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Nano and microencapsulation of bacteriocins for food applications: A review

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ABSTRACT

The increasing demand for prolonging food shelf life and inhibiting food spoilage and pathogenic microorganisms lead to development of novel biopreservatives and conservation methods. Antimicrobial peptides derived from microorganisms like bacteriocins are considered as a major alternative to the application of chemical preservatives in food products. Direct addition of purified antimicrobial peptides into food matrix results in reduced antibacterial activity due to the interaction of peptides with food components. Nano- or microencapsulation process can protect biopreservatives against many adverse conditions and controlled release of the agents efficiently prevent microbial contamination and food spoilage. This review focuses on the most frequently used chemical, physical and mechanical encapsulation methods for various antimicrobial bacteriocins including liposome entrapment, coacervation, emulsification, spray-drying, and vibrating technology. The most recent applications of nano- or microencapsulated antimicrobial bacteriocins with emphasis on nisin and pediocin in various food products like dairy, meat products and fruit juice have been discussed.

1. Introduction

Foods are known to be complex ecosystems with various microbial mixtures. Microorganisms are rarely evenly distributed in food matrices but tend to form microcolonies in solid foods or biofilms on food surfaces (Gálvez, Abriouel, Lopez, & Ben Omar, 2007). Biofilms can form on food surfaces as well as in processing areas by attachment and growth of foodborne pathogens on these surfaces, contributing to the main source of food contamination (Abebe, 2020). Biopreservation is defined as extending the shelf life of foods and improving their safety by using microorganisms and/or their metabolites (Singh, 2018) such as bacteriocins. The safety and microbial quality of food have been studied following the addition of bacteriocins mainly produced by Gram-positive bacteria. The benefits of using bacteriocins in foods as natural biopreservatives include (i) extending the shelf life of foods, (ii) providing additional protection in high temperature processes, (iii) reducing the use of chemical agents, (iv) limiting the transmission of foodborne pathogens through the food chain, and (v) preventing the loss of nutritional value (Bastos & Ceotto, 2011). However, further studies are needed to find promising solutions to reduce the high expense of

bacteriocin purification (Daba & Elkhateeb, 2020).

Application of antimicrobial peptides especially bacteriocins as safe food preservatives has gained great attraction in recent years due to their easy digestion by the human gastrointestinal tract (Anumudu, Hart, Miri, & Onyeaka, 2021; Silva, Silva, & Ribeiro, 2018). Bacteriocins, made up of 10–50 amino acid residues, have shown the ability to prevent color loss and lipid oxidation, to extend the shelf life of various food products along with providing food safety (León Madrazo & Segura Campos, 2020). Consumer demands for high quality and safe food products free from any chemical preservatives like benzoic acid, nitrites and sulfur dioxide can be provided by using antimicrobial peptides as natural food preservatives. The main advantages of using antimicrobial peptides are the preservation of foods without altering their quality as well as their harmlessness for human health (Wang, Zeng, Yang, & Qiao, 2016; Zhang, Luo, Sun, & Ma, 2021). Both Gram-positive and Gram-negative bacteria can produce bacteriocins that most of them are small cationic peptides having amphiphilic or hydrophobic properties and displaying antimicrobial activity against related or non-related bacteria species (Darbandi et al., 2022). A few approaches are used for the application of bacteriocins in foods as bio-preservatives including

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inoculation of food products with the bacteriocin-producer strain, addition of purified or semi-purified bacteriocins as food additives, and incorporation of a fermented product containing the bacteriocin-producing strain in other food products (Silva et al., 2018). The application of bacteriocins in food could be restricted mainly because of the narrow spectrum of the antibacterial activity of some bacteriocins, the loss of the antimicrobial activity due to detrimental effects of enzymes and pH of the food matrix, along with the resistance of *Listeria monocytogenes* and *Staphylococcus aureus* strains to some molecules like nisin and plantaricin (Liu, Nie, Liu, & Mehmood, 2022). In fact, different food-related parameters significantly affect the antibacterial activity of directly added bacteriocins such as interacting with fat or protein particles naturally present in foods, proteolytic degradation or oxidation leading to the biological activity destabilization, and heterogeneous distribution of biopreservatives inside the food matrix (Coma, 2008; da Silva Malheiros, Daroit, & Brandelli, 2010). Moreover, the emergence of bacteriocin resistant bacteria might be occurred by direct incorporation of nisin in food systems (Kaur, Singh, Malik, Bhardwaj, & De, 2014). Several reports have illustrated that higher concentrations of bacteriocin should be added to food for exhibiting required inhibitory activity compared to *in vitro* experiments (Bastos & Ceotto, 2011). Thus, encapsulation could be an alternative to minimize the drawbacks of direct addition of antimicrobial peptides.

Nano- or microencapsulation technology can contribute to various beneficial applications in the food industries. The process of nano- or microencapsulation technology is based on producing capsules containing active agents as core material which is packed within a solid or liquid immiscible compound named wall material (encapsulant) (Ghosh, Sarkar, Das, & Chakraborty, 2021). The method is named as microencapsulation when the size of capsules ranges from 3 to 800 μm while nanoencapsulation is referred to encapsulation process of active agents when the particle size ranges from 10 to 1000 nm (Kwak, Al Mijan, & Ganesan, 2014). The nano/microencapsulation can preserve different active compounds such as antimicrobial peptides from adverse environmental conditions and deliver them to the controlled target site (Reque & Brandelli, 2021).

The objective of this review is to describe various antimicrobial bacteriocins, their frequently used encapsulation methods, and the applications of encapsulated bacteriocins as natural biopreservatives in different food products. Discussing the incorporated nano- or microencapsulated bacteriocins in food will allow us to achieve conclusions about the most recent research approaches and help the improvement of new strategies for further food preservation.

2. Antimicrobial bacteriocins

Bacteriocins are ribosomally biosynthesized peptides that only few of them have been used in the food industry probably because of their high salt sensitivity and susceptibility to proteolysis (Etchegaray & Machini, 2013). Lactic acid bacteria (LAB) such as *Lactococcus lactis*, *Pediococcus acidilactici*, *Lactobacillus helveticus*, *Lactobacillus plantarum*, and *Lactobacillus bulgaricus* have been extensively studied for their use in fermented products (converting lactose to lactic acid) and production of antimicrobial molecules such as bacteriocins that are highly effective in inhibiting food spoilage and contamination with foodborne pathogens (Cleveland, Montville, Nes, & Chikindas, 2001; Egan et al., 2016; Trejo-González, Gutiérrez-Carrillo, Rodríguez-Hernández, Del Rocío López-Cuellar, & Chavarría-Hernández, 2021). The LAB and their metabolites have been given Generally Recognized as Safe (GRAS) status by the American Food and Drug Administration (FDA) that enables direct usage of LAB as starters in the food products with the purpose of natural bacteriocin production (Punia Bangar, Chaudhary, Singh, & Özogul, 2022). Bacteriocins produced by LAB are resistant to high temperatures and having antimicrobial activity against foodborne pathogens (Ahmad et al., 2017) and do not have any negative effect on the gut microbiota because of being sensitive to digestive proteases like trypsin and

chymotrypsin (Egan et al., 2016). Branched or cyclic structure of non-ribosomal peptides can consist of non-proteinogenic amino acids like D-amino acids, and can be acylated, halogenated, glycosylated, or hydroxylated. The cationic peptide polymyxin B (produced by *Bacillus polymyxa*) and the noncationic glycopeptide vancomycin (produced by *Amycolatopsis orientalis*) are both FDA-approved antibiotics (Zhang & Gallo, 2016). Fewer investigated bacteriocins are those obtained from Gram-negative bacteria such as microcins that are produced by *E. coli* for two main reasons: i) showing antibacterial activity against a narrow spectrum of microorganisms, and ii) the source bacteria is considered as a serious threat for food safety (Liu et al., 2022).

Molecular mass, mode of action, antimicrobial activity, chemical structure, thermal and enzymatic stability, and the presence of post-translationally modified amino acid residues are among the major parameters for classifying bacteriocins (Gharsallaoui, Oulahal, Joly, & Degraeve, 2016). Various classification schemes have been proposed for bacteriocins according to their biosynthesis mechanism, structure as well as their biological activity (Soltani et al., 2021). Three main classes of bacteriocins include Class I (small post-translationally modified peptides, lantibiotics), Class II (unmodified peptides), and Class III (large peptides, > 10 kDa, heat labile). The fourth class of bacteriocins has been eliminated from classification and named as bacteriolysins (leuconocin S and lactocin 27) which are large proteins and their activity depends on complexation with essential lipid or carbohydrates (Kumariya et al., 2019).

Class I peptides is subdivided into two groups: Class Ia (nisin and subtilin) and Class Ib (lactacin) are post-translationally modified bacteriocins or lantibiotics with 19–37 amino acids (Yang, Lin, Sung, & Fang, 2014) which are recognized by the presence of the unusual amino acids lanthionine and methyllanthionine, dehydrated amino acids, head-to-tail cyclized peptides like enterocin AS-48, glycosylation and could also be classified based on the modifying enzymes suggested by Montalbán-López et al. (2021). Class II bacteriocins are small, heat stable and non-modified peptides subdivided into four groups of class IIa containing 36–58 amino acid residues (pediocin PA-1, bifidocin B, and acidocin A), class IIb made up of two various peptides containing 25–62 amino acids (lactococin G and plantaricins), class IIc with 58–70 amino acids (amylocyclicin, lactocyclin Q, and acidocin B) (Yi et al., 2022) and class IId lactococin A (LcnA) as well as lactococin B (LcnB) (Tan, Moore, & Nodwell, 2019). Plantaricin LPL-1 is a novel class IIa bacteriocin produced by *Lactobacillus plantarum* LPL-1 that showed high bactericidal activity against *L. monocytogenes*. It can be used as natural food preservative for its pH, high thermal, and surfactant stability as well as its easy degradation by proteases (Wang, Qin, Zhang, Wu, & Li, 2019). Class IIa bacteriocins does not show antibacterial activity against *Lactococcus* spp but are active against *Clostridium difficile* (Zimina et al., 2020). Scanty information is available regarding the large and heat sensitive class III bacteriocins. Examples of this group are including helveticin J, helveticin M, colicin, and enterolysin A produced by *Lactobacillus helveticus*, *Lactobacillus crispatus*, *E. coli*, and *E. faecalis*, respectively (Kumariya et al., 2019).

Currently, the commercialized bacteriocins in the USA and Canada include nisin (produced by *L. lactis*), pediocin PA1 (produced by *P. acidilactici*), and Micocin® (produced by *Carnobacterium maltaromaticum* UAL307). Micocin® is a mixture of three bacteriocins (carnocyclin A, carnobacteriocin BMI, and piscicolin 126). FDA approved nisin has also been commercialized in more than 48 countries (Zou, Jiang, Cheng, Fang, & Huang, 2018).

2.1. Nisin

Nisin is a class Ia bacteriocin produced by a certain strain of Gram-positive bacteria *Lactococcus lactis* (Małaczewska & Kaczoruk-Lukowska, 2021). Nisin, approved as a food preservative (E234) is recognized as safe by the Joint Food and Agriculture Organization/World Health Organization (Favaro, Barretto Penna, & Todorov, 2015).

It was granted a generally recognized as safe designation in 1988 by the FDA in the United States (US) for use in processed cheese (Eghbal, Chihib, & Gharsallaoui, 2020). Nisin A is the originally defined variant of nisin which is produced by *Lactococcus lactis* and contains 34 amino acids. Nisin and other lantibiotics have an antimicrobial activity against a wide spectrum of the mostly Gram-positive bacteria with low cellular cytotoxicity at the usual antimicrobial concentrations (Cotter, Ross, & Hill, 2013; Shin et al., 2015, 2016). Nisin Z is the closest variant of nisin A, differing by a single amino acid residue at position 27, asparagine instead of histidine (Mulders, Boerrigter, Rollema, Siezen, & de VOS, 1991). Other natural nisin variants have been isolated from various bacterium strains such as nisin F and Q (from *Lactococcus lactis*), nisin U, U2 and H (from some Streptococci), nisin J (from *Staphylococcus capitis*), and nisin O (from *Blautia obeum*) (O'Sullivan et al., 2020; Shin et al., 2016). Nisin can disrupt the transglycosylation process by targeting the lipid II of vegetative forms of Gram-positive bacteria, thus creating pores in the cell membrane and preventing biosynthesis of the cell wall. The growth of germinated bacterial spores can be inhibited by nisin through impeding the initiation of a membrane potential and oxidative metabolism (Modugno et al., 2018). The outer membrane of Gram-negative bacteria prevents the accessibility of nisin to the cytoplasmic membrane consequently making them resistant to this bacteriocin (Khan, Vu, Riedl, & Lacroix, 2015). Applying high-pressure treatment (HP), as a non-thermal process in combination with nisin can inactivate nisin-resistant Gram-negative bacteria and spores due to imposing sub-lethal harms to the cell membrane (Aouadhi, Méjri, & Maaroufi, 2015; Black, Kelly, & Fitzgerald, 2005; Modugno et al., 2018). Also, the addition of chelators like ethylene diaminetetraacetic acid (EDTA) can enhance nisin resistant bacteria inactivation as a result of partial disruption of the lipopolysaccharide layer which permits nisin to reach the cytoplasmic membrane (Prudêncio, dos Santos, & Vanetti, 2015). In this relation, it was found that nisin could significantly decrease the growth of Gram-negative bacteria such as *Vibrio parahaemolyticus*, *Pseudomonas fluorescens*, and *Salmonella enterica* in broth when combined with EDTA (Liang, Hsiao, & Jhang, 2020).

2.2. Pediocin

Pediocin PA-1, a class IIa bacteriocin exhibiting antibacterial activity against the pathogen *Listeria monocytogenes*, is produced by *Pediococcus* spp. and *Lactobacillus plantarum* (Kuniyoshi et al., 2022) and is commercially available as Alta 2341TM or MicrogardTM (Darbandi et al., 2022). The two main species of *Pediococcus pentosaceus* and *Pediococcus acidilactici* are used for the production of pediocin and as a starter in fermentation processes for inhibition of contamination (Porto, Kuniyoshi, Azevedo, Vitolo, & Oliveira, 2017). The structure of this small and cationic polypeptide contains a variable hydrophobic/amphiphilic C-terminal region capable of cell recognition and a conserved hydrophilic and charged N-terminal part (Papagianni & Anastasiadou, 2009). Inhibitory activity of class IIa bacteriocins is due to their interaction with components of the mannose phosphatransferase system embedded in bacterial membrane leading to disruption of the membrane (Bédard et al., 2018). Pediocin has higher antimicrobial activity against some Gram-positive foodborne pathogens like *L. monocytogenes* (Liu, Liu, & Zhang, 2019) and Gram-negative bacteria like *Pseudomonas* and *Escherichia coli* in comparison to nisin (Jamuna & Jeevaratnam, 2004). Studies on the biosynthesis and antibacterial activity of pediocin are increasing for controlling the food-born pathogen *L. monocytogenes* which lead to listeriosis regarded as a serious and mortal illness (Khorshidian, Khanniri, Mohammadi, Mortazavian, & Yousefi, 2021). The ability of the pathogen to grow at low temperature can contribute to contamination majorly by ready-to-eat foods because of the possibility of containing high pathogen counts (Wilking et al., 2021). Kumar, Kaur, Shahi, Kairam, & Tyagi. (2017) have reported that the count of *Listeria innocua* reduced significantly in minimally processed raw goat meat emulsion treated with pediocin and *Murraya*

koenigii berries during storage at 4 °C for 9 days indicating comparable antilisterial activity of pediocin to nitrite.

3. Encapsulation techniques used for antimicrobial bacteriocins

The properties of capsules containing active agents like solubility, particle size, size distribution, shape, and encapsulation efficiency can be modified according to the encapsulation technique and the used wall materials. Thus, the appropriate technique should be applied by taking into account both nano- and microencapsulation requirements. It is noteworthy that methods used for nanoencapsulation are more complicated in comparison to microencapsulation methods (Kwak, 2014). The capsule size plays an important role in the release properties and the delivery of active substances to the target site. The microcapsules formed by suitable wall material have good delivery properties (Hosseini & Jafari, 2020) but decreasing the particle size to the nanosize provides more surface area per unit volume leading to production of improved delivery systems (Reza Mozafari, Johnson, Hatziantoniou, & Demetzos, 2008; Shegokar & Müller, 2010). Solubility, bioavailability, and specific targeting of the encapsulated active agents can be significantly enhanced by applying nanoencapsulation which could also result in using reduced amounts of active compounds compared to microencapsulation (Boelter & Brandelli, 2016). Recently, Brum, dos Santos, Zimnoch Santos, & Brandelli. (2022) used structured silica-based materials for the encapsulation of nisin by applying sol-gel method through acid-catalyzed route for the first time. Encapsulated nisin within nano silica xerogels exhibited antimicrobial activity against both Gram-positive and Gram-negative bacteria. Here, inhibitory activity against Gram-negative bacteria like *E. coli* can be attributed to the formation of hydrogen bonds between hydroxyl groups of silica nanoparticles and the saccharides of bacteria's outer membrane.

Irreversible aggregations and leakage of active agents are two main drawbacks of the formed micro- or nanocapsules. Therefore, drying techniques like freeze-drying and spray-drying are mostly used for the development of physical and chemical properties of nanocapsules, sustainable release of the agents from the capsules and preservation of their activity under long-term storage conditions (Degobert & Aydin, 2021; Guterres, Beck, & Pohlmann, 2009). The most frequent techniques used for the encapsulation of antimicrobial bacteriocins are presented in Table 1 and the most used ones are summarized in the following sections.

3.1. Chemical encapsulation processes

3.1.1. Liposome entrapment

Liposomes, as micro- and nanocarrier systems are mostly used by food, pharmaceutical, and cosmetic industries for the protection of chemically labile substances from undesirable interactions with outside conditions before release and the efficient delivery of bioactive substances (Mohammadi, Jafari, Sadeghi Mahoonak, & Ghorbani, 2021; Pinilla & Brandelli, 2016). Several reasons make liposomes suitable for use in the food industry including being well characterized, simple to produce, adjustable carrier properties, biocompatible, and granted GRAS status (Imran et al., 2015; Xia & Xu, 2005). Liposomes, self-assembled vesicular structures are composed of one or several bilayer membranes of phospholipids that can encapsulate a volume of aqueous media as a core material (Reza Mozafari et al., 2008). Encapsulation of bacteriocins into liposomes is mainly reported to be achieved by the thin-film hydration method (Fig. 1). Phospholipids are the major components of liposomes which are amphiphilic substances with hydrophilic head section (water soluble) and hydrophobic tail section (lipid soluble). Various types of active compounds (water soluble or lipid soluble) can be encapsulated within liposomes consisting of both aqueous and lipid phases in their structure (Aguilar-Pérez, Avilés-Castriello, Medina, Parra-Saldivar, & Iqbal, 2020). Amphiphilic compounds such as nisin can be entrapped in both the core and the bilayers of

Table 1
Most applied encapsulation methods for antimicrobial bacteriocins.

Encapsulation method	Encapsulant material	Antimicrobial agent	References
Liposome entrapment	Pro-lipo®H, S, C, DUO	Nisin Z	Laridi et al. (2003)
Liposome entrapment	PC, PC-chol, PC/PG/ chol	Nisin and lysozyme	Were et al. (2004)
Liposome entrapment	PC, PC/PG	Nisin	Taylor et al. (2007)
Liposome entrapment	PC, lecithin. Hybrid	Pediocin	Narsaiah et al. (2013)
Spray-drying	Alg-nanoliposomes MD/porous starch	lipopeptide from <i>Bacillus amyloliquefaciens</i>	Wang et al. (2014)
Spray-drying	zein	nisin	Xiao et al. (2014)
Single emulsion diffusion	PLA	nisin	Ji et al. (2014)
Emulsification	Alg and Alg/starch	nisin	Hosseini et al. (2014)
Liposome entrapment	Soy lecithin and marine lecithin	nisin	Imran et al. (2015)
Vibrating technology	alginate	nisin	Maresca et al. (2016)
Complex coacervation	Collagen/gelatin and collagen/pectin	Nisin and avocado extract peel	Calderón-Oliver et al. (2017)
Spray drying	Pectin or alginate	nisin	Ben Amara et al. (2017)
Spray drying	exopolysaccharides	nisin	Abid et al. (2019)
Liposome	Phosphatidylcholine	pediocin	Suganthi et al. (2021)

PC: phosphatidyl choline; PG: phosphatidylglycerol; chol: cholesterol; EDTA: ethylene diaminetetraacetic acid; MD: maltodextrin; PLA: poly lactic acid; Alg: alginate; CNC: cellulose nanocrystal.

liposome (Imran et al., 2015). Liposome encapsulation has the great advantage of keeping water soluble compounds stable in food products having high water activity (da Silva Malheiros et al., 2010). Uncontrolled polydispersity in size, lamellarity and low encapsulation efficiency (EE) can be obtained by using traditional liposome formation encompassing organic solvents. Therefore, homogeneous liposomes can be produced through the addition of some post-processing steps like solvent elimination and membrane extrusion (Jahn, Vreeland, DeVoe, Locascio, & Gaitan, 2007). Various physical methods such as membrane emulsification, sonication, microfluidisation, high-pressure homogenization, and colloid mill as well as chemical techniques like reversed phase evaporation and depletion of mixed detergent-lipid micelles have been applied for the production of nanoliposomes (Tontul, Eroglu, & Topuz, 2017). The size of nanoliposomes has shown distinctive effect on the core agent retention during encapsulation process (Narsaiah et al., 2013). Bioactive compounds encapsulated in liposomes were protected against enzyme degradation, reactive chemical substances, changes of pH, temperature, ion concentration, and therapeutic effectiveness can be enhanced (Reza Mozafari et al., 2008; Taylor, Gaysinsky, Davidson, Bruce, & Weiss, 2007). Increased stability of the antimicrobial activity of nisin entrapped in liposome could occur as a result of interactions between nisin and phospholipid membranes contributing to the structural changes in nisin through β -turns formation (Were, Bruce, Davidson, & Weiss, 2004). In this relation, higher amount of nisin encapsulated in phosphatidyl choline (PC) and PC-cholesterol (chol) liposomes reduced the growth of *L. monocytogenes* more than 2 log CFU (colony-forming unit)/mL compared to nisin entrapped in PC/phosphatidylglycerol (PG)/chol liposomes and free nisin (Were et al., 2004). According to Laridi et al. (2003), the encapsulation capacity of liposomes depends on lipid composition and net charge which also determines nisin affinity for liposome membrane. Increasing negatively charged lipids and unsaturated phospholipids enhance the insertion of nisin in lipid membrane

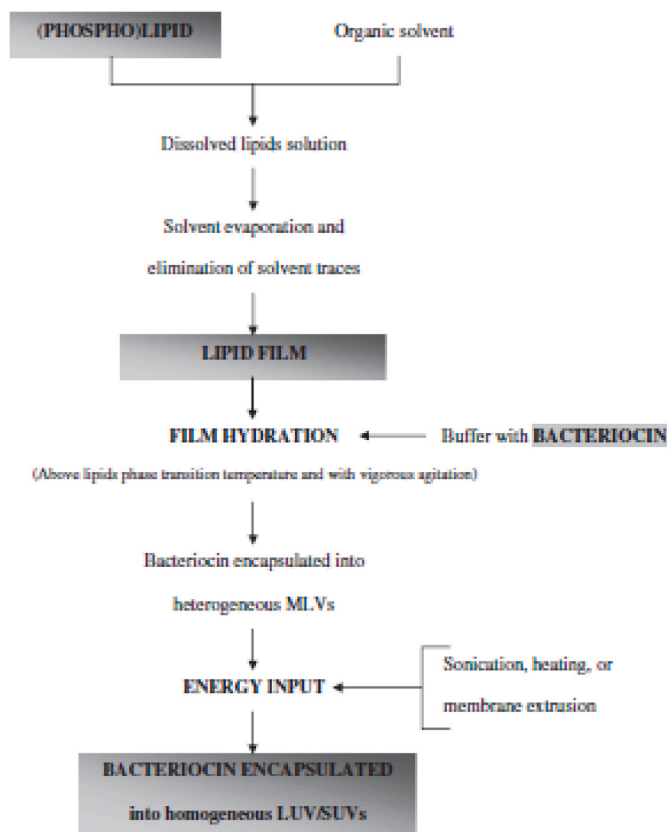


Fig. 1. Process of liposomal encapsulation of bacteriocins (Malheiros et al., 2010).

causing pore formation and leakage (El-Jastimi & Lafleur, 1997).

Efficient preservation of the encapsulated nisin in liposomes can be achieved by using phospholipids in liposome formulation which maintain their charge at different pH values. Entrapped nisin (5 μ g/mL) in PC/PG (8:2 and 6:4) liposomes produced more packed and smaller vesicles (184 \pm 13.3, and 122.7 \pm 6.2 nm) in pH range from 5.5 to 11.5 at 25 $^{\circ}$ C compared to PC liposomes (310.1 \pm 104.9 nm) mainly because of the electrostatic interactions between oppositely charged nisin and lipids (Taylor et al., 2007). Selecting the lipid composition of liposomes and optimizing the encapsulation procedure could limit the interactions between nisin and phospholipid membranes. Nisin was encapsulated in nanoliposomes produced from soy lecithin and marine lecithin (151 \pm 4 to 181 \pm 5 nm) and the highest encapsulation efficiency of 47% was obtained for soy lecithin liposomes containing higher omega-6 and polar lipids than pro-liposomes. Mixture of free and encapsulated nisin (1:1) showed stronger antimicrobial activity against *L. monocytogenes* in comparison with free or 100% encapsulated nisin alone (Imran et al., 2015). The stability as well as sustained release of extracted pediocin from *Pediococcus pentosaceus* KC692718 was shown to be enhanced by nano-liposome encapsulation that could decrease 2 log of *L. monocytogenes* in TSB (tryptic soy broth) plus YE (yeast extract) broth during 48 h of incubation (Suganthi, Ethiraj, Anbalagan, Siddique, & Vaithilingam, 2021). It is noteworthy that using polysaccharides like alginate and chitosan as wall materials in the encapsulation of bacteriocins into nano-liposomes can produce efficient delivery systems (Niaz et al., 2018). Incorporation of pediocin loaded PC nanoliposomes within alginate/guar gum hybrid capsules was shown to be an excellent delivery system for controlled release of pediocin by prolonging growth inhibition of *L. innocua* due to prevention of pediocin leakage from alginate/guar gum matrix (Narsaiah et al., 2013).

3.1.2. Coacervation

Coacervation refers to the separation of colloidal systems into two liquid phases and the component concentrated phase is coacervate formed from oppositely charged biopolymers (Eghbal & Choudhary, 2018). Entrapment of active agent (core) within wall materials can occur through the addition of electrolytes, non-solvent substances, or by changing the processing factors such as temperature and pH (Mehta et al., 2022). There are two types of coacervation based on their formation process including simple coacervation and complex coacervation. Coacervation phase separation can be obtained for a macromolecular solute through mixing water with different alcohols to various mixing ratios. Water soluble macromolecules solution is separated into two phases at a specific range of water/alcohol ratios or other added dehydrating components like salts. One phase is containing high concentration of macromolecules in equilibrium with a second dilute phase of macromolecule having high water concentration. This type of coacervation is defined as simple coacervation (Veis, 2011; Zhou, Shi, Li, & He, 2020). Complex coacervation refers to associative phase separation of oppositely charged biopolymers into a dense phase known as coacervates and a dilute phase known as supernatant as a result of established electrostatic interactions (Sing & Perry, 2020). Material properties such as total biopolymer concentration, biopolymer mixing weight ratio, biopolymer molecular weight, biopolymers charge density and process parameters such as pH, pressure/stirring, and temperature greatly influence the process of complex coacervation (Hashemi Gahruie et al., 2022; Naderi, Keramat, Nasirpour, & Aminifar, 2020). Coacervates can be used as encapsulants, emulsifiers, additives, and viscosity modifiers in food industry (Sing and Perry, 2020). Emulsification, coacervation, gelation, and hardening are four main steps of encapsulation via complex coacervation. The encapsulation efficiency of bioactive agents is directly affected by the combination of wall materials and drying processes like lyophilization and atomization used for obtaining microcapsules for complex coacervation method. Atomization is known as low-cost and well adapted industrial drying method but degradation of active agents during encapsulation process can be occurred due to the applied high temperature ranges 70–150 °C. Lyophilization as a low temperature method can stabilize the heat-labile active compounds during the encapsulation process (Rutz et al., 2017). The efficiency of complex coacervation in encapsulation of sensitive agents can be attributed to the use of mild temperatures during the process and lack of organic solvents, also the produced active capsules have good controlled release properties (Yan & Zhang, 2014). The formation of nano- or microparticles is strongly affected by the intensity of electrostatic interactions. Highly negatively charged polysaccharides can not produce nanoparticles due to the formation of insoluble complexes or precipitated coacervates. Moderately charged protein/polysaccharide mixtures can produce functional nanoparticles (Eghbal & Choudhary, 2018; Lv, Yang, Zhang, & Abbas, 2014). The functional properties of nano-coacervates are contingent on the chemical and surface characteristics of the biopolymers, agitation rate, rate of dropwise polymer solution addition, and solubilities of bioactive and biopolymers in solution (Fathi, Martín, & McClements, 2014).

A mixture of an avocado peel extract as antioxidant agent and nisin as antimicrobial peptide were co-encapsulated in collagen/gelatin and collagen/pectin microcapsules obtained by complex coacervation. The final characteristics of the microcapsules including encapsulation efficiency, encapsulation loading, water activity, and moisture were shown to depend on various factors like the core dispersion method (emulsified or not), drying method (freeze- or spray-drying), and their interactions (Calderón-Oliver, Pedroza-Islas, Escalona-Buendía, Pedraza-Chaverri, & Ponce-Alquicira, 2017).

3.2. Mechanical or physical encapsulation processes

3.2.1. Emulsification

Emulsification is defined as the equilibrium of at least two

incompatible liquids like water and oil by applying an emulsifier (Comunian, Silva, & Souza, 2021). Kinetically stable emulsions are thermodynamically unstable systems that are ultimately breaking into separate phases with time (Rao & McClements, 2012). The microemulsions are thermodynamically stable isotropic liquids prepared by mixing water (W), oil (O), and surfactants (S) together. One, two, three, or more separate phases in equilibrium with each other can be produced through mixing W, O, and S forming water-continuous, oil-continuous, or bi-continuous phases based on nature, arrangement, and concentrations of substances (McClements, 2012). The same components used for the formation of microemulsions can be applied for the preparation of nanoemulsions containing very small particles (radius <100 nm). Thermodynamic stability is regarded as the main difference between nano- or microemulsions: nanoemulsions are thermodynamically unstable requiring addition of surfactants for their droplet formation while microemulsions are thermodynamically stable. Reduction in the size of particles increases their long-term stability, optical clarity and bioavailability (McClements, 2012; Seng & Loong, 2019). Double emulsions such as W₁/O/W₂ or water-in-oil (W/O) emulsions are applied to the encapsulation of sensitive bioactive compounds like vitamins and enzymes (Leister & Karbstein, 2020). However, contrary to common coacervation methods, hydrophilic/aqueous cores can be encapsulated directly in the aqueous suspension by emulsification method (Trojer, Nordstierna, Nordin, Nydén, & Holmberg, 2013). Charged biopolymers used for the layering of dispersed phase droplets provide the surface of droplets with electrical charge permitting them to accept another layer of polyelectrolytes in further steps. Deposition of layers can be occurred by the electrostatic interactions between oppositely charged biopolymers (Anandharamakrishnan & Ishwarya, 2015). Encapsulation of bioactive compounds by emulsification is carried out through various techniques like, microfluidization, ultrasonication, high-pressure homogenization, colloid mill, phase inversion, and spontaneous emulsification methods (Anandharamakrishnan, 2014).

Various operational conditions of single emulsion method like emulsification steps and temperature have important effects on formed particles morphology. Manipulating temperature of the final emulsification steps (W/O/W) formed hollow structured nisin/poly lactic acid (PLA) microspheres (1–5 µm) at high temperature (~60 °C), and solid nisin/PLA nanospheres (50–200 nm) at room temperature (Ji et al., 2014). According to the authors, nisin encapsulation was enhanced by applying more emulsification steps as a result of increased electrostatic interactions between nisin and PLA at the interface. Particles of alginate, the most studied wall material for the production of nano- or microcapsules containing nisin (Hosseini et al., 2014; Maresca et al., 2016) has porous structure and poor physical properties leading to rapid diffusion of water and other fluids in and out of the capsules (Chan et al., 2011; Gouin, 2004; Martín, Lara-Villoslada, Ruiz, & Morales, 2013). To overcome the drawbacks of alginate used as a wall material, alginate-resistant starch and alginate microparticles prepared by W/O emulsion external crosslinking procedure was used for the encapsulation of nisin (Hosseini et al., 2014). Homogeneous distribution of starch in alginate matrix contributed to the formation of more packed and spherical microparticles without having obvious crack on their surfaces and increased EE due to the stabilizing effect of starch on the alginate matrix. Release rate of nisin which is controlled by diffusional behavior, showed significant reduction in microparticles containing starch.

3.2.2. Spray-drying

Applying spray-drying as an encapsulation technique has numerous advantages such as its flexible nature with readily available equipment, being appropriate for heat-sensitive compounds, producing dried and stable capsules in a single-step continuous process, and being cost-effective (Arpagaus, Collenberg, Rütli, Assadpour, & Jafari, 2018; Reis, Ambrosi, & Luccio, 2022). Basically spray-drying involves four process steps including heating of the drying gas, formation of droplets, drying of the droplets, and collection of the formed particles.

Atomization of the liquid feed in a nozzle generates droplets in the drying chamber, continuous flow of a hot drying gas removes the solvent from the sprayed droplets. Formed dried particles are collected in a collection vessel after being separated from the gas stream (Arpagaus et al., 2018). Morphology and size of particles obtained by spray-drying can be controlled through optimizing the process parameters like inlet as well as outlet temperature, feed rate and feed formulation (Wolska, 2021). Nanoscale particles can be generated using spray-drying method by modifying the experimental setup of conventional spray-dryers (Arpagaus, John, Collenberg, & Rütli, 2017). Choosing proper wall material for spray-drying encapsulation process has an essential effect on achieving high encapsulation efficiency and stable microcapsules. The most important property of the selected wall material is its solubility in water, as spray-drying process is mostly carried out in aqueous feed preparations and directly affects the release mechanism of the core material (Gouin, 2004). Thanks to their good emulsifying ability, proteins are used as wall material for the encapsulation of agents through spray-drying technique. Another major property of a wall material is its film-forming ability for hardening around the core material to create oxygen barrier and keeping temperature of the core below 100 °C despite the application of high temperature during spray-drying (Anandharamakrishnan & Ishwarya, 2015). Wall material suspension should have low viscosity at high solid concentrations for avoiding formation of larger microcapsules with lower specific surface area along with accelerating atomization process. Maltodextrins (MD) are used as a wall material for the encapsulation of hydrophilic active agents for producing low viscosity solutions at high solid amounts. Also, in combination with other polymers, MD can decrease the viscosity of all the material suspension (Prata, Garcia, Tonon, & Hubinger, 2013). Common shell materials for microencapsulation of food active agents are polysaccharides such as gum acacia, maltodextrins with different dextrose equivalence, hydrolyzed starch, and anhydride modified starch (Ramakrishnan, Adzahan, Yusof, & Muhammad, 2018), or proteins like gelatin, milk protein, and soy protein or the combination of polysaccharides with proteins (Timilsena, Haque, & Adhikari, 2020).

Spray-drying has been used to improve delivery systems of bacteriocins like nisin (Abid et al., 2019; Ben Amara, Kim, Oulahal, Degraeve, & Gharsallaoui, 2017; Xiao, Liu, Zhu, Zhou, & Niu, 2014). The exopolysaccharides (EPS) produced by *Bacillus tequilensis*-GM and *Leuconostoc citreum*-BMS have been used for the encapsulation of nisin through electrostatic complexation by applying spray-drying method. During spray-drying, nisin structure did not undergo any significant alterations exhibited by UV spectra that can be interpreted by the high protective effect of the used EPS (Abid et al., 2019). In another study, the effect of the type of used polysaccharides including pectin or alginate as encapsulants (wall materials) (2 g/L concentration) was investigated on retaining structure and antimicrobial activity of nisin (5 g/L concentration). Data achieved from UV-Vis absorption and fluorescence spectroscopy were used for illustrating the excellent efficacy of the polysaccharides in maintaining physical and microbial properties of the spray-dried peptide (Ben Amara et al., 2017). The release kinetics of encapsulated agents depends on their interactions with carrier polymers and structure of the formed capsules. Nisin was encapsulated in zein microcapsules by applied spray-drying method at inlet temperatures ranging from 75 to 120 °C (Xiao et al., 2014). Almost whole specific activity of nisin was retained at an inlet temperature of 95 °C and above due to the rapid drying of particles resulting in a very low effect on its stability.

3.2.3. Vibrating technology

The theory of laminar jet break-up through the application of a vibrational frequency with specific amplitude to the extruded jet is the base of the vibrating nozzle technique (Whelehan & Marison, 2011). Polymer beads with controlled size can be produced by using special nozzles through which the feed is pumped in the form of laminar jet (Menin et al., 2018). High production rate of uniform sized beads with

an average diameter <300 µm is the major reason for the production of alginate beads by vibrating nozzle technology (Homar, Šuligoj, & Gašperlin, 2007). Nozzle (jet) diameter, surface tension, viscosity of fluid, jet velocity, and frequency of disturbance have distinctive effects on the size of droplet (De Prisco, Maresca, Ongeng, & Mauriello, 2015). Labile bioactive agents can be highly preserved by using this method because of its mild and nontoxic conditions (Homar et al., 2007) but the formation of capsules from highly viscous polymers is limited (Whelehan & Marison, 2011). Nisin has been encapsulated in alginate microbeads by vibrating technology for the first time by Maresca et al. (2016). Homogeneous microcapsules in shape with an average diameter of 118 ± 3 µm showed high nisin encapsulation efficiency (EE) (75%) which was attributed to the electrostatic interactions between opposite charges of nisin and alginate. The residual activity of encapsulated nisin against the growth of *Brochothrix thermosphacta* 7R1 was highest for the microcapsules formulated at pH 6.0 and stored at 4 °C (Maresca et al., 2016).

An interesting alternative for modulating nisin release is the immobilization of nisin producing LAB (*L. lactis* subsp. *lactis*) by encapsulation within various biopolymer beads. In fact, direct addition of bacteria strains to food can negatively affect the efficiency of the strain in producing bacteriocin because of the interactions between LAB and food ingredients. Encapsulation of *L. lactis* in alginate-pectin hydrogel microspheres by vibrating technology led to higher reduction of *L. monocytogenes* counts in TSB medium after the 5th day of storage at 30 °C compared to free LAB strain (Bekhit, Sánchez-González, Ben Messaoud, & Desobry, 2016). The authors have detected significant concentration of nisin in alginate-pectin microbeads with a ratio of 75/25 (with glucose-enriched M17) containing encapsulated LAB in exponential state after 7 days of storage at 30 °C.

4. Food biopreservation by encapsulated antimicrobial bacteriocins

Prevention of food spoilage and growth of foodborne pathogens could be achieved via applying nano- or microencapsulated antimicrobial peptides in food systems without losing the food quality and its nutritional value (Benech, Kheadr, Lacroix, & Fliss., 2002; Xiao, Davidson, & Zhong, 2011; Malheiros, Sant'Anna, Barbosa, Brandelli, & Franco, 2012; Huq, Riedl, Bouchard, Salmieri, & Lacroix, 2014; Huq, Vu, Riedl, Bouchard, & Lacroix, 2015; Cui, Wu, Li, & Lin, 2016; Pinilla & Brandelli, 2016; Pradhan, Goyal, Malik, & Narsaiah, 2018; Zimet et al., 2018; Feng, Ibarra-Sánchez, Luu, Miller, & Lee, 2019; Hassan et al., 2020; Calderón-Oliver, Escalona-Buendía, & Ponce-Alquicira, 2020; Lin, Luo, Li, Chen, & Cui, 2022). The range of antibacterial activity of bacteriocins against different microorganisms as well as their compatibility and interactions with produced microcapsules and the ability of microcapsules in protecting entrapped agents and releasing core materials at the desired rate should be evaluated. Also, it is possible to increase food shelf life through applying active antimicrobial packaging based on microencapsulated agents (Boelter & Brandelli, 2016; Ribeiro-Santos, Andrade, & Sanches-Silva, 2017). Slow release of antimicrobial bacteriocins from nano- or microcapsules can improve their antimicrobial activity by reducing their contact time with some food components involving their partial inactivation. Encapsulated antimicrobials alone or together with other processes were applied to improve the quality of food products like dairy, meat products, and fruit juices (Table 2).

4.1. Dairy products

Some of the most important human bacterial pathogens such as *L. monocytogenes*, *Salmonella* spp., *Staphylococcus aureus*, and *E. coli* can be conveyed to milk. The growth of these bacteria takes place thanks to the nutrients provided by raw milk. The potential pathogenic bacteria can be damaged by pasteurization process, but recontamination of dairy products can occur as a result of postpasteurization steps (Arqués,

Table 2
Antibacterial activity of encapsulated bacteriocins in food products.

Bacteriocin	Encapsulant	Food	Target microorganism	Antimicrobial activity (log reduction)	Storage conditions	References
Nisin Z	Proliposome H	Cheddar cheese	<i>L. innocua</i>	Below the detection limit in 6 month-old cheese	Ripened at 7°C for 6 months	Benech et al. (2002)
Nisin	Zein	milk	<i>L. monocytogenes</i>	~2 log CFU/mL (compared to control at 4 h incubation)	Incubation at 25 °C for 48 h	Xiao et al. (2011)
nisin	DPPC liposome	Cooked ground beef	<i>L. monocytogenes</i> , <i>L. innocua</i> , <i>P. acidilactici</i> , <i>C. sporogenes</i>	20-25 mm inhibition zones	24 h incubation at 30°C	Boualem et al. (2013)
Nisin	Alginate- CNC microbeads	RTE ham	<i>L. monocytogenes</i>	3.04 log CFU/g (beads containing 63 µg/mL nisin)	28 d of storage at 4°C	Huq et al. (2014)
Nisin + cinnamon EO	Alginate- CNC microbeads	RTE cooked ham	<i>L. monocytogenes</i>	4.04 log CFU/g	35 d of storage at 4°C	Huq et al. (2015)
Nisin + garlic extract	nanoliposome	Whole milk	<i>L. monocytogenes</i> , <i>S. enteritidis</i> , <i>E. coli</i> , <i>S. aureus</i>	5-6 log CFU/mL (Gram-positive), 3-4 log CFU/mL (Gram-negative)	24 h incubation at 37°C	Pinilla and Brandelli (2016)
nisin	Silica liposome	cheese	<i>L. monocytogenes</i>	4-5 log CFU/mL	25 d at 7°C	
Pediocin	Nanoliposome-alginate hybrid	milk	<i>L. innocua</i> , <i>L. monocytogenes</i>	ND at 5 d ND at 6 d	25°C, 7 d 4°C, 15 d	Cui et al. (2016)
nisin	Alginate-chitosan nanoparticles	Lean beef	<i>L. monocytogenes</i>	No reduction	48h at 4 and 37°C	Pradhan et al. (2018)
nisin	γ-PGA/PLL nanoparticles	Fresh pork meat	<i>S. aureus</i>	2.8 log CFU/g (sample containing 800 IU/g nisin loaded particles at 24th d)	Stored at 4°C for 24 d	Zimet et al. (2018)
nisin	Chitosan nanoparticles	Orange juice	<i>S. aureus</i>	1.5 log CFU/g 1.2 log CFU/g	4°C for 14 d 25°C for 7 d	Cui et al. (2018)
nisin	Amaranth protein isolate/pullulan	Apple juice	<i>Salmonella</i> Typhimurium <i>L. monocytogenes</i> <i>L. mesenteroids</i>	3.84 log CFU/mL 3.54 log CFU/mL 3.44 log CFU/mL	Incubation at 25°C for 48 h	Lee et al. (2018)
nisin	zein	Queso fresco cheese	<i>L. monocytogenes</i>	4-6 log CFU/mL	Incubation for 96 h	Soto et al. (2019)
Nisin A	Alginate/hi-maize resistant starch	Cheddar cheese	<i>C.tyrobutyricum</i>	2 log CFU/g	Storage at 4°C for 28 d	Feng et al. (2019)
Nisin + avocado peel extract	Collagen/pectin	Ground beef	Mesophiles Coliforms LAB	1.4 log CFU/g	Storage at 4 for one week	Hassan et al. (2020)
Nisin + rosemary essential oil	Lyceum barbarum polysaccharides	Ground beef	<i>S. aureus</i> <i>E. coli</i> O157:H7	1.83 log CFU/g 0.3 log CFU/g 0.6 log CFU/g	Storage at 4°C for 10 d	Calderón-Oliver et al. (2020)
				2.0 log CFU/g at 4°C 2.37 log CFU/g at 25°C 1.31 log CFU/g at 4°C 1.80 log CFU/g at 25°C	Storage at 4°C and 25°C for five days	Lin et al. (2022)

d: day; ND: not detected; DPPC: Dipalmitoylphosphatidylcholine; RTE: ready-to-eat; CNC: cellulose nanocrystal; γ-PGA: poly-γ-glutamic acid; PLL: poly-L-lysine; *Listeria monocytogenes*; *Listeria innocua*; *Staphylococcus aureus*; *Escherichia coli* O157.H7; *Salmonella enteritidis*; *Clostridium sporogenes*; *Pediococcus acidilactis*.

Rodríguez, Langa, Landete, & Medina, 2015). Inhibition of clostridial spoilage causing the late-blowing defect in cheese was the earliest nisin application in dairy products (Delves-Broughton, Blackburn, Evans, & Hugenholtz, 1996). Besides nisin and pediocin has also been used in dairy products for their promising antilisterial activity, stability, and activity in a wide range of pH values. Addition of a dried preparation of pediocin PA1 produced by *Pediococcus acidilactici* in cottage cheese, cream, and cheese sauce systems could reduce *L. monocytogenes* counts (Pucci, Vedamuthu, Kunka, & Vandenbergh, 1988). Being active against Gram-positive bacteria, bacteriocins can be applied by incorporating the strains that produce them as starters in fermented milk products to improve their safety. These starter cultures capable of producing bacteriocins can be used directly in foods without the need for regulatory approval or label declaration (Arqués et al., 2015; Silva et al., 2018). However, it is possible to widen antimicrobial activity of bacteriocins by combination with various bioactive agents or other preservation treatments in order to avoid food spoilage and contamination. Combination of nisin with natural biopreservatives reuterin and pediocin had potential of decreasing initial bacterial load besides coliform counts of raw milk resulting in its shelf life extension by 6 h at 37 °C (Kumar, Kumar, Waheed, & Pradhan, 2020). The application of encapsulated antimicrobial peptides like nisin in dairy products is an attractive strategy for further prevention of growth of pathogens and avoiding interactions of

bacteriocins with food components. Negatively charged casein micelles at the pH of milk (around 6.6) electrostatically interact with positively charged nisin. Nisin loaded-zein microcapsules (400 IU/mL) were more effective in decreasing *L. monocytogenes* population in milk containing 2 g/100 g fat incubated at 25 °C compared with free nisin (400 IU/mL) due to the reduction in the interactions of nisin with milk components and its slow release (Xiao et al., 2011). Microfluidic technique was used for the encapsulation of nisin in zein as a wall material to extend the shelf life of Queso Fresco (a type of cheese). Stability and antimicrobial activity of nisin can be preserved during encapsulation by microfluidic process because of no heat generation. Despite of *L. monocytogenes* regrowth in cheese after 7 days of cold storage, the high flow rate (0.3 mL/h) and low loading of nisin (5.94 µg/mg) in the zein microcapsules were highly efficient in reducing *L. monocytogenes* counts by 2 log CFU/g compared to the other treated and untreated cheese (Feng et al., 2019). The encapsulation of nisin A in biodegradable, nontoxic and low cost alginate/non gelatinized starch beads produced by ionotropic gelation vibration technology resulted in improved shelf life of cheddar cheese during two months of storage due to the prevention of *C. tyrobutyricum* growth within cheese at large-scale production (Hassan et al., 2020).

Several researches have been carried out to study the application of bacteriocin loaded liposomes in real food systems especially dairy products. The concentration of milk fat has major effect on the stability

of liposomes added to the milk as increasing of milk fat content could increase the amount of released nisin Z (Laridi et al., 2003). Liposomal encapsulated nisin Z exhibited higher antibacterial activity against bacterial cells of *L. innocua* compared with *in situ* produced nisin by *Lactococcus lactis* subsp. *lactis* biovar diacetylactis UL 719 in cheddar cheese matrix during 6 months ripening (Benech, Kheadr, Lacroix, & Fliss, 2002). The activity of nisin was preserved by 90% in liposome compared to only 12% retained activity of produced peptide by nisinogenic starter in 6 months ripened cheese. However, according to Kheadr, Vuillemand, and El Deeb (2000), liposomes can be destabilized by being placed between fat globules and casein matrix in Cheddar cheese due to the establishment of interactions between liposome and fat globule membrane. Targeting Gram-negative pathogens can be enhanced by using bacteriocins in combination with other antimicrobial agents exhibiting synergistic antibacterial effects or hurdles (Sobrinho-López & Martín-Beloso, 2008; Mills, Stanton, Hill, & Ross, 2011). Co-encapsulated nisin and garlic extract in PC nanoliposomes (with size of 150–200 nm) could reduce 3–4 log CFU/mL of Gram-negative bacteria and 5–6 log CFU/mL of Gram-positive strains in whole milk at 37 °C for 24 h compared to control treatments (Pinilla & Brandelli, 2016).

Nanoliposomes made up of polysaccharides and phospholipids are alternative systems for the encapsulation and controlled delivery of bacteriocins such as nisin and pediocin. Very few studies have been carried out to investigate the efficacy of liposome-polysaccharide systems for the encapsulation of antimicrobial bacteriocins (Lopes, Pinilla, & Brandelli, 2017) and controlled release of agents into food matrix (Pradhan et al., 2018). However, lower rate of nisin release (active against five *Listeria* strains) was achieved for PC-pectin and PC-polygalacturonic acid liposomes compared to PC liposomes due to the interactions of nisin with the negatively charged polysaccharide molecules (Lopes et al., 2017) (Fig. 2). Pediocin encapsulated in nanoliposome-alginate hybrid system did not have any effect on *L. innocua* and *L. monocytogenes* in milk during 48 h storage at 4 and 37 °C (Pradhan et al., 2018).

In addition to incorporating encapsulated liposomes into the food matrix, edible coatings, as liposome carriers, can be used for even distribution of liposomes on food surfaces to prevent aggregation and rupture of lipid membranes. Sustained antibacterial activity against *L. monocytogenes* was obtained for chitosan coated nisin-silica liposomes (with size range of 138.7 – 149.2 nm and 5 mg/mL nisin) at 25 °C for 5 days and at 4 °C for 6 days in comparison to pure chitosan coating (Cui et al., 2016). In addition, the chitosan-coated nisin liposomes did not show adverse effect on the sensory properties of cheese. Using silica in liposome encapsulation of nisin significantly increased EE (65.5 to 75.7%) which was ranged from 43.8 to 53.6% for free silica liposomes (Cui et al., 2016). Silica has the potential to improve the encapsulation efficiency of bacteriocins due to its high surface area, biocompatibility, and encapsulation capacity (Bitar, Ahmad, Fessi, & Elaissari, 2012).

4.2. Meat products

Contamination of meat products by spoilage bacteria negatively influences their shelf life, flavor, color, textural and sensory qualities (Jayasena & Jo, 2013). Three main foodborne pathogens including *L. monocytogenes*, *Salmonella* spp., and *E. coli* O157:H7 can contaminate raw and processed meat products and lead to serious human illnesses regarding as major public health menace (Huang, Hwang, & Fang, 2019). Growing of *L. monocytogenes* at refrigerated temperature contributes to high risks of contamination of a refrigerated ready-to-eat (RTE) meat or poultry product (US FDA, 2017). The transmission of this pathogen can be occurred by infected raw and cooked meat products (Kumar, Kaur, Shahi, Kairam, & Tyagi, 2017). Application of chemical preservatives such as nitrite, nitrate and sodium chloride does not have the potential to inhibit the growth of *L. monocytogenes* under storage conditions at the refrigeration temperature (Coma, 2008). Pediocin produced by *P. acidilactici* used on raw Spanish meat (1000 or 5000 bacteriocin unit/mL (BU/mL)) inhibited the growth of *L. monocytogenes* effectively at 4 °C (2.5 or 3.5 log cycles, respectively) during 21 days compared to 15 °C (2 or 3 log cycles, respectively) during 72 h (Nieto-Lozano, Reguera-Useros, Peláez-Martínez, & Hardisson de la Torre, 2006). Direct addition of natural antimicrobial peptides such as nisin into various meat products results in reduction or loss of its antimicrobial activity because of its inactivation by formation of a nisin-glutathione adduct, interaction with food components like lipids, and decrease of its solubility (Rose et al., 2003). Consequently, antimicrobial peptides can be applied through mixing with other bioactive agents or technological hurdles (Calderón-Oliver et al., 2020; Liu et al., 2021, 2022). For example, a combination of nisin at 1000 IU/g with 0.6% of thyme essential oil (EO) had the potential of lowering *L. monocytogenes* counts below the official limit of the European Union defined at 2 log CFU/g in treated minced beef meat during storage at 4 °C. Enhanced bacterial membrane damage could be attributed to the synergistic effect of nisin and thyme EO because of their ability to act on the cytoplasmic membrane (Solomakos, Govaris, Koidis, & Botsoglou, 2008). In another study, it was shown that the vacuum-packaged ground beef containing nisin and avocado peel extract microcapsules elaborated by complex coacervation undergone the highest reduction in the growth of mesophiles (30%), coliforms (38%), and the oxidation of proteins (~45%) compared with the beef samples treated by permeable packaging and separately encapsulated biopreservatives (Calderón-Oliver et al., 2020).

Functional nanoparticles prepared by proteins and polysaccharides known as food grade biological macromolecules have gained great attraction as new food additives for having large specific surface area as well as flexible structure, biocompatibility, and easy fabrication process (Zhang, Zhou, et al., 2021). Nanoencapsulated nisin can exhibit more efficient antibacterial activity in meat products because of its high delivery properties and being preserved from establishing interactions with meat ingredients. Recently, nisin, rosemary essential oil, and

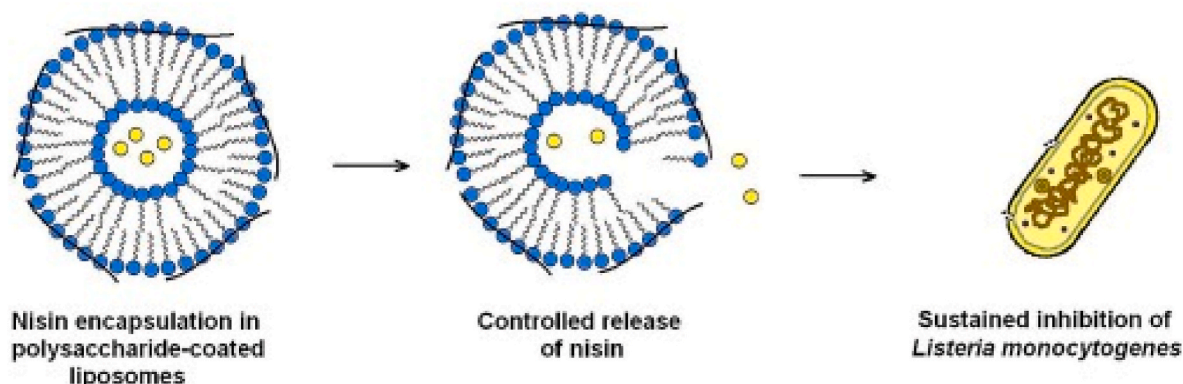


Fig. 2. Antilisterial activity of released nisin from polysaccharide coated liposome (Lopes et al., 2017).

Lycium barbarum polysaccharides were used to produce novel ternary nanoparticles (TNP) with encapsulation efficiency of 86.6% and average capsule size of 211.5 nm. The produced TNPs were applied on beef exhibiting acceptable preservation properties against *S. aureus* and *E. coli* O157:H7 during 5 days of storage at 4 and 25 °C with no changes in color and texture of the meat (Lin et al., 2022). Liposomes and natural biopolymers have been investigated as nisin delivery systems for improving safety of different meat products (Boualem, Subirade, Desjardin, & Saucier, 2013; Cui, Dai, & Lin, 2018; Huq et al., 2014, 2015; Zimet et al., 2018). Dipalmitoylphosphatidylcholine (DPPC) liposomes containing nisin (average size of 495 nm and nisin concentration of 83.3 µg/g) exhibited inhibition zones (20–25 mm) in cooked ground beef (71 °C) and no inhibition zone in raw meat both inoculated with indicator organisms like *L. monocytogenes* (Boualem et al., 2013). According to the authors, nisin was released at the cooking temperature due to melting of the liposomes and simultaneously denaturation of glutathione (nisin inactivation compound) resulted in enhanced antibacterial activity of the bacteriocin.

Polysaccharide/protein encapsulation systems can be formed by electrostatic interactions between anionic and cationic functional groups of macromolecules (Eghbal et al., 2016). Using negatively charged biopolymers like alginate and pectin can have some benefits in nisin encapsulation. Firstly, the susceptibility of nisin to proteolytic attack can be reduced through its complexation with negative charges of biopolymers that the degree of negatively charged groups directly affects nisin stability (Gruskiene, Krivorotova, & Sereikaite, 2017). Electrostatic repulsion between the cationic nisin and divalent cations of the surface of bacterial cell can decrease the antimicrobial activity of the bacteriocin. Secondly, higher negative charges of nisin-loaded anionic biopolymers can enhance its antibacterial effect by reducing electrostatic repulsion between peptide and bacteria cell (Krivorotova, Staneviciene, Luksa, Serviene, & Sereikaite, 2017). Biodegradability, biocompatibility, and formation of heat-resistant hydrogels in the presence of divalent cations in an aqueous medium make alginate an appropriate polymer for production of nisin capsules. Alginate based delivery systems were shown to be able to release the bioactive compound at the desired rate through diffusional process from pores that might be accelerated by the degradation of polymeric network (Goh, Heng, & Chan, 2012). Nisin-loaded alginate/chitosan nanoparticles (with size of 66.4 ± 8.9 nm) inhibited the growth of *L. monocytogenes* in vacuum-sealed, refrigerated lean beef samples for 10 days (400 IU/g) and for 24 days (800 IU/g) (Zimet et al., 2018). Alginate and cellulose nanocrystals (CNC) microbeads containing 63 µg/mL nisin inhibited the growth of *L. monocytogenes* in RTE meat by 3.04 log reduction after 28 days of storage at 4 °C through increasing the lag phase of bacterial growth while free nisin (31 and 63 µg/mL concentrations) increased bacterial lag phase only for 7 days (Huq et al., 2014). Gamma (γ) irradiation known as the post packaging decontamination method, is applied on RTE cooked food processing for inactivating the DNA of the pathogenic bacteria like *Listeria* spp (Farkas, 2006). Meat irradiation has been approved by the FDA and can be performed at doses up to 10 kGy without showing any side effect (Ha & Kang, 2015; Lacroix & Ouattara, 2000). Huq et al. (2015) showed synergistic antilisterial effect of encapsulated EOs and nisin in alginate-CNC microbeads in combination with γ-Irradiation process (at 1.5 kGy) on RTE cooked ham. According to the authors, the highest antibacterial activity was obtained for the cooked ham treated with encapsulated cinnamon EO (250 µg/mL)-nisin (16 µg/mL)-γ (at 1.5 kGy) after 35 days of storage at 4 °C.

Another worldwide major food hazard is contamination of food with *Staphylococcus aureus* that endangers food safety. Fresh pork meat is mostly contaminated by *S. aureus* pathogen (Cui, Zhao, & Lin, 2015). Staphylococcal food poisoning (SFP) is engendered by staphylococcal enterotoxins (SEs) which are secreted through vast growth of *S. aureus* in food matrix. SEs are produced under a wide range of pH, temperature, sodium chloride concentration, and water activity (a_w). Insufficient heating process, poor storage conditions, and manual handling can lead

to SFP that is mostly related to protein-rich food like meat and dairy products (Lin et al., 2018). The foodborne *staphylococcal* illness is related to *Staphylococcal* Enterotoxin A (Wallin-Carlquist, Márta, Borch, & Rådström, 2010). Encapsulated nisin in poly-γ-glutamic acid (γ-PGA)/poly-L-lysine (PLL) nanoparticles (average particle size 451.1 nm) produced by polyelectrolyte self-assembly technique as a new delivery system could significantly control the growth of *S. aureus* in fresh pork meat (up to 14 days at 4 °C and 7 days at 25 °C) without having negative effect on its quality (Cui et al., 2018).

4.3. Fruit juice

Fruit juices containing foodborne pathogens can be associated with outbreaks of diseases which is an increasing public health hazard since the early 1990s (Danyluk, Goodrich-Schneider, Schneider, Harris, & Worobo, 2012). Heat processing is the most used method for inactivation of microorganisms and enzymes in fruit juice and prolonging their shelf life (Petruzzi et al., 2017). However, it is obvious that thermal processing can negatively affect the organoleptic and nutritional properties of these products. Non-thermal processing methods including pulsed electric, high-pressure processing, cold plasma, ultrasound, and radiation processing have gained attention to enhance food juice shelf life without influencing sensory properties, nutritional value, and safety (Bevilacqua et al., 2018). Various microorganisms such as *L. monocytogenes* and *Alicyclobacillus acidoterrestis* can contaminate raw fruits and fruit juices (Barbosa, Silva de Araújo, Matos, Carnellosi, & Almeida de Castro, 2013; Oteiza, Soto, Alvarenga, Sant'Ana, & Giannuzzi, 2014). Regarding the antibacterial activity of nisin against such microorganisms, it can be used in fruit products for inhibiting growth of foodborne pathogens. There is limited information on nisin application in fruit juices. Incubation of different fruit juices with nisin during 24 h at 37 °C and 40 °C (depending of the strain) could reduce up to 10⁷ CFU/mL of inoculated *S. aureus*, *A. acidoterrestis*, *L. monocytogenes*, and *B. cereus* strains (de Oliveira Junior, Silva de Araújo Couto, Barbosa, Carnellosi, & de Moura, 2015). Nanodelivery systems of bacteriocins can be offered as an alternative to the applications of pulsed electric fields and high hydrostatic pressures for decontamination of fruit juice. Encapsulation of nisin in amaranth protein isolate/pullulan nanofibers (average diameter of 120 nm) through the electrospinning method led to improvement in microbial quality of apple juice because of displaying bactericidal activity against *L. monocytogenes*, *L. mesenteroides*, and *S. Typhimurium* inoculated in the juice after 20, 48 and 48 h, respectively (Soto, Hernández-Iturriaga, Loarca-Piña, Luna-Bárceñas, & Mendoza, 2019). According to the authors, increased antimicrobial activity of nisin can be related to the stabilization its β-conformation by having intermolecular interactions with the pullulan and the amaranth protein throughout the electrospinning process. Nisin loaded chitosan nanoparticles (with an average size of 147.93 ± 2.9 nm) could prominently decrease 3.84 log CFU/mL of *S. aureus*, 3.54 log CFU/mL of *L. monocytogenes*, and 3.44 log CFU/mL of *E. coli* O157:H7 than nisin, chitosan, and chitosan nanoparticles in orange juice after 48 h of incubation at 25 °C (Lee, Khan, & Oh, 2018). The effectiveness of nisin/chitosan nanoparticles against *S. aureus* and *E. coli* can be attributed to the release of intracellular compounds from the cell membrane generated by disruption of the bacterial cell membrane integrity in the presence of nanoparticles (Alishahi, 2014; Lee et al., 2018).

5. Conclusion and future perspectives

This review particularly pointed out that extending the shelf life of food products could be achieved by applying encapsulated bacteriocins without contributing to significant losses in their nutritional value and organoleptic quality. However, the selection of the best encapsulation technique and the most suitable encapsulation materials for a bacteriocin requires the evaluation of certain parameters, such as encapsulation efficiency, release kinetics, biological properties of entrapped

bacteriocins as well as their compatibility and their interactions with the prepared microcapsules. The most recent trends in the use of nano- or microencapsulated antimicrobial bacteriocins with focus on commercialized bacteriocins including nisin and pediocin in diverse food products have been highlighted. The efficiency of direct added bacteriocins in food systems can be weakened because of enzymatic degradation, poor solubility, heterogeneous distribution in food, and partial inactivation through interacting with food components. Successful incorporation of encapsulated bacteriocins in food matrices could control food safety against pathogens by protecting biopreservative from harmful parameters and releasing at desired rate within food matrix. Application of encapsulated bacteriocins have been focused mainly on some dairy products like raw milk and cheese, and meat products for their ability in inhibiting growth of *Listeria monocytogenes* which is associated with listeriosis. Despite the efficiency of these antimicrobial peptides against wide spectrum of foodborne pathogens, very limited studies have been done on the use of encapsulated bacteriocins for the conservation of fruit, fruit juices, vegetables, and some dairy products like cream and yoghurt.

On the other hand, a combination of antimicrobial peptides of microorganisms with other natural active agents or other preservation processes as hurdle technologies can assure extended shelf life of food products. A very few research works have demonstrated the synergistic antimicrobial activity of co-encapsulated bacteriocins like nisin with essential oils in real food systems. Also, edible polymer films can be used as a carrier of encapsulated bacteriocins especially for liposomes containing biopreservative for diminishing aggregation and rupture of lipid membrane. In the latter cases, further investigations are indispensable to examine the combined activity of encapsulated antimicrobial peptides with various bioactive compounds and efficacy of prepared biopolymer coatings and films in preserving active nano- or microcapsules and enhancing food safety.

Declaration of competing interest

The authors declare no conflicts of interest that pertain to this manuscript.

Data availability

Data will be made available on request.

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