**BACTERIOCIN BASED ACTIVE FOOD PACKAGING IN NANOTECHNOLOGICAL PERSPECTIVE – AN OVERVIEW**

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**Abstract**

Bacteriocins are antimicrobial peptides or protein materials produced by bacteria against pathogens. These molecules have high potency and specificity and are endowed with many properties useful in food-related applications such as preservatives and food additives, as well as biomedical applications such as alternatives to current antibacterial, antiviral, anticancer and antibiofilm agents. Despite their advantages as alternative therapeutics over existing strategies, several limitations of bacteriocins, such as high cost of isolation and purification, narrow spectrum of activity, low stability and solubility, and easy enzymatic degradation, need to be improved. This review addresses nanotechnological innovations to improve the antimicrobial properties and applications of bacteriocins through active food packaging systems. In this sense, nanotechnological techniques are described as alternatives for the physicochemical stabilization of bacteriocins and for increasing their antimicrobial properties. Incorporation of nano-encapsulated bacteriocins into active packaging systems needs to be explored to expand the potential in the food industry.

**Key words:** Bacteriocin, Lactic Acid bacteria, Nanotechnology and Food Packaging

**INTRODUCTION**

Bacteriocins are a group of ribosomally synthesized peptides that are secreted extracellularly by various Gram-positive and Gram-negative bacteria (Cotter et al., 2005), although most reported bacteriocins are produced by the former, especially lactic acid bacteria (LAB). (Hassan et al., 2012). Extensive studies have been carried out on bacteriocins due to their excellent antibacterial activity, which is closely related to the species producing strains. Moreover, bacteriocins have received considerable research attention in the field of biomedicine due to their Generally Recognized as Safe (GRAS) status and because they are safe for human consumption due to their degradation by gastrointestinal proteases Silva et al., 2018. is modified to improve the antibacterial spectrum. Most of the well-known bacteriocins are produced by Gram-positive bacteria, while only a few of Gram-negative bacteria have been characterized (Ansari et al., 2015). The activities of these small bacteriocins consisting of cationic molecules (30–60 amino acids) vary across the antibacterial spectrum, mainly due to their amphiphilic helices. Bacteriocins are widely used as natural food preservatives that inhibit the growth of microorganisms in the food industry because they are easily degraded by enzymes produced in the human gastrointestinal tract. The high quality and safety profile of bacteriocins as natural food preservatives is possible without the use of chemical preservatives, which is strictly regulated by government agencies such as the Food and Drug Administration (FDA) in the United States due to their safety concerns. Bacteriocins can generally be added directly to foods or incorporated into foods during cultivation using bacteriocin-producing bacterial strains.

Commercially available bacteriocins approved by the Food and Drug Administration (FDA) for use as biopreservatives are nisin and pediocin PA-1. These peptides can be added to foods by incorporating (i) a purified bacteriocin, (ii) a starter culture of a producing microorganism, or (iii) fermentation containing the bacteriocin. Nisin and pediocin have an antimicrobial spectrum of action limited to Gram-positive bacteria. In addition, they can show instability in acidic and basic media and reduce solubility in a wide range of pH 3.4 In addition, bacteriocins are sensitive to proteolytic enzymes. Because of this, bacteriocins lose their antimicrobial activity due to the breaking of peptide bonds by proteases. Thus, new systems to improve their stability are promising research. The use of nanotechnology becomes an innovative alternative to bacteriocins to increase their stability when applied to food.

Active packaging can be developed with antimicrobial compounds that interact with the food and/or the internal atmosphere of the product. There is a reduction in spoilage, an improvement in the quality of food and the promotion of an extension of the shelf life. In addition, active packaging can be used as a barrier to oxygen, moisture and more. The release of antimicrobial agents controls the growth of undesirable microorganisms in food. Antimicrobial packages can be (a) sachets containing antimicrobial compounds; (b) incorporating antimicrobial agents into the polymer matrix; or c) use of antimicrobial polymeric raw materials. Nanoencapsulation of bacteriocins for subsequent application as an active coating can thus extend the properties of food protection. As nanomaterials exhibit different physicochemical properties, they can contribute to thermal stability, mechanical resistance, biodegradability and flexibility. In addition, nanoencapsulated bacteriocins can be used as absorption systems and active release compounds to promote a moisture and gas exchange barrier. These properties can be given to packaging through emerging and innovative technologies such as nanotechnology.

Nanotechnology is applied in the development of antimicrobial packaging containing various encapsulated compounds that are physically and chemically unstable. Examples of encapsulated compounds include metallic silver nanoparticles, zinc oxide (Ceballos 2021, gold Yadav et al., 2021), Sahoo G2017 copper, and titanium dioxide (Esmailzadeh, 2021). Compounds obtained from natural sources have also been studied. Salama (2020), such as garlic essential oil, biomass of Spirulina sp. (Kamkar et al., 2021). phycocyanin (Kuntzler et al., 2020), acai extract (Terra et al., 2021). However, there are few studies on the nanoencapsulation of bacteriocins (Silva et al., 2017). Nisin and pediocin belong to class I and II. Both are electrostatically attracted to the cellular phospholipid membrane and act on its permeability, according to the specificity of each class. The high content of anionic lipids present in Gram-positive cells explains the specificity of these bacteriocins. Therefore, nanomaterials with active functions provided by bacteriocins are becoming promising alternatives for the development of food packaging and need to be further investigated and presented in the literature.

**Nanomaterials in combination with bacteriocins**

The potential of nanotechnology for the application of bacteriocins in active packaging can be developed using antimicrobial compounds that interact with food and/or the product's internal atmosphere.

There is a reduction in spoilage, an improvement in the quality of food and the promotion of an extension of the shelf life. In addition, active packaging can be used as a barrier to oxygen, moisture, and more (Almasi, et al., 2020). The release of antimicrobial agents controls the growth of undesirable microorganisms in food. Antimicrobial packages can be (a) sachets containing antimicrobial compounds; (b) incorporating antimicrobial agents into the polymer matrix; or c) use of antimicrobial polymeric raw materials. Thus, nanoencapsulation of the bacteriocin for subsequent application as an active coating can extend the food protection properties (Almasi et al., 2020). As nanomaterials exhibit different physicochemical properties, they can contribute to thermal stability, mechanical resistance, biodegradability and flexibility. In addition, nanoencapsulated bacteriocins can be used as absorption systems and active release compounds to promote a moisture and gas exchange barrier (Singh et al., 2017). These properties can be given to packaging through emerging and innovative technologies such as nanotechnology. Nanotechnology studies and manipulates materials with dimensions on the nanoscale and can therefore produce packaging with different physicochemical properties. The differentiated physicochemical properties are related to the high surface-to-volume ratio and the increase in the number of reactive sites in the nanomaterial. Thanks to these properties, the use of nanotechnological methods has shown promising results in the yield and stabilization of bioactive compounds (Sahoo et al., 2021). Nanoencapsulation can protect bacteriocins from inactivation by proteolytic and lipolytic enzymes and thus extend the shelf life of foods. In addition, nanoencapsulation of bacteriocins can lead to the reduction of high doses of the antimicrobial agent in food.

**Table – 1. Antimicrobial spectrum and bacteriocin stability**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Bacteriocin** | **Producer microorganism** | **Bacteriological activity** | **Stability** | **Instability** | **Reference** |
| Bac23 | *Lactobacillus plantarum*PKLP5 | Gram-positives andGram-negatives | pH (2–12)Temperature 20–100°C | Proteolytic enzymes (trypsin andproteinase-K) | Sidhu and Nehra K. 2021. |
| ZFM54 | *Lactobacillus paracasei*ZFM54 | Gram-positives andGram-negatives | Acidic mediumThermostable (30min, 100°C) | Proteolytic enzymes (trypsin andproteinase K)Fungi | Ye *et al.,* 2021 |
| AMYX6 | *Bacillus amyloliquefaciens*JDF-17 | Gram-negative | Heat (121°C, 15 min)Acid-base (pH 2–10) | Trypsin, Protease K, Papain andPepsin | Xiang *et al.*, 2021 |
| PlantacillinB21AG | *Lactiplantibacillus**plantarum* B21 | Gram-positive | up until 80°C | Proteinase KGram-negative | Golneshin *et al.,* 2020 |
| Nisin | *Lactobacillus lactis spp. Lactis* | Gram-positive | Heat | Gram negativesInteracts with proteins, lipids andcarbohydrates being inactivatedProteolytic enzymes | Ibarra-Sanchez *et al.,* 2020 |
| PediocinPA-1 | *Pediococcus pentosaceus*NCDC 273 | Gram-positive | Heat (90°C, 5–10 min) | Gram negativesFungiProteolytic enzymes | Ibarra-Sanchez *et al.,* 2020 |

**Nanotechnological approaches for encapsulation of active compounds**

Nanotechnology systems have a controlled release of compounds. Composite nanoencapsulation processes are different for each type of nanostructures, such as nanoemulsions, nanofibers, or nanocapsules. In addition to the food properties, the nanostructure system used is related to the properties of the encapsulated antibacterial agent and the materials used. Nanostructures have potential for application in food Table – 2

**Table - 2. jNano structures and their potential for application in food**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Nanostructures** | **Benefit** | **Disadvantages** | **Potential application in food** | **Techniques** | **References** |
| Nanoliposomes. | Encapsulation of hydrophilicand lipophilic molecules.Physicochemical stabilizationof encapsulated compounds.Controlled release system. | Low capacity to encapsulateActive substances. DispersionOf nanoliposomes during thestorage period | Embedding in coating material. | Microfluidization, ultrasoundand rotor-stator | Beltran *et al.,* 2020 |
| Cold high-pressurehomogenization | Bianchi *et al.,* 2020 |
| Lipid film hydration | Bianchi *et al.,* 2019 |
| Heckler *et al.,* 2020 |
| Nanoemulsions | Increase solubility andpermeability of activesubstances. Permeate onrough surfaces. | Need for high amountsof surfactant, which canchange productcharacteristics such astexture, flavor or makethe food unsuitable forconsumption. | Direct mix,Spraying or soaking,Incorporation into coatingmaterial,Embedding in edible film | Spontaneous emulsification | Lina *et al.,* 2020 |
| Ultra-high pressurehomogenization | Xu *et al.,* 2017 |
| Phase inversion composition | Li *et al.,* 2021 |
| Emulsion inversion pointPhase inversion temperature | Hien *et al.,* 2021 |
| Nanofibers. | Use of low temperaturesthat do not degrade theactive compounds.They can be obtained withdifferent conformations,sizes and polymers | High initial cost.Environment needs to havehigh temperature andhumidity control. | Pads or sachets.Incorporation ofantimicrobial agents into thepolymeric coating or ediblematrix. | Electrospinning | Terra *et al.,* 2021 |
| Dry spinning | Tian *et al.,* 2017 |
| Wet spinning | Abe & Utsumi 2020 |
| Gel spinning | Abe & Utsumi 2020 |
| Melt spinning | Abe K, Utsumi |
| Nanospheres | Active compounds aredispersed or solubilizedwithin the polymer matrix,allowing physicochemicalstabilization.Controlled release ofcompounds. | Low ability to encapsulatelipophilic compounds.High initial cost.Environment needs to havehigh temperature andhumidity control | Direct mixing.Spraying or dipping.Embedding in coatingmaterial.Embedding in Edible Film | Electrospray | Coppock *et al.,* 2021 |
| High-pressurehomogenization technologyand emulsion–solventevaporation | Zhang *et al.,* 2021 |
| Nanocapsules | Allows different active compounds to be adsorbedor dissolved in the polymermatrix. | High initial cost.Environment needs to havehigh temperature andhumidity control | Direct mixing.Spraying or dipping.Embedding in coatingmaterial.Embedding in edible film. | Electrospray | Rodrigues *et al.,* 2019 |
| High-pressurehomogenization technology | Cui *et al.,* 2020 |
| Emulsification-diffusion | Galindo-Perez *et al.,* 2017 |
| Nanoprecipitation | Ramos *et al.,* 2021 |

**NANOLIPOSOMES**

Liposomes are spherical structures that may contain a lipid bilayer or multiple bilayers (Fig. 1), and are classified as unilamellar and multilamellar. The difference between unilamellar and multilamellar liposomes is the size of the vesicle, which can range from 20 nm to 167 nm. The formation of vesicles occurs spontaneously due to the hydrophilic interaction of the polar part of the phospholipid with water. Nanoliposomes have the same structure and components as liposomes. However, they do not arise spontaneously (Maja, et al., 2020). Nanoliposomes can be applied as carriers of bioactive substances, such as antimicrobials and antioxidants, which promote physicochemical stabilization of the molecule. Nanolipozymes can be added directly to the polymer film-forming solution to participate in packaging production. In this context, Cui et al. used chitosan and Artemisia Annua Oil (AAO) liposomes to control the growth of E. coli 0157:H7. To prepare the film, agar and chitosan were dissolved in water and acetic acid. Liposomes were incorporated into this solution at 45°C. The prepared solution was stirred and degassed by ultrasound to remove air bubbles. The authors concluded that this film presented potential for use as an active packaging, as the incorporation of AAO liposomes had a bacteriostatic effect. Nanoliposomes can thus perform a controlled release of these compounds into the system (food), thus extending the shelf life of the product. Other studies have also demonstrated the potential of nanoliposomes for application in active packaging. Sarabandi and Jafari produced betanin nanoliposomes added to isolated whey protein and chitosan films with potential application as an active coating. The authors found a reduction in water vapor permeability from 7.38 g Pa-1s-1m-1 to 5.46 g Pa-1s-1m-1. In addition, nanoliposome films showed antibacterial activity against Staphylococcus aureus (63.45%). (Hamadou et al., 2020) evaluated the oxidation of β-carotene when encapsulated in lipid nanoliposomes composed of marine phospholipids (MPL) and egg phosphatidylcholine. The greatest inhibition of lipid peroxidation was achieved with MPL nanowires with b-carotene (42.9 – 2.18%). Thus, it was found that MPL nanoliposomes can be used to extend the shelf life of foods. Furthermore, according to Lopez-Polo et al., the combined use of nanoliposomes with edible coatings is an innovative approach to improve the physical properties of these coatings. Therefore, this combination contributes to increasing the applicability of nanoliposomes in food packaging systems, improving food shelf life through the gradual and controlled release of the encapsulated compound from the polymer matrix.



**Fig. 1. Structural characteristics of nanoliposomes.**

**NANOEMULSIONS**

A nanoemulsion (Fig. 2) is a dispersion of two immiscible solutions stabilized by surfactants or surfactant systems. An oil-in-water (O/W) nanoemulsion corresponds to a system where the particles have a hydrophobic part facing inwards, an oil and a hydrophilic part facing outwards in contact with water. A water-in-oil (W/O) nanoemulsion has the hydrophobic part of the particles facing outwards with oil and the hydrophilic part facing inwards with water. High-energy or low-energy processes can be used to produce Amin and Das nanoemulsions (2019). High-energy methods include high-pressure homogenization, sonication, and microfluidization. Low-energy techniques use the chemical energy stored in the components, such as spontaneous emulsification and phase inversion temperature (Naseema et al., 2020).

Nanoemulsions have potential for use in food production because the droplets are stable to gravity separation and aggregation of the encapsulated components. Nanoencapsulated bioactive substances have an enhanced effect due to a large contact surface area, thereby providing greater bioavailability. (Aswathanarayan 2019, Moreira et al., 2019). Nanoemulsions can be used in solutions to coat foods such as fruit. When it comes to their use in food packaging, nanoemulsions must be solidified. In this case, the active compounds are dispersed in the continuous phase formed by the film-forming matrix. This procedure requires Bacteriocin.



Giant. 2. Microscopic representation of the nanoemulsion oil structure and the hydrophilic part facing outwards when in contact with water. A water-in-oil (W/O) nanoemulsion has the hydrophobic part of the particles facing outwards with oil and the hydrophilic part facing inwards with water. High-energy or low-energy processes can be used to produce nanoemulsions. High-energy methods include high-pressure homogenization, sonication, and microfluidization. Low-energy techniques use the chemical energy stored in the components, such as spontaneous emulsification and phase inversion temperature. Nanoemulsions have potential for use in food production because the droplets are stable to gravity separation and aggregation of the encapsulated components. Nanoencapsulated bioactive substances have an enhanced effect due to a large contact surface area, thereby providing greater bioavailability. Nanoemulsions can be used in solutions to coat foods such as fruit. When it comes to their use in food packaging, nanoemulsions must be solidified. In this case, the active compounds are dispersed in the continuous phase formed by the film-forming matrix. This process requires the addition of an emulsifier and the introduction of energy through homogenization or other processes. Finally, casting of film-forming formulations at a controlled thickness is applied to obtain a uniformly dry layer. Xiong et al. evaluated the effect of oregano essential oil (OEO), encapsulated in a resveratrol nanoemulsion (RES) and incorporated into a pectin matrix (PEC), in the preservation of fresh pork tenderloin. The results showed that OEO coatings on RES nanoemulsions significantly extended the shelf life of pork meat. The pH values ​​and color variations were stable. Oxidation of lipids and proteins was minimized, thereby preserving meat tenderness and inhibiting microbial growth. The authors found that an edible nanoemulsion-loaded biopolymer coating has the potential to produce active packaging for fresh meat.

**NANO FIBERS**

Nanofibers can be obtained by several methods, such as drawing, template synthesis, self-assembly, and phase separation. Nanofibers can be developed with bioactive compounds, producing porous materials with a large surface area relative to the volume. Compared to macroscale materials, nanofibers have high surface functionality, tensile strength, and stiffness depending on the polymer matrix. They can be used in the synthesis of multilayer films, which promote the improvement of the mechanical properties of the polymer film. The nanometer-scale diameter provides greater flexibility and malleability of the material. However, some nanotechnology methods have disadvantages, including: complex and expensive preparation steps; additional techniques for drying materials; creating particles with a high distribution of diameters; the use of extreme temperatures and chemical solvents. In this sense, the electrospinning technique has the potential to encapsulate cells using a simple one-step processing. The principle of this process is the application of high voltage in a polymer solution leading to the formation of nanofibers by electrostatic repulsion of charges and stretching of the solution (Fig. 3). In addition, this technique has advantages such as (i) the use of organic and inorganic solvents, (ii) it does not use high temperatures, (ii) it has a uniform size distribution of the nanofibers, which promotes the stability of controlled-release bioactive compounds, and (iv) it produces dry nanofibers without agglomerates. These properties make nanofibers promising materials for food packaging applications through nanoencapsulation of various bioactive compounds. Nanofibers can be fabricated to produce components for application to primary packaging, such as a label, pouch, or sticker, to promote improved stability and controlled release of bioactive compounds. The application of nanofibers in active packaging can be done internally to preserve food quality. In this sense, Yang et al. developed nanofibrous films filled with pullulan/ethylcellulose-cinnamaldehyde (CA) with inhibition against Gram-negative (Escherichia coli) and Gram-positive (Staphylococcus aureus) bacteria. The authors therefore proposed the use of films composed of nanofibers with CA as a potential material for application in active (antimicrobial) food packaging.

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**Fig. 3. Schematic representation of the production of nanofibers using the electrospinning technique.**

NANOSPHERES AND NANOCAPSULES

Nanocapsules (Fig. 4a) and nanospheres (Fig. 4b) differ in composition and structural organization. Nanocapsules consist of polymers that surround a core. Hence there is nuclear differentiation. However, nanospheres that do not contain oil in their structure are composed of a polymer matrix in which there is no core differentiation and are composed of a more uniform matrix. Compounds can be encapsulated, adsorbed or dispersed in nanoparticles. Nanocapsules and nanospheres can be added directly to the packaging material synthesis. Another alternative is to incorporate them into a polymer solution, adding them to smaller components (bags, labels, others) for addition to food packaging. Several nanoparticles composed of different materials (including lipids, inorganic materials, natural and synthetic polymers) have been developed (Pereira et al., 2020). These differentiated nanoparticle systems have resulted in delivery systems with different physicochemical properties that provide several applications in the pharmaceutical, biomedical, food, and other industries. There are various methods for the development of polymeric microparticles and nanoparticles, such as electrospray (Jarai et al., 2017), coacervation by spray dryer, and polymerization (Assis et al., 2017).

An electrospray technique, known as electrohydrodynamic atomization, is widely used to obtain nanoparticles. Like electrospinning, electrospray is a procedure based on the application of an electric potential, generating an electric field between the capillary and the collector (Ibili et al., 2019). The characteristics of nanospheres produced by electrospraying can vary due to parameters such as flow rate, electrostatic potential, capillary-to-collector distance, and solution parameters (viscosity, density, and concentration). Environmental factors such as temperature and pressure will also affect the properties of the nanospheres. In this context (Schmatz et al., 2019) developed polymeric nanoparticles to encapsulate phycocyanin using the electrospray technique. Electrospray nanoencapsulation maintains the pigment's antioxidant activity at high temperatures (up to 216°C). These results showed the potential of this nanoencapsulated phycocyanin for future applications as an ingredient in food formulations. Nanoencapsulated bacteriocins for potentiation as an active ingredient Bacteriocins have antibacterial activity in their isolated form. However, they have low molecular stability when exposed to foods with high protein and lipid content, which does not contribute to long-term microbiological stability (Schmatz et al., 2019). Bacteriocins, when added to a food matrix or polymer matrix, have some disadvantages such as (i) enzymatic inactivation in the presence of proteolytic enzymes (ii) interaction with food components/polymer matrix and (iii) neutralization of anionic charges. These disadvantages can be overcome by nanoencapsulation of bacteriocins to protect them from other media components. Nanoencapsulation allows gradual rel . At the nanoscale, materials have unique properties. The large surface area, potential for biological activity, and controlled release of compounds make nanotechnology a strategy to increase the stability of bacteriocins (Gedarawatte et al., 2020). Most of these nanoencapsulation systems are used for bioactive substances (Radaic et al., 2020). However, there are few Fig. 3. Schematic representation of the production of nanofibers using the electrospinning technique (Yousefi et al., 2019). Fig. 4. Structural characteristics of (a) nanocapsules and (b) nanospheres. Studies with nanoencapsulated bacteriocins applied in food packaging and this exact deficit is becoming a promising research alternative. Nisin was encapsulated in microemulsions containing dittany essential oil. The zone of inhibition formed during the development of Bacillus cereus was better compared to the free nisin solution.

On plates contaminated with Bacillus cereus (6.8 log cfu ml-1), encapsulated nisin promoted overall inhibition of the microorganism, while free nisin did not show an inhibition zone. In another study, polysaccharide-coated liposomes were used for nanoencapsulation of lysozyme and nisin. Phosphatidylcholine liposomes were coated with pectin with an average particle size of 77 nm and an encapsulation efficiency of 77–87% (Chatzidak et al.,,2019). Co-encapsulation of lysozyme and nisin reduced the microbial count of Listeria monocytogenes in whole milk by 3 log CFU ml-1 and 2 log CFU ml-1 after 4 and 10 h, respectively. The data demonstrated controlled release of nisin and lysozyme. Nisin liposomes entrapped in phosphatidylcholine and embedded in gelatin and cellulose films also showed high activity against L. monocytogenes (Lopes et al., 2019). The phospholipid must interact well with the bacteriocin and not reduce its antimicrobial effect (Boelter et al., 2016). Therefore, liposomes are a promising source for encapsulating bacteriocins for application in an active material, as they provide stability and protection against molecular degradation (Sidhu et al., 2019). Nanoencapsulation of bacteriocins can be performed with nanoparticles and promote the controlled release of the antimicrobial agent. In a study developed by (Lee et al., 2018), chitosan nanoparticles were added with nisin through ionic interactions between the positively charged amino groups of chitosan and the negatively charged tripolyphosphate ions of nisin. Free nisin reduced 2.73 log CFU ml-1 of Staphylococcus aureus in orange juice, while nisin-encapsulated nanoparticles reduced it by 3.82 log CFU ml-1.98 In another study, nisin was added to amaranth protein isolate to form by electrospinning pullulan nanofibers (Lee et al., 2018). The release of nisin encapsulated in pullulan nanofibers was faster within 12 h of analysis, resulting in 81.4% in acetate buffer at pH 3.4 (apple juice simulant). Thus, the release gradually decreased until it reached a constant accumulation. Encapsulated nisin nanofibers showed bactericidal activity against Listeria monocytogenes inoculated in apple juice after 20 hours. According to Cui et al. nanofibers containing nisin-containing nanoparticles show activity against Listeria monocytogenes. Moreover, these nanostructures did not change the sensory properties of the product (Soto et al., 2019).

Thus, studies prove that nanoencapsulation methods contribute to the protection of bacteriocins and increase their bacteriostatic effects. (Cui et al., 2017). In addition, nanoencapsulation controls the release of the active compound, which enables product stability and subsequently improves food preservation.



Nanocapsules (Fig. 4a) Nanospheres (Fig. 4b)

**Conclusion**

Several studies have been carried out to determine different bacteriocin-producing strains with a broad spectrum of antibacterial activity. However, the application of bacteriocins in food is limited due to the low physicochemical stability of the molecule. The use of innovative techniques such as nanoencapsulation from nanoliposomes, nanoemulsions, nanoparticles, and nanofibers is becoming an alternative for bacteriocins to have greater physicochemical stability when applied to foods. Research in the literature reports nanotechniques applied to bacteriocins with direct application in the food matrix. This review article presents techniques applicable to bacteriocins with potential for incorporation into active packaging. The mentioned techniques are applied to bacteriocins and show a high potential for inclusion in an active envelope. Nanoliposomes and nanofibers stand out due to their low price and improved mechanical properties of the packaging, respectively. In addition, both contribute to avoiding a single and high dose of the compound, which ensures the microbiological stability of the food over a long period of time. However, these technologies are little explored in the literature for bacteriocin encapsulation, especially for its application in active food packaging. Future perspectives of the application of nanotechnology in the encapsulation of bacteriocins show several advantages. Nanoencapsulation of bacteriocins can be used to extend the shelf life of foods, increase the safety of industrial foods, and produce more effective nutricosmetics, pharmaceuticals, and biomedical materials. These applications will be a reality soon after further studies focus on the molecular stability and controlled release of encapsulated bacteriocin from nanotechnology.

**References**

1. Abe K, Utsumi M. Wet spinning of cellulose nanofibers via gelation by alkaline

treatment. Cellulose 2020;27(17):10441-10446. https://doi.org/10.1007/

s10570-020-03462-6.

1. Assis RQ, Lopes SM, Costa TMH, et al. 2017.Active biodegradable cassava starch films incorporated lycopene nanocapsules. Ind Crops Products;109:818- 827. <https://doi.org/10.1016/j.indcrop.2017.09.043>.
2. Almasi H, Oskouie MJ, Saleh A. 2020.A review on techniques utilized for design of controlled release food active packaging. Crit Rev Food Sci Nutr;1-21.
3. Ansari, A. 2015. Bacteriocin from LAB for medical and health applications. In Beneficial Microorganisms in Medical and Health Appli-cations. Microbiology Monographs; Liong, M.T., Ed.; Springer: Cham, Switzerland,; Volume 28, pp.199–221.
4. Aswathanarayan JB, Vittal RR. 2019.Nanoemulsions and their potential applications in food industry. Front Sustain Food Syst;3:1-21. <https://doi.org> 10.3389/fsufs.2019.00095
5. Amin N, Das B.2019. Review on formulation and characterization of herbosome complex. Int J Curr Pharm Res 201911(4):42-46. https://doi.org/10.22159/

ijcpr.2019v11i4.34948

1. Beltra´n JD, Ricaurte L, Estrada KB, et al. 2019.Effect of homogenization methods on the physical stability of nutrition grade nanoliposomes used for encapsulating high oleic palm oil. LWT 2020;118:108801. <https://doi.org/10.1016/> j.lwt..108801
2. Bianchi A, Velot E´, Kempf H, *et al*.,.2020. Nanoliposomes from agro-resources as promising delivery systems for chondrocytes. Int J Molecul Sci ;21(10).

<https://doi.org/10.3390/ijms21103436>.

1. Beltra´n JD, Ricaurte L, Estrada KB, et al. 2019..Effect of homogenization methods on the physical stability of nutrition grade nanoliposomes used for encapsulating high oleic palm oil. LWT 2020;118:108801. [https://doi.org/10.1016/j.lwt. 108801](https://doi.org/10.1016/j.lwt.%20108801)
2. Boelter JF, Brandelli A. Innovative bionanocomposite films of edible proteins

containing liposome-encapsulated nisin and halloysite nanoclay. Colloids

Surfaces B: Biointerfaces 2016;145:740-747. https://doi.org/10.1016/

j.colsurfb.2016.05.080

 11.Ceballos RL, Bilderling C, Guz L, et al. 2021.Effect of greenly synthetized

 silvernanoparticles on the properties of active starch films obtained by extrusion

and compression molding. Carbohydr Polym;261:117871.

1. Cui H, Yuan L, Li W, et al. 2017.Edible film incorporated with chitosan and Artemisia annua oil nanoliposomes for inactivation of Escherichia coli O157:H7 on cherry tomato. Int J Food Sci Technol;52:687-698. 10.1111/ijfs.13322.
2. Cui J, Sun C, Wang A, et al.2020. Dual-functionalized pesticide nanocapsule delivery system with improved spreading behavior and enhanced bioactivity. Nanomaterials ;10(2):220. <https://doi.org/10.3390/nano10020220>.
3. Cui H, Wu J, Li C, et al. 2017. Improving anti-listeria activity of cheese packaging via nanofiber containing nisin-loaded nanoparticles. LWT - Food Sci Technol ;81:233-242
4. Cotter, P.D.; Hill, C.; Ross, R.P. 2005. Bacteriocins: Developing innate immunity for food. Nat. Rev. Microbiol., 3, 777–788.
5. Chatzidaki MD, Balkiza F, Gad E, et al. Reverse micelles as nano-carriers of

nisin against foodborne pathogens. Part II: The case of essential oils. Food

Chem;278:415-423. <https://doi.org/10.1016/j.foodchem.2018.11.078>

1. Coppock J, Waxter Q, Hannan J. High temperature measurements of levitated

gold nanospheres derived from gold suspensions. J Quantitat Spectrosc

Radiative Transfer 2021;270:107645. https://doi.org/10.1016/

j.jqsrt.2021.107645.

17. Duong VA, Nguyen TTL, Maeng HJ, et al. 2019.Nanostructured lipid carriers

 containing ondansetron hydrochloride by cold high-pressure homogenization

 method: Preparation, characterization, and pharmacokinetic evaluation. J

 Drug Delivery Sci Technol ;53:101185. <https://doi.org/10.1016/>

 j.jddst.2019.101185.

18. Esmailzadeh H, Sangpour P, Shahraz F, et al. CuO/LDPE nanocomposite for

active food packaging application: A comparative study of its antibacterial

activities with ZnO/LDPE nanocomposite. Polym Bullet 2021;78(3):1671-1682.

19.Espitia PJP, Fuenmayor CA, Otoni CG. 2019. Nanoemulsions:

 Synthesis,characterization, and application in bio-based active food packaging.

 Compr Rev Food Sci Food Safety;18:264-285.

20. Gedarawatte STG, Ravensdale JT, Al-Salami H, et al. 2021.Antimicrobial efficacy of nisin-loaded bacterial cellulose nanocrystals against selected meat spoilage lactic acid bacteria. Carbohydr Polym;251:117096. https://doi.org/

10.1016/j.carbpol.2020.117096.

21. Gopi S, Balakrishnan P. 2021.Liposomal nanostructures: Properties and

applications. In: Sabu Thomas and Preetha Balakrishnan (Eds.). Nanoscale

Processing: Micro and Nano Technologies;163-179. https://doi.org/

10.1016/B978-0-12-820569-3.00006-

22.Galindo-Pe´rez MJ, Quintanar-Guerrero D,Cornejo-Villegas M,*et al*. 2017.Optimization of the emulsification-diffusion method using ultrasound to

prepare nanocapsules of different food-core oils. LWT - Food Sci Technol

2018;87:333-341. <https://doi.org/10.1016/j.lwt..09.008>

23. Gor MC, Golneshin A, Van TTH, et al. 2020.Cloning and functional expression of a

 food-grade circular bacteriocin, plantacyclin B21AG, in probiotic Lactobacillus

 plantarum WCFS1. PLoS ONE;15:1-18. <https://doi.org/10.1371/>

 journal.pone.0232806.

24. Gupta A, Badruddoza AZM, Doyle PS. 2017;.A general route for nanoemulsion

 synthesis using low-energy methods at constant temperature.

 Langmuir33(28):7118-7123.

25. Golneshin A, Gor MC, Williamson N, et al. 2020.Discovery and characterisation of

 circular bacteriocin plantacyclin B21AG from Lactiplantibacillus plantarum B21. 26. Heliyon;6(8):e04715. <https://doi.org/10.1016/j.heliyon.2020.e04715>

27. Hien LTM, Dao DTA. Black pepper essential oil nanoemulsions formulation

using EPI and PIT methods. J Food Process Preserv 2021;45(3):e15216. https://

doi.org/10.1111/jfpp.15216.

28.Hassan, M.; Kjos, M.; Nes, I.F.; Diep, D.B.; Lotfipour, F. 2012.Natural antimicrobial peptides from bacteria: Characteristics and potential applications to fight against antibiotic resistance. J. Appl. Microbiol., 113, 723–736.

29. Hamadou AH, Huang W-C, Xue C, et al.2020. Comparison of b-carotene loaded marine and egg phospholipids nanoliposomes. J Food Eng 2020;110055.

https://doi.org/10.1016/j.jfoodeng.2020.110055

30. Hassan H, Gomaa A, Subirade M, et al. 2019.Novel design for alginate/resistant

starch microcapsules controlling nisin release. Int J Biol Macromol 2020;153:

1186-1192. https://doi.org/10.1016/j.ijbiomac..10.248.

31. Ibili H, Dasdemir M.2019. Investigation of electrohydrodynamic atomization (electrospraying) parameters’ effect on formation of poly (lactic acid) nanoparticles. J Mater Sci ;54:14609-14623.

32. Ibarra-Sa´nchez LA, El-Haddad N, Mahmoud D, et al. 2020.Invited review: Advances in nisin use for preservation of dairy products. J Dairy Sci;103(3):2041-2052. https://doi.org/10.3168/jds.2019-17498

33. Jarai BM, Kolewe EL, Stillman ZS, et al. 2020.Polymeric nanoparticles. In: E. J. Chung, L. Leon, & C. Rinaldi (Eds.). Nanoparticles for Biomedical Applications: Fundamental Concepts, Biological Interactions and Clinical Applications 303-324. https://doi.org/10.1016/B978-0-12-816662-8.00018-7

34. Heckler C, Marques MSC, Cacciatore AF, et al. Thymol and carvacrol in

nanoliposomes: Characterization and a comparison with free counterparts

against planktonic and glass-adhered Salmonella. Lwt 2020;127:109382.

<https://doi.org/10.1016/j.lwt.2020.109382>

35. Kuntzler SG, Costa JAV, Brizio APDR, et al. 2020.Development of a colorimetric pH indicator using nanofibers containing Spirulina sp. LEB 18. Food Chem;32:126768.

36. Kamkar A, Molaee-aghaee E, Khanjari A, et al. 2021. Nanocomposite active packaging based on chitosan biopolymer loaded with nano-liposomal essential oil: Its characterizations and effects on microbial, and chemical properties of refrigerated chicken breast fillet. Int J Food Microbiol;342:

109071.

37. Kirtonia K, Salauddin M, Bharadwaj KK, et al. 2021. Bacteriocin: A new strategic antibiofilm agent in food industries. Biocatal Agric Biotechnol;36:

102141. https://doi.org/10.1016/j.bcab.2021.102141

38. Lopes LQS, Vaucher AR, Giongo JL, et al. 2019.Characterisation and anti-biofilm activity of glycerol monolaurate nanocapsules against Pseudomonas aeruginosa. Microb Pathogen 2019;130:178-185. <https://doi.org/10.1016/> j.micpath..03.007

39. Lee EH, Khan I, Oh DH. 2018.Evaluation of the efficacy of nisin-loaded chitosan nanoparticles against foodborne pathogens in orange juice. J Food Sci Technol;55(3):1127-1133.

 40.Lahiri D, Chakraborti S, Jasu A, et al. 2020.Production and purification of bacteriocin from Leuconostoc lactis SM 2 strain. Biocatal Agric Biotechnol ;30:101845. https://doi.org/10.1016/j.bcab.2020.101845

41.Li HW, Xiang,YZ, Zang M, et al. 2021.A novel bacteriocin from *Lactobacillus salivarius* against Staphylococcus aureus: Isolation, purification, identification, antibacterial and antibiofilm activity. LWT;140:110826.https://doi.org/10.1016/j.lwt.2020.110826

42.Li H, Lu H, Zhang Y, et al. 2021.Oil-in-water nanoemulsion with reversible charge prepared by the phase inversion composition method. J Mol Liquids 2021;336: 116174. https://doi.org/10.1016/j.molliq.2021.116174.

43.Lopes LQS, Vaucher AR, Giongo JL, et al. Characterisation and anti-biofilm

activity of glycerol monolaurate nanocapsules against Pseudomonas

aeruginosa. Microb Pathogen 2019;130:178-185. https://doi.org/10.1016/

j.micpath.2019.03.007

44.Lina EC, Tama DP, Nelly N, et al. 2020.Design of nanoemulsion of Tephrosia vogelii extract as botanical insecticide to control cabbage pest. IOP Conference Series: Earth and Environmental Science;583(1). https://doi.org/10.1088/

1755-1315/583/1/012024

45.PLopes NA, Pinilla CMB, Brandelli A. 2019. Antimicrobial activity of lysozyme-nisin co-encapsulated in liposomes coated with polysaccharides. Food Hydrocoll 93:1-9. https://doi.org/10.1016/j.foodhyd.2019.02.009

46.Mel JU, Gupta S, Willner L, et al. 2021.Manipulating phospholipid vesicles at the nanoscale: A transformation from unilamellar to multilamellar by an n-Alkylpoly( ethylene oxide). Langmuir;37(7):2362-2375. https://doi.org/

10.1021/acs.langmuir.0c03302

47.Maja L, Zˇ eljko K, Mateja P. 2020.Sustainable technologies for liposome preparation.J Supercrit Fluids 2020;165. https://doi.org/10.1016/j.supflu..104984

Moreira JB, Morais MG, Morais EG, et al. 2018. Electrospun Polymeric Nanofibers in Food Packaging. In A. M. Grumezescu, & A. M. Holban (Eds.). Handbook of Food Bioengineering, Impact of Nanoscience in the Food Industry;387- 417.

48. Moreira JB, Goularte PG, Morais MG, et al. 2019.Preparation of beta-carotene nanoemulsion and evaluation of stability at a long storage period. Food Sci Technol 2019;39(3):599-604. <https://doi.org/10.1590/fst.31317>.

49.Moreira JB, Kuntzler SG, Terra ALM, et al. 2020. Electrospun nanofbers fundamentals, food packaging technology, and safety. In: S. M. Rangappa, P.

 Jyotishkumar, S. M. K. Thiagamani, S. Krishnasamy, & S. Siengchin (Eds.), Food Packaging: Advanced Materials, Technologies, and Innovations (1st ed.). CRC Press..

50.Moreira JB, Lim LT, Zavarezec ER, et al. 2019.Antioxidant ultrafine fibers developed with microalga compounds using a free surface electrospinning. Food Hydrocoll;93:131-136.

51.Naskar, A.; Lee, S.; Lee, Y.; Kim, S.; Kim, K.-s. 2020. A new nano-platform of erythromycin combined with Ag nano-particle ZnO nano-structure against methicillin-resistant Staphylococcus aureus. Pharmaceutics, 12, 841.

52.Naseema A, Kovooru L, Behera AK, et al. A critical review of synthesis

procedures, applications and future potential of nanoemulsions. Adv Colloid Interface Sci 2021;287:102318. https://doi.org/10.1016/j.cis.2020.102318

53.Prakash A, Vadivel V, Rubini D, et al. 2019. Antibacterial and antibiofilm activities of linalool nanoemulsions against Salmonella Typhimurium. Food Biosci; 28:57-65. https://doi.org/10.1016/j.fbio.2019.01.018

54.Radaic A, Jesus MB, Kapila YL. 2020.Bacterial anti-microbial peptides and nanosized drug delivery systems: The state of the art toward improved bacteriocins. J Controlled Release;321:100-118. https://doi.org/10.1016/j.jconrel.2020.02.001

55.Ramos R, Koh K, Gabryelczyk B, et al.2021. Nanocapsules produced by

nanoprecipitation of designed suckerin-silk fusion Proteins. ACS Macro Lett 2021;10(5):628-634. https://doi.org/10.1021/acsmacrolett.1c00171

56.Razavi S, Janfaza S, Tasnim N, *et al*. 2021.Nanomaterial-based encapsulation for controlled gastrointestinal delivery of viable probiotic bacte´ria. Nanoscale Adv;3:2699-2709.

57.Rodrigues RM, Ramos PE, Cerqueira MF, et al. 2020..Electrosprayed whey proteinbased nanocapsules for b-carotene encapsulation. Food Chem ;314:126157. https://doi.org/10.1016/j.foodchem.2019.126157ANTAGES

58.Pereira DCS, Teixeira NS, Zin IM, et al. 2020.Active and intelligent packaging:

Security, legal aspects and global Market. Brazilian J Dev;6:61766-61794. DOI:10.34117/bjdv6n8-164 d Their Potential for Application in FoUCTUBENEFITS

59.Silva, C.C.G.; Silva, S.P.M.; Ribeiro, S.C. 2018. Application of bacteriocins and protective cultures in dairy food preservation.Front. Microbiol., 9, 594.

60.Sidhu PK, Nehra K. 2021. Purification and characterization of bacteriocin Bac23 extracted from Lactobacillus plantarum PKLP5 and its interaction with silver nanoparticles for enhanced antimicrobial spectrum against food-borne

pathogens. Lwt;139:110546. <https://doi.org/10.1016/j.lwt.2020.110546>

61.Shin, J.M.; Gwak, J.W.; Kamarajan, P.; Fenno, J.C.; Rickard, A.H.; Kapila, Y.L. 016.Biomedical applications of nisin. J. Appl. Microbiol., 120, 1449–1465.

62.Salama HE, Aziz AMS. Optimized carboxymethyl cellulose and guanidinylated

chitosan enriched with titanium oxide nanoparticles of improved UV-barrier

properties for the active packaging of green bell pepper. Int J Biol Macromol

2020;165:1187-1197.

63.Sahoo G, Sarkar N, Swain SK. 2017. Antimicrobial properties of nano-gold imprinted starch bionanocomposites. Polymer-Plastics Technol Eng;56(3):334-345.

Sahoo M, Vishwakarma S, Panigrahi C, et al. 2021.Nanotechnology: Current applications and future scope in food. Food Front;2(1):3-22.

64.Soto KM, Herna´ndez-Iturriaga M, Loarca-Pin˜a G, et al. 2019.Antimicrobial effect of nisin electrospun amaranth: Pullulan nanofibers in apple juice and fresh

cheese. Int J Food Microbiol295:25-32.

65.Singh T, Shukla S, Kumar P, et al. 2017.Application of nanotechnology in food science: Perception and overview. Front Microbiol 8:1-7.

66.Schmatz DA, Mastrantonio DJS, Costa JAV, et al. 2019.Encapsulation of phycocyanin by electrospraying: A promising approach for the protection of sensitive compounds. Food Biopr Process :119:206-215. https://doi.org/10.1016/j.fbp.2019.07.008

67.Sidhu PK, Nehra K. 2019.Bacteriocin-nanoconjugates as emerging compounds for enhancing antimicrobial activity of bacteriocins. J King Saud Uni Sci;31:758-767. https://doi.org/10.1016/j.jksus.2017.12.007

68.Sarabandi K, Jafari SM.2020. Effect of chitosan coating on the properties of

nanoliposomes loaded with flaxseed-peptide fractions: Stability during spraydrying.Food Chem;310:125951. <https://doi.org/10.1016/>

69.Silva CKD, Mastrantonio DJDS, Costa JAV, et al. 2019.Innovative pH sensors developed from ultrafine fibers containing ac¸aı´ (Euterpe oleracea) extract.Food Chem 294:397-404.

70.Tian Q, Xu Z, Liu Y, et al. Dry spinning approach to continuous graphene fibers

with high toughness. Nanoscale 2017;9(34):12335-12342. https://doi.org/

10.1039/c7nr03895j.

71.Terra ALM, Moreira JB, Costa JAV, et al. 2021. Development of time-pH indicator nanofibers from natural pigments: An emerging processing technology to monitor the quality of foods. Lwt;142:111020. <https://doi.org/10.1016/> j.lwt.2021.111020.

72.Tripepi M, Ferrauto G, Bennardi PO, et al.2020. Multilamellar LipoCEST agents

obtained from osmotic shrinkage of paramagnetically loaded giant

unilamellar vesicles (GUVs). Angewandte Chemie ;132(6):2299-2303.

https://doi.org/10.1002/ange.201912327.

73.Vilela C, Kurek M, Hayouka Z, et al. 2018.A concise guide to active agents for active food packaging. Trends Food Sci Technol;80:212-222.

74.Xiong Y, Li S, Warner RD, et al. 2020.Effect of oregano essential oil and resveratrol nanoemulsion loaded pectin edible coating on the preservation of pork loin in modified atmosphere packaging. Food Control;114:107226.

75.Xu J, Mukherjee D, Chang SKC. 2018.Physicochemical properties and storage

stability of soybean protein nanoemulsions prepared by ultra-high pressure

homogenization. Food Chem;240:1005-1013. https://doi.org/10.1016/

j.foodchem.2017.07.077

76.Xiang YZ, Zhang YM, Liu YY, et al. 2021. Purification, characterization, and

antibacterial and antibiofilm activity of a novel bacteriocin against Salmonella

enteritidis. Food Control 127:108110. https://doi.org/10.1016/

j.foodcont.2021.108110

77.Yousefi M, Ehsani A, Jafari SM. 2019.Lipid-based nano delivery of antimicrobials to control food-borne bacteria. Adv Colloid Int Sci 2019;270:263-277. https:// doi.org/10.1016/j.cis..07.005

78.Ye P, Wang J, Liu M, et al. 2021.Purification and characterization of a novel

bacteriocin from Lactobacillus paracasei ZFM54. LWT 2021;143:111125. https://doi.org/10.1016/j.lwt..111125.

79.Yadav S, Mehrotra GK, Dutta PK. 2020.Chitosan based ZnO nanoparticles loaded gallic-acid films for active food packaging. Food Chem 2021;334.

 80.Zamani-Ghaleshahi A, Rajabzadeh Gh, Ezzatpanah H, et al. 2020.Biopolymer coated nanoliposome as enhanced carrier system of perilla oil. Food Biophys; 15:273-287. <https://doi.org/10.1007/s11483-019-09621-y>.

81.Zhang K, Zhao W, Liu Q, et al. 2021.A new magnetic melt spinning device for

patterned nanofiber. Sci Rep;11(1):8895. https://doi.org/10.1038/

s41598-021-88520-0