.

Esfandyari-Manesha, M., Ghaedib, Z., Asemic, M., Khanavic, M., Manayic, A., Jamalifard, H., Atyabia, F., and Dinarvand, R. (2013). Study of antimicrobial activity of anethole and carvone loaded PLGA nanoparticles. *J. Pharm. Res.* 7: 290-295.

Espitia, P.J.P., Soares, N.F.F., and Teófilo, R.F. (2013). Physical-mechanical and antimicrobial properties of nanocomposite films with pediocin and ZnO nanoparticles. *Carbohydr. Polym.* 94: 199-208.

Ghayempour, S., Montazer, M., and Mahmoudi Rad, M. (2015). Tragacanth gum as a natural

polymeric wall for producing antimicrobial nanocapsules loaded with plant extract. *Int. J. Biol. Macromol.* 81: 514-520.

Gomes, C., Moreira, R.G., and Castell-Perez, E. (2011). Poly(DL-lactide-co-glycolide) (PLGA) nanoparticles with entrapped *trans*-cinnamaldehyde and eugenol for antimicrobial delivery applications. *J. Food Sci.* 76: S16-S24.

Gorbenko, G.P., Ioffe, V.M., and Kinnunen, P.K.J. (2007). Binding of lysozyme to phospholipids bilayers: Evidence for protein aggregation upon membrane association. *Biophys. J.* 93: 140-153.

Guan, R., Ma, J., Wu, Y., Lu, F., Xiao, C., Jiang, H., and Kang, T. (2012). Development and characterization of lactoferrin nanoliposome: cellular uptake and stability. *Nanoscale Res. Lett*. 7: 679.

Heunis, T., Bshena, O., Klumperman, B., and Dicks, L. (2011). Release of bacteriocins from nanofibers prepared with combinations of poly(D,L-lactide) (PDLLA) and poly(ethylene oxide) (PEO). *Int. J. Mol. Sci.* 12: 2158-2173.

Hill, L.E., Taylor, T.M., and Gomes, C. (2013). Antimicrobial efficacy of poly (DL-lactide-co-glycolide) (PLGA) nanoparticles with entrapped cinnamon bark extract against *Listeria monocytogenes* and *Salmonella typhimurium*. *J. Food Sci.* 78: N626-N632.

Ibarguren, C., Naranjo, P.M., Stötzel, C., Audisio, M.C., Sham, E.L., Torres, E.M.F., and Müller, F.A. (2014). Adsorption of nisin on raw montmorillonite. *Appl. Clay Sci.* 90: 88-95.

Imran, M., Revol-Junelles, A.-M., Rene, N., Jamshidian, M., Akhtar, M.J., Arab-Tehrany, E., Jacquot, M., and Desobry, S. (2012). Microstructure and physico-chemical evaluation of

nano-emulsion-based antimicrobial peptides embedded in bioactive packaging films. *Food Hydrocolloids*. 29: 407-419.

Imran, M., Revol-Junelles, A.M., Paris, C., Guedon, E., Linder, M., and Desobry, S. (2015). Liposomal nanodelivery systems using soy and marine lecithin to encapsulate food biopreservative nisin. *LWT Food Sci. Technol*. 62: 341-349.

Liu, Y., Sun, Y., Xu, Y.X., Feng, H., Fu, S.D., Tang, J.W., Liu, W., Sun, D.C., Jiang, H., and Xu, S.S. (2013). Preparation and evaluation of lysozyme-loaded nanoparticles coated with poly-γ-glutamic acid and chitosan. *Int. J. Biol. Macromol.* 59: 201-207.

Liu, Y., Liu, D., Zhu, L., Gan, Q., and Le, X. (2015). Temperature-dependent structure stability and in vitro release of chitosan-coated curcumin liposome. *Food Res. Int.* 74: 97-105.

Lu, Q., Lu, P.M., Piao, J.H., Xu, X.L., Chen, J., Zhu, L., and Jiang, J.G. (2014). Preparation and physicochemical characteristics of an allicin nanoliposome and its release behavior. *LWT Food Sci. Technol.* 57: 686-695.

Madureira, A.R., Pereira, A., Castro, P.M., and Pintado, M.E. (2015). Production of antimicrobial chitosan nanoparticles against food pathogens. *J. Food Eng.* 167: 210-216.

Malheiros, P.S., Micheletto, Y.M.S., Silveira, N.P., and Brandelli, A. (2010). Development and characterization of phosphatidylcholine nanovesicles containing the antimicrobial peptide nisin. *Food Res. Int.* 43: 1198-1203.

Malheiros, P.S., Sant'Anna, V., Micheletto, Y.M.S., Silveira, N.P., and Brandelli, A. (2011). Nanovesicle encapsulation of antimicrobial peptide P34: physicochemical characterization and mode of action on *Listeria monocytogenes*. *J. Nanopart. Res.* 13: 3545-3552.

Malheiros, P.S., Sant'Anna, V., Barbosa, M.S., Brandelli, A., and Franco, B.D.G.M. (2012). Effect of liposome-encapsulated nisin and bacteriocin-like substance P34 on *Listeria monocytogenes* in Minas frescal cheese. *Int. J. Food Microbiol*. 156: 272-277.

Malheiros, P.S., Cuccovia, I.M., and Franco, B.D.G.M. (2016). Inhibition of *Listeria monocytogenes* in vitro and in goat milk by liposomal nanovesicles containing bacteriocins produced by *Lactobacillus sakei* subsp. *sakei* 2a. *Food Control*. 63: 158-164.

Manohar, C.M., Prabhawathi, V., Sivakumar, P.M., and Doble, M. (2015). Design of a papain immobilized antimicrobial food package with curcumin as a crosslinker. *PLoS One*. 10: e121665, doi: 10.1371/journal.pone.0121665.

Mascheroni, E., Figoli, A., Musatti, A., Limbo, S., Drioli, E., Suevo, R., Talarico, S., and Rollini, M. (2014). An alternative encapsulation approach for production of active chitosan-propolis beads. *Int. J. Food Sci. Technol.* 49: 1401-1407.

Meira, S.M.M., Zehetmeyer, G., Jardim, A.I., Scheibel, J.M., Oliveira, R.V.B., and Brandelli, A. (2014). Polypropylene/montmorillonite nanocomposites containing nisin as antimicrobial food packaging. *Food Bioprocess Technol*. 7: 3349-3357.

Meira, S.M.M., Jardim, A.I., and Brandelli, A. (2015). Adsorption of nisin and pediocin on nanoclays. *Food Chem.* 188: 161-169.

Meira, S.M.M., Zehetmeyer, G., Scheibel, J.M., Werner, J.O., and Brandelli, A. (2016). Starch-halloysite nanocomposites containing nisin: characterization and inhibition of *Listeria monocytogenes* in soft cheese. *LWT Food Sci. Technol*. 68: 226-234.

Mello, M.B., Malheiros, P.S., Brandelli, A., Silveira, N.P., Jantzen, M.M., and Motta, A.S. (2013). Characterization and antilisterial effect of phosphatidylcholine nanovesicles containing the antimicrobial peptide pediocin. *Probiotics Antimicrob. Prot.* 5: 43-50.

Moghadamtousi, S.Z., Kadir, H.A., Hassandarvish, P., Tajik, H., Abubakar, S., and Zandi, K. (2014). A review on antibacterial, antiviral, and antifungal activity of curcumin. *BioMed Res. Int.* 2014: 186864.

Motta, A.S., Cannavan, F.S., Tsai, S.M., and Brandelli, A. (2007). Characterization of a broad range antibacterial substance from a new *Bacillus* species isolated from Amazon basin. *Arch. Microbiol*. 188: 367-375.

Munin, A, and Edwards-Lévy, F. (2011). Encapsulation of natural polyphenolic compounds; a Review. *Pharmaceutics*. 3: 793-829.

Narsaiah, K., Jha, S.N., Wilson, R.A., Mandge, H.M., Manikantan, M.R., Malik, R.K., and Vij, S. (2013). Pediocin-loaded nanoliposomes and hybrid alginate-nanoliposome delivery systems for slow release of pediocin. *BioNanoScience*. 3: 37-42.

Noudoost, B., Noori, N., Amo Abedini, Gh., Gandomi, H., Akhondzadeh Basti, A., Jebeli Javan, A., and Ghadami, F. (2015). Encapsulation of green tea extract in nanoliposomes and evaluation of its antibacterial, antioxidant and prebiotic properties. *J. Med. Plants*. 14: 66-78.

Otoni, C.G., Pontes, S.F., Medeiros, E.A., and Soares, N.F. (2014). Edible films from methylcellulose and nanoemulsions of clove bud (*Syzygium aromaticum*) and oregano (*Origanum vulgare*) essential oils as shelf life extenders for sliced bread. *J. Agric. Food Chem.* 62: 5214-5219.

Pan, K., Zhong, Q., and Baek, S.J. (2013). Enhanced dispersibility and bioactivity of curcumin by encapsulation in casein nanocapsules. *J. Agric. Food Chem.* 61: 6036-6043.

Pei, J., Yue, T., and Yuan, Y. (2014). Control of *Alicyclobacillus acidoterrestris* in fruit juices by a newly discovered bacteriocin. *World J. Microbiol. Biotechnol.* 30: 855-863.

Peng, S., Zou, L., Liu, W., Gan, L., Liu, W., Liang, R., Liu, C., Niu, J., Cao, Y., Liu, Z., and Chen, X. (2015). Storage stability and antibacterial activity of eugenol nanoliposomes prepared by an ethanol injection-dynamic high-pressure microfluidization method. *J. Food Protec.* 78: 22-30.

Pinho, E., Grootveld, M., Soares, G., and Henriques, M. (2014). Cyclodextrins as encapsulation agents for plant bioactive compounds. *Carbohydr. Polym*. 101: 121-135.

Pinilla, C.M.B., and Brandelli, A. (2016). Antimicrobial activity of nanoliposomes co-encapsulating nisin and garlic extract against Gram-positive and Gram-negative bacteria in milk. *Innov. Food Sci. Emerg. Technol*. 36: 287-293.

Pinilla, C.M.B., Noreña, C.P.Z., and Brandelli, A. (2017). Development and characterization of phosphatidylcholine nanovesicles containing garlic extract with antilisterial activity in milk. *Food Chem*. 220: 470-476.

Piras, A.M., Maisetta, G., Sandreschi, S., Gazzarri, M., Bartoli, C., Grassi, L., Esin, S., Chiellini, F., and Batoni, G. (2015). Chitosan nanoparticles loaded with the antimicrobial peptide temporin B exert a long-term antibacterial activity in vitro against clinical isolates of *Staphylococcus epidermidis*. *Front. Microbiol.* 6: 372.

Piras, A.M., Sandreschi, S., Maisetta, G., Esin, S., Batoni, G., and Chiellini, F. (2015b). Chitosan nanoparticles for the linear release of model cationic peptide. *Pharm. Res.* 32: 2259-2265.

Prombutara, P., Kulwatthanasal, Y., Supaka, N., Sramala, I., and Chareonpornwattana, S. (2012). Production of nisin-loaded solid lipid nanoparticles for sustained antimicrobial activity. *Food Control*. 24: 184-190.

Rai, M., Ingle, A.P., Gupta, I., and Brandelli, A. (2015). Bioactivity of noble metal nanoparticles decorated with biopolymers and their application in drug delivery. *Int. J. Pharm*. 496: 159-172.

Ranjan, S., Dasgupta, N., Chakraborty, A.R., Samuel, S.M., Ramalingam, C., Shanker, R., and Kumar, A. (2014). Nanoscience and nanotechnologies in food industries: opportunities and research trends. *J. Nanopart. Res.* 16: 2464.

Rhim, J-W., Park, H-M., and Ha, C-S. (2013). Bio-nanocomposites for food packaging applications. *Progr. Polym. Sci.* 38: 1629-1652.

Rieger, K.A., and Schiffman, J.D. (2014). Electrospinning an essential oil: cinnamaldehyde enhances the antimicrobial efficacy of chitosan/poly(ethylene oxide) nanofibers. *Carbohydr. Polym*. 113: 561-568.

Rijo, P., Matias, D., Fernandes, A.S., Simões, M.F., Nicolai, M., and Reis, C.P. (2014). Antimicrobial plant extracts encapsulated into polymeric beads for potential application on the skin. *Polymers.* 6: 479-490.

Ruengvisesh, S., Loquercio, A., Castell-Perez, E., and Taylor, T.M. (2015). Inhibition of bacterial pathogens in medium and on spinach leaf surfaces using plant-derived antimicrobials loaded in surfactant micelles. *J. Food Sci.* 80: 2522-2529.

Saini, S., Sillard, C., Belgacem, M.N., and Bras, J. (2016). Nisin anchored cellulose nanofibers for long term antimicrobial active food packaging. *RCS Adv*. 6: 12422.

Salvia-Trujillo, L., Rojas-Graü, A., Soliva-Fortuny, R., and Martín-Belloso, O. (2015). Physicochemical characterization and antimicrobial activity of foodgrade emulsions and nanoemulsions incorporating essential oils. *Food Hydrocolloids*. 43: 547-556.

Salmieri, S., Islam, F., Khan, R.A., Hossain, F.M., Ibrahim, H.M.M., Miao, C., Hama, W.Y., and Lacroix, M. (2014). Antimicrobial nanocomposite films made of poly(lactic acid)-cellulose nanocrystals (PLA-CNC) in food applications: part A - effect of nisin release on the inactivation of *Listeria monocytogenes* in ham. *Cellulose.* 21: 1837-1850.

Sant'Anna, V. Malheiros, P.S., and Brandelli, A. (2011). Liposome encapsulation protects bacteriocin-like substance P34 against inhibition by Maillard reaction products. *Food Res. Int.* 44: 326-330.

Sari, T.P., Mann, B., Kumar, R., Singh, R.R.B., Sharma, R., Bhardwaj, M., and Athira, S, (2015). Preparation and characterization of nanoemulsion encapsulating curcumin. *Food Hydrocolloids*. 43: 540-546.

Satishkumar, R., and Vertegel, A.A. (2011). Antibody-directed targeting of lysostaphin adsorbed onto polylactide nanoparticles increases its antimicrobial activity against *S. aureus* in vitro. *Nanotechnology*. 22: 505103.

Sava, G., Benetti, A., Ceschia, V., and Pacor, S. (1989). Lysozyme and cancer: role of exogenous lysozyme as anticancer agent (review). *Anticancer Res*. 9: 583-591.

Sharma, T.K., Sapra, M., Chopra, A., Sharma, R., Patil, S., Malik, R., Pathania, R., and Navani, N. (2012). Interaction of bacteriocin-capped silver nanoparticles with food pathogens and their antibacterial effect. *Int. J. Green Nanotechnol*. 4: 93-110.

Silva, I.M., Boelter, J.F., Silveira, N.P., and Brandelli, A. (2014). Phosphatidylcholine nanovesicles coated with chitosan or chondroitin sulfate as novel devices for bacteriocin delivery. *J. Nanopart. Res*. 16: 2479.

Sinha, M., Kayshik, S., Kaur, P., Sharma, S., and Singh, T.P. (2013). Antimicrobial lactoferrin peptides: the hidden players in the protective function of a multifunctional protein. *Int. J. Pept*. 2013: 1-12.

Taylor, T.M., Davidson, P.M., Bruce, B.D. and Weiss, J. (2005). Liposomal nanocapsules in food science and agriculture. *Crit. Rev. Food Sci. Nutr*. 45: 587-605.

Taylor, T.M., Gaysinsky, S., Davidson, P.M., Bruce, B.D., and Weiss, J. (2007). Characterization of antimicrobial-bearing liposomes by ζ-potential, vesicle size, and encapsulation efficiency. *Food Biophys.* 2: 1-9.

Taylor, T.M., Bruce, B.D., Weiss, J., and Davidson, P.M. (2008). *Listeria monocytogenes* and *Escherichia coli* O157:H7 inhibition *in vitro* by liposome-encapsulated nisin and ethylene diaminetetraacetic acid. *J. Food Safety*. 28: 183-197.

Teixeira, M.L., Santos, J., Silveira, N.P., and Brandelli, A. (2008). Phospholipid nanovesicles containing a bacteriocin-like substance for control of *Listeria monocytogenes*. *Innov. Food Sci. Emerg. Technol*. 9: 49-53.

Thirumurugan, A., Ramachandran, S., and Gowri, A.S. (2013). Combined effect of bacteriocin with gold nanoparticles against food spoiling bacteria - an approach for food packaging material preparation. *Int. Food Res. J*. 20: 1909-1912.

Tiwari, U. and Cummins, E. (2013). Factors influencing levels of phytochemicals in selected fruit and vegetables during pre- and post-harvest food processing operations. *Food Res. Int*. 50: 497-506.

Torres, N.I., Noll, K.S., Xu, S., Li, J., Huang, Q., Sink, P.J., Wachsman, M.B., and Chikindas, M.L. (2013). Safety, formulation, and *in vitro* antiviral activity of the antimicrobial peptide subtilosin against herpes simplex virus type 1. *Probiotics Antimicrob. Prot*. 5: 26-35.

Vaucher, R.A., Giongo, J.L., Bolzan, L.P., Corrêa, M.S., Fausto, V.P., Alves, C.F.S., Lopes, L.Q.S., Boligon, A.A., Athayde, M.L., Moreira, A.P., Brandelli, A. Raffin, R.P., and Santos, R.C.V. (2015) Antimicrobial activity of nanostructured Amazonian oils against *Paenibacillus* species and their toxicity on larvae and adult worker bees. *J. Asia-Pacific Enthomol*. 18: 205-210.

Vega-Lugo, A.C., and Lim, L.T. (2009). Controlled release of allyl isothiocyanate using soy protein and poly(lactic acid) electrospun fibers. *Food Res. Int*. 42, 933-940.

Were, L.M., Bruce, B.D., Davidson, P.M., and Weiss, J. (2004). Encapsulation of nisin and lysozyme in liposomes enhances efficacy against *Listeria monocytogenes*. *J. Food Protec*. 67: 922-927.

Wu, Q.X., Zhang, Q.L., Lin, D.Q., and Yao, S.J. (2013). Characterization of novel lactoferrin loaded capsules prepared with polyelectrolyte complexes. *Int. J. Pharm.* 455: 124-131.

Xu, W., Jin, W., Zhang, C., Li, Z., Lin, L., Huang, Q., Ye, S., and Li, B. (2014). Curcumin loaded and protective system based on complex of κ-carrageenan and lysozyme. *Food Res. Int*. 59: 61-66.

Zhang, Y., Niu, Y., Luo, Y., Ge, M., Yang, T., Yu, L.L., and Wang, Q. (2014). Fabrication,

characterization and antimicrobial activities of thymol-loaded zein nanoparticles stabilized by sodium caseinate-chitosan hydrochloride double layers. *Food Chem.* 142: 269-75.

Zohri, M., Alavidjeh, M.S., Haririan, I., Ardestani, M.S., Ebrahimi, S.E., Sani, H.T., and Sadjadi, S.K. (2010). A comparative study between the antibacterial effect of nisin and nisin-loaded chitosan/alginate nanoparticles on the growth of *Staphylococcus aureus* in raw and pasteurized milk samples. *Probiotics Antimicrob. Prot*. 2: 258-266.

Table 1. Characteristics of principal nanostructures available to encapsulate natural antimicrobials.

Nanostructure	**Characteristics**	**Advantages**
Nanoliposomes	Vesicles consisting of one or more phospholipidic bilayer, with an aqueous core	Produced using natural ingredients on an industrial scale and can encapsulate compounds with different solubilities
Polymeric nanoparticles	Depending upon the method of preparation, nanospheres or nanocapsules can be obtained in which compounds are either dissolved, entrapped, encapsulated or attached to the nanoparticle matrix	Relatively easy to prepare and can form complexes with polysaccharides, lipids or other biopolymers
Metallic nanoparticles	Composed of metals, such as copper, silver, zinc, palladium, or titanium, and can be used to produce nanostructures with varying size, shape, and porosity	Can be easily incorporated into numerous materials such as textiles and plastics
Nanofibers	Nanostructures of small diameter sizes and large surface areas with improvement of physical, chemical and biological properties	Produced from various organic and inorganic materials
Nanotubes	Nanotubes are of two types single walled	May be organic or inorganic in

	and double walled carbon tubes	composition

Table 2. Examples of nanostructured bacteriocins and antimicrobial proteins.

Antimicrobial	Nanostructure	Size (nm)	Zeta potential (mV)	Reference
Nisin	SLN	159 to 167	-28.3 to -29.2	Prombutara et al. 2012
Nisin	Liposome	122 to 310	-7.6 to -70.2	Taylor et al. 2007
Nisin	Liposome	148 to 190	-54.5 to -55.8	Malheiros et al. 2010
Pediocin	Liposome	190	-44.0	Mello et al. 2013
Peptide P34	Liposome	150	-27.4	Malheiros et al. 2011
Nisin	Chitosan/ carageenan nanocapsules	397 to 1106	ND	Chopra et al. 2014
Lactoferrin	Liposome	100 to 200	-14.6	Balcão et al. 2013
Lysozyme	Chitosan/ poly-γ-glutamic acid nanoparticles	244 to 609	-41.9 to -60.6	Liu et al. 2013

ND= not determined

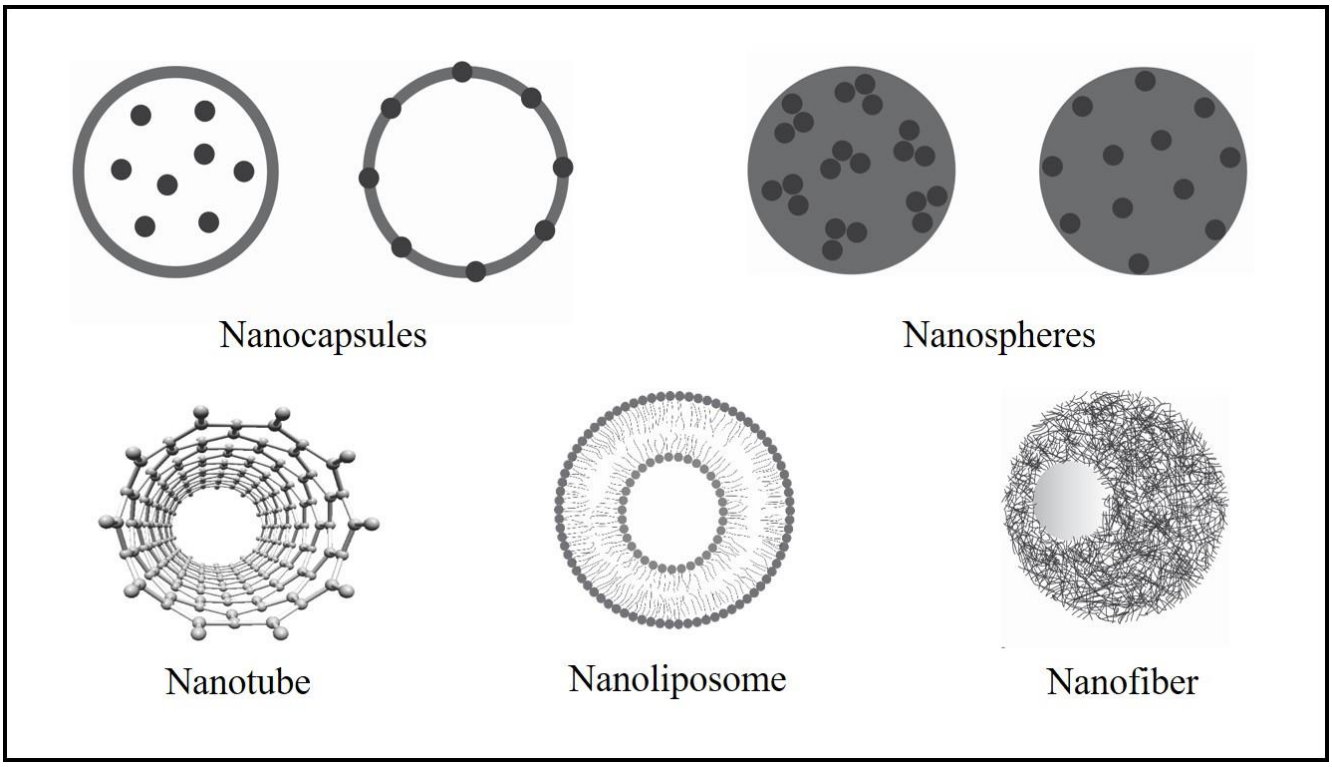

Fig. 1. Schematic representation of different types of nanostructures.

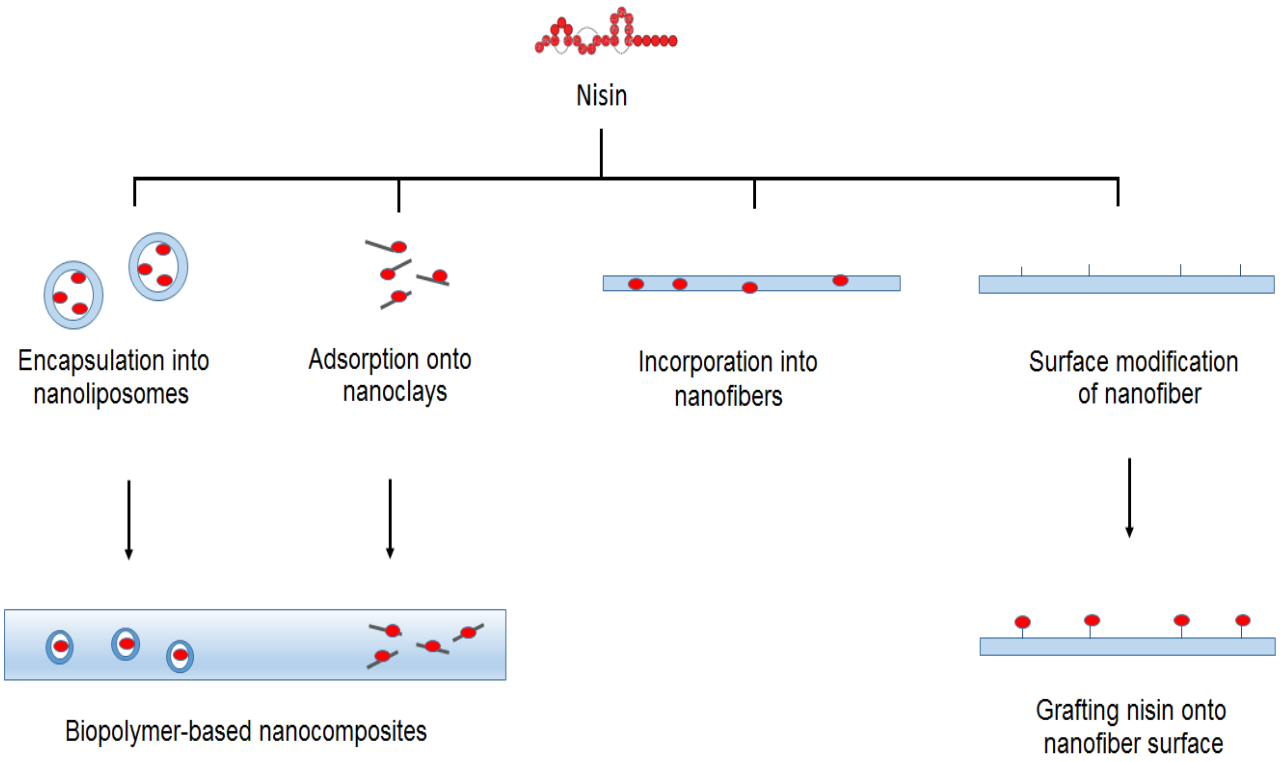

Fig. 2. Innovative strategies to delivery and controlled release of nisin incorporated into nanocomposites. Nisin can be encapsulated into liposomes or adsorbed to nanoclays and these nanostructrures can be incorporated into nanocomposite films intended for food packaging. Nisin can be also directly encapsulated into nanofibers or grafted on the surface of nanofibers that undergone a surface-modification process.

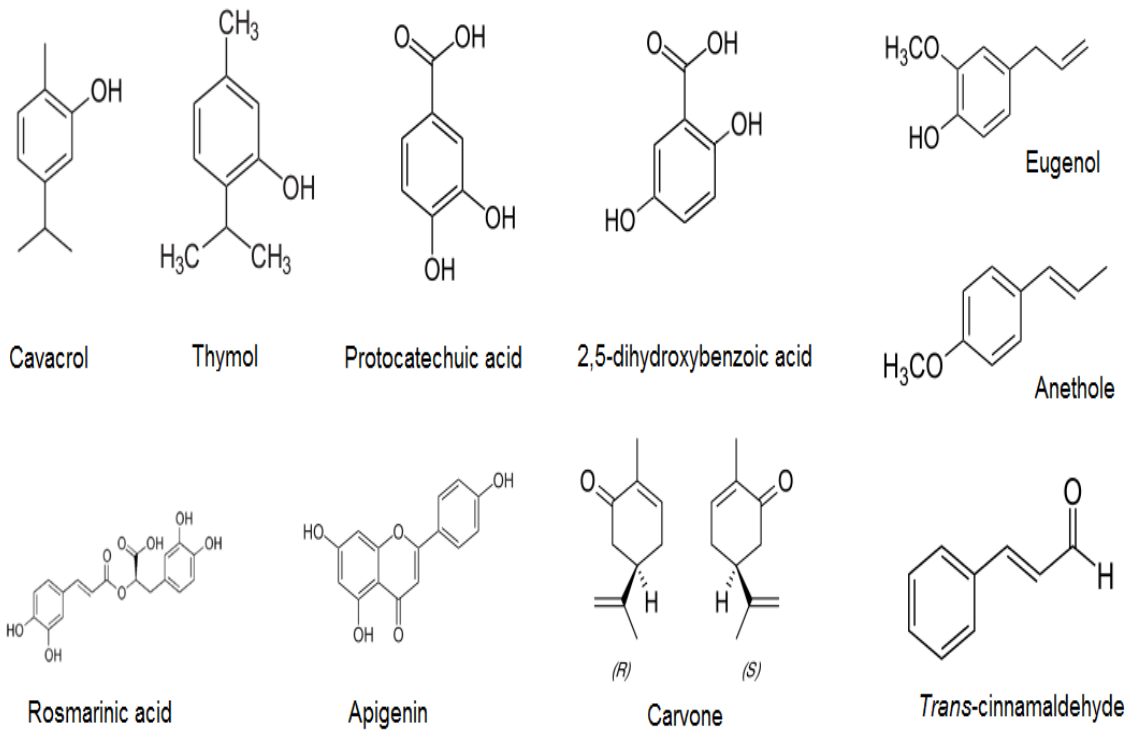

Fig. 3. Chemical structures of antimicrobial phytochemicals that have been encapsulated into nanostructures.

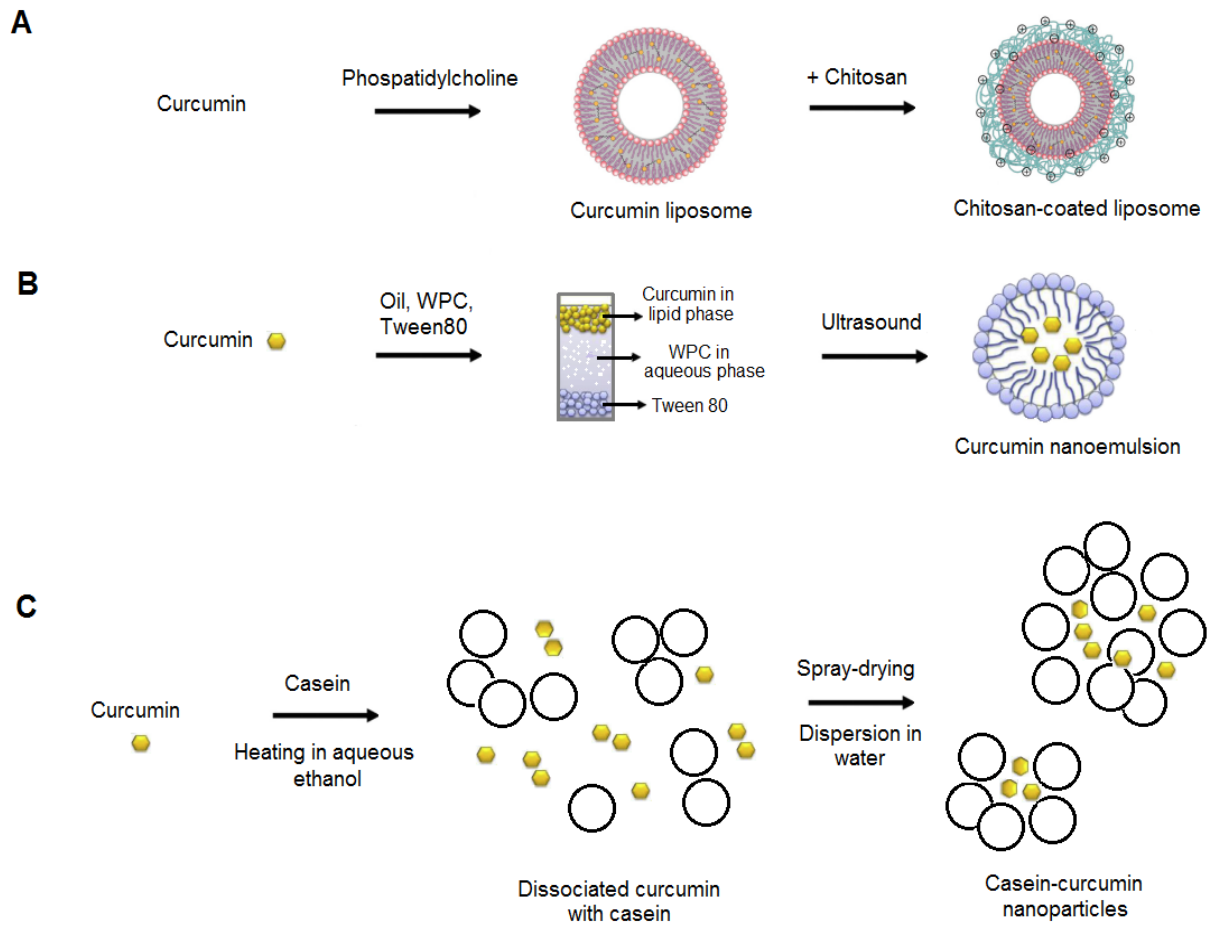

Fig. 4. Development of nanostructures for curcumin delivery. (A) Encapsulation of curcurmin into phosphatidylcholine nanoliposomes and chitosan-coated liposomes (adapted with permission from Liu et al. 2015). (B) Development of curcumin nanoemulsions with oil, whey protein concentrate and Tween 80 (adapted with permission from Sari et al. 2015). (C) Casein-curcumin nanoparticles developed from (adapted with permission from Pan et al. 2013).