Applications of (Nano)encapsulated Natural Products by Physical and Chemical Methods

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Abstract Natural products that originate from fungal, bacteria, plant, marine, and animal sources have a wide variety of applications. Numerous studies have highlighted natural products in different areas for medicinal purposes, such as antimicrobial, antioxidant, anti-infammatory, and anticancer agents. Although they are fascinating from an applied point of view, natural products can be unstable and fragile. A key issue for using these natural products and biomolecules is their bioavailability and stability, depending on the context in which they will be applied. In this context, encapsulation is a viable alternative to protect active compounds against the deterioration of environmental conditions, maintaining their natural compounds. Many encapsulation methods can be used, whether physical or chemical, and their use is intrinsically linked to their application. Among them, we can highlight electrospinning methods and micelles' formation. These encapsulation methods allow their application internally or externally to living organisms, bringing a series of distinct benefts. In an increasingly effcient search for new drugs, the

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[©] The Author(s), under exclusive license to Springer Nature Switzerland AG 2023 J. N. Cruz (ed.), *Drug Discovery and Design Using Natural Products*, [https://doi.org/10.1007/978-3-031-35205-8_11](https://doi.org/10.1007/978-3-031-35205-8_11#DOI)

encapsulation of natural products can enhance delivery and use. This chapter will emphasize physical and chemical methods and the characterization of nanoencapsulated natural products for different applications.

Keywords Encapsulation · Natural products · Biomolecules

1 Introduction

Biomolecules are biological compounds derived from cells, comprising nucleic acids, lipids, and structural proteins that lead to important antioxidant, antiinfammatory, anticarcinogenic, and antimicrobial properties, being available in fruits and vegetables, yeasts, bacteria, animals, and even marine and freshwater organisms that have gained notoriety due to its potential of interacting with designed systems to enhance therapeutic activity and/or technological applications (e.g., drug delivery and bioremediation) (Rajput et al. [2022](#page-47-0); Saeed et al. [2022](#page-48-0)).

However, biomolecules extracted from their natural matrices exhibit some degree of instability when exposed to changes in temperature, pH, light, and oxygen concentration once their native structures do not protect them. As the biomolecules can deteriorate by polymerization (Jakobek [2015\)](#page-43-0) with other molecules and oxidation reactions, the challenge for Science and Industry for scaling up the technological production lies in preserving the desired biological effects of bioactive compounds, mainly, their antioxidant activity and bioavailability by improving its stability. In this context, the literature has drawn attention to (nano)encapsulation as an important prospective method for addressing this challenge (Giaconia et al. [2020](#page-42-0)).

Encapsulation consists of an active substance known as core material entrapped into a coating material (Gupta et al. [2016](#page-42-1)). This combination allows changes in solubility (Chaari et al. [2018\)](#page-40-0), chemical and thermogravimetric stability (Centurion et al. [2021](#page-40-1); Schmatz et al. [2020\)](#page-49-0), and inhibition of bacterial growth (Malheiros et al. [2016\)](#page-45-0), corroborating to several specifc purposes. Encapsulating biomolecules has several benefts, such as maintaining their functionality, preventing degradation through processing and storing, controlling its release in living tissues, and keeping food safe along shelf-life (Pereira et al. [2022\)](#page-47-1). Along these lines, this chapter is underpinned by examining nanoencapsulated natural products from microbes, plants, animals, and marine and freshwater organisms over physical and chemical methods and their characterization for different applications.

2 Encapsulation Methods

Bioactive compounds have several biological properties such as anti-infammatory, anticarcinogenic, and antimicrobial antioxidant; however, these properties are affected when these biomolecules are exposed to changes in physical and chemical factors such as temperature, pH, light, and oxygen concentration, these conditions being frequent in processes industrial and biological (Ramos et al. [2020](#page-47-2)). Faced with these limitations for the application of these compounds, encapsulation is a powerful tool to overcome these drawbacks as it provides protection to a wide variety of compounds by coating the molecules with a matrix (Ramos et al. [2022\)](#page-48-1).

Due to the diversity of properties that bioactive compounds present, different techniques can be used to guarantee protection and maintenance of the biomolecule, the performance of the selected matrix, and mainly the bioavailability to exert the necessary action. Each technique for forming encapsulated materials (nano- or microencapsulation) depends on a series of factors that related to the material to be protected, since the matrix-forming substance and the conditions in which the techniques will be applied are also fundamental in the process. Choosing the most appropriate methodology includes analyzing several parameters such as physicochemical characteristics of the substances for encapsulation, particle size (or structure) and release rate, in addition to the economic aspect (Giaconia et al. [2020\)](#page-42-0).

Nanoemulsions are kinetically stable solutions composed of two dispersed insoluble liquid phases and in the presence of surfactants, in which it is possible to guarantee the bioavailability of lipophilic bioactive compounds in aqueous medium (oil-in-water (o/w)) or hydrophilic compounds in oil phase (water-in-oil (w/o)). The formation of nanoemulsions can occur by high energy, such as homogenization under high pressure and sonication, or low energy, such as phase inversion temperature or solvent displacement, the latter being more applied since it ensures greater control in particle formation and low cost (Giaconia et al. [2020](#page-42-0)). Nanoemulsions consist of the formation of nanometric droplets that have excellent stability, which is why they have been widely used as a delivery system due to their protective effect on bioactive compounds against hostile environments provided, mainly in biological systems and industrial processes, improving their stability, solubility, and bioavailability (Saini et al. [2019](#page-48-2)).

The nanoemulsion formation by solvent evaporation is a traditional technique that involves the emulsifcation of a polymer in the liquid phase and the consequent evaporation of the solvent, resulting in precipitation of the polymer as nanoparticles with a spherical shape and its size is related to properties such as viscosity of the solution polymer used, agitation rate and temperature (Sabjan et al. [2019\)](#page-48-3). Researchers evaluated the bacterial activity of lavender essential oil before and after incorporation into a nanoemulsion using ethanol and acetone as organic solvents, and subsequent dispersion in an aqueous phase, under agitation. The nanoemulsion of lavandula essential oil formed after solvent evaporation showed greater activity against bacteria when compared to its crude form (Garzoli et al. [2020\)](#page-42-2). Nanoemulsions are commonly used for delivery systems, and thus, pesticide microcapsules have been synthesized using solvent evaporation system from dichloromethane as solvent, polylactic acid as carrier material, and polyvinyl alcohol as emulsifer. The microcapsules containing β-cypermethrin as a pesticide had a smooth, spherical shape with an encapsulation efficiency greater than 80%, in addition to an effective release mechanism (Feng et al. [2018](#page-41-0)).

Nanoemulsions is associated with the change in the surfactant spontaneous curvature during the emulsifcation process. This change can be achieved in two different ways: maintaining a constant composition while varying the temperature (phase inversion temperature method—PIT method) and maintaining a constant temperature while varying the composition (phase inversion composition method—PIC method). Both methods imply phase inversion (Ren et al. [2019\)](#page-48-4).

In the PIT method, at low temperatures, temperature-sensitive surfactants are water soluble and the spontaneous curvature of the surfactant layer at the micelle interface is positive, and at high temperatures, these surfactants are oil soluble and the spontaneous curvature of the surfactant layer at the micelle interface becomes negative. At an intermediary temperature (PIT), the surfactants have the same affnity for the oil and aqueous phase, and the spontaneous curvature of the surfactant layer at the micelle interface is zero (Jintapattanakit [2018](#page-43-1)). Nanoemulsions of cinnamon oil were produced by PIT method using nonionic surfactant and water. The mixture was heated above the PIT of the system, and then rapidly cooled with continuous stirring, which led to the spontaneous generation of small oil droplets $(107.30 \text{ nm and } 100.70 \text{ nm})$ were formed in the condition using 40:60 wt% of cinnamon oil and surfactant in total lipid phase (Chuesiang et al. [2018](#page-40-2)). The PIC method does not have restriction on surfactant characteristics, as in the PIT method which requires thermosensitive surfactants. The procedure for forming the emulsion in the PIC method consists of adding water, at constant temperature, to a mixture containing the oil phase and surfactant. As the oily mixture (w/o) is added to water, generally slowly or stepwise, there is a change in the spontaneous curvature of the surfactant and consequent change of the mixture to o/w (Feng et al. [2020\)](#page-41-1).

The spontaneous emulsifcation technique is used to obtain nanoemulsions with properties similar to obtained by physical methods, but this method, in addition to being simpler and faster, has a lower cost. This technique takes place through spontaneous emulsifcation of an oil phase with surfactant that has affnity for the organic phase. An organic solvent is used to dissolve the oil phase and then this phase is poured into an aqueous phase, consisting of water and hydrophilic surfactant, under agitation. The next step consists of removing the solvent through evaporation under reduced pressure. After adding the oil phase to the water phase, the diffusion of solvents promotes the formation of droplets (Bouchemal et al. [2004](#page-40-3)). Carotenoidsloaded nanoemulsions were prepared by spontaneous emulsifcation, and the optimized conditions of the process produced particles with diameters of 50 nm (Zhang and Li [2022](#page-51-0)).

One of the most used methods for nanoencapsulation is the electrospun technique, in which a polymeric solution subjected to a high voltage, promotes the evaporation of the solvent from the solution and the formation of nanostructures. Electrospun does not require specifc reagents or extreme temperatures and therefore is closely related to food and pharmaceutical applications (Ramos et al. [2021\)](#page-48-5).

The electrospun technique can lead to the formation of two types of structures: nanoparticles (electrospray) or nanofbers (electrospinning). In both techniques, a polymeric matrix is used that will protect the bioactive compound and, consequently, increase its properties and application viability. The polymeric solution is subjected to a potential difference when it is ejected through a metal needle until it reaches a collector that is also metallic. The solid nanostructure is deposited in the collector since the solvent evaporated during the process. The diameter size of the structures formed depends on the fow rate and the applied voltage. However, the polymer type, solute concentration, and nozzle to collector distance are also important regulators of the process (Walia et al. [2019\)](#page-50-0).

To attest to the use of nanoencapsulation of natural pigments in the face of digestive processes, nanofber composite with incorporated anthocyanins from jussara pulp using polyethylene oxide was developed through electrospinning. The polymeric solution and composites produced maintained the antioxidant activity, showing their protective effect on bioactive compounds and the composite improved thermal stability of the anthocyanins (Kalsoom et al. [2020](#page-43-2)). Nanofbers containing hydroxyapatite dispersed in polycaprolactone/chitosan were developed by electrospinning aiming application in tendon and ligament tissue engineering. The nanofbers showed signifcant mechanical and biological properties (Wu et al. [2018\)](#page-51-1).

3 Nanoencapsulation of Biomolecules from Microbes

Biological, ubiquitous, and diverse organisms classifed as viruses, bacteria, archaea, fungi, or protists, considered pathogenic, beneficial, or neutral, are called microbes. They can be found in almost all habitats and are adapted to survive extreme conditions (Berg et al. [2020\)](#page-39-0). Microorganisms are critical cellular factories for synthesizing proteins, small and large metabolites, and the production of single-cell proteins. Microbial biotechnology includes methods and strategies for producing and using prokaryotic and eukaryotic microorganisms in essential applications such as industrial, pharmaceutical, medical, agricultural, energy, food and feed, biocatalysis, mining, and biomaterials (Amaning Danquah et al. [2022;](#page-39-1) Kalsoom et al. [2020](#page-43-2)).

The recombinant DNA technology made it possible to modern microbial biotechnology to include fermentation, microbial physiology, screening of new metabolites and strain improvement, bioreactor design and processing, cell immobilization, cell fusion, metabolic engineering, and directed evolution of enzymes (Adrio and Demain [2010](#page-39-2)). However, for these microbial cells to function optimally, they must fnd suitable growth and metabolic conditions while being protected from the threatened environmental conditions to which they are exposed. Furthermore, in the case of microbial cells producing commercially essential substances, it is often desirable to achieve high cell densities for higher product yields, retention of activity over more extended periods, and ease of cell recovery from the product (Cho et al. [2022\)](#page-40-4). The encapsulation of microbial cells or their products has been proposed to attain these goals for different commercially advantageous applications (Fig. [1](#page-5-0)).

Fig. 1 Nanoencapsulation of biomolecules from microbe. Created in Biorender

3.1 Nanoencapsulation of Biomolecules from Bacteria

Bacteria are unicellular prokaryote organisms, generally classifed into fve groups according to their basic shapes: spherical (cocci), rod (bacilli), spiral (spirilla), comma (vibrio), or corkscrew (spirochaetes). They can be found as single cells, in pairs, chains, colonies, or bioflms. They are rich in bioactive compounds such as carbohydrates, proteins, enzymes, genetic material, antimicrobial peptides, virulence factors, resistance genes, etc. Due to the facility of manipulation and engineering, they can display specifc functions for desired applications. This way, bacterial secondary metabolites, extracts, or biomolecules are potential bioactive compounds that can be encapsulated and used in various industries. In the pharmaceutical feld, they can be applied in probiotic delivery, improving survival, resistance, and targeted release of sensitive microorganisms. In the food industry, encapsulation increases important molecules' functional properties and antimicrobial activities to avoid product spoilage (Bagheri Darvish et al. [2020](#page-39-3); Freschlin et al. [2022\)](#page-41-2). The results presented in this section are summarized in Table [1](#page-6-0).

In medical research, a vaccine delivery system is a novel application for encapsulated bacterial products. Outer membrane vesicles (OMV), a spherical, nonreplicant structure naturally produced by Gram-negative bacteria, were encapsulated in sodium alginate nanoparticles using the unique gelation method. OMVs extracted from Bordetella pertussis are used as an adjuvant molecule to induce a stronger immune response in mice (Rami et al. [2021\)](#page-47-3). Alginate is a hydrophilic linear

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polysaccharide obtained from brown algae, composed of two interconnected monosaccharide chains: $1-4$ β-D-mannuronic (M) and α -L-guluronic (G). The gelling mechanism of alginate occurs when the G chains link on opposite sides to form a hydrophilic cavity that binds Ca2+ using oxygen atoms from the carboxyl group. The ratio and sequence of G and M residues achieved the physical properties of alginate hydrogels: the more G chains, the more rigid and porous the gel will be. In comparison, the more M chains form soft gels that disintegrate more easily. Alginate has become an excellent support for the administration of biomolecules, mainly due to the processing under mild conditions by crosslinking ions at room temperature and the good permeability of alginate gels, which facilitates the exchange of air, nutrients, and the release of metabolites (Wang et al. [2022](#page-50-1)).

In the food industry, strict control of food safety and microbiological quality is one of the fundamental requirements present in all stages of production, storage, and distribution. Bacteria and fungi contaminate different food products and cause several adverse effects on the sensory properties of foods, such as taste, color, and texture, as well as nutritional and economic losses (Delshadi et al. [2021\)](#page-41-5). They are responsible for food deterioration, causing a decrease in shelf life and disease transmission and impairing food safety and quality. Microbial growth in food products can be affected by internal factors such as pH, oxygen, and amount of water present in the food; and external factors such as light, temperature, and humidity. Despite the recent exploration of chemical or artifcial preservatives to prevent these losses, their growing association with adverse health implications, such as carcinogenic effects and allergies, has resulted in arisen of the bio-preservatives, which includes the use of microorganisms or their natural products (Kaur and Kaur [2021](#page-43-5)).

Among microbial compounds capable of controlling undesired microorganisms in food systems, bacteriocins naturally produced by some bacterial species are the best studied. Bacteriocins are antimicrobial peptides capable of prolonging the shelf life of food by inhibiting spoilage by pathogenic Gram-positive and Gram-negative bacteria (Kumariya et al. [2019\)](#page-44-6). Bacteriocins are mainly bactericidal, acting by forming pores in the membranes of microorganisms. At the same time, some are bacteriostatic, making them useful in the food and pharmaceutical sectors, especially where fermentation is undesirable. Although the producer organisms are generally recognized as safe, as proteolytic enzymes degrade them in the human intestinal tract, the direct incorporation of bacteriocins to the surface of food has some limitations, generally associated with the interaction with food compounds, which result in partial or total loss of antimicrobial activity (Mokoena et al. [2021;](#page-45-3) Kaur and Kaur [2021](#page-43-5)). Thus, the encapsulation of antimicrobial peptides in nanostructures has been used to protect them against degradation and improve their bioavailability. Encapsulated bacteriocins produced by *Lactobacillus sakei* subsp. *sakei* 2a in phosphatidylcholine and cationic 1,2-dioleoyloxy-3-trimethylammoniumpropane (DOTAP) nanovesicles were characterized and evaluated for their effect against *Listeria monocytogenes,* in vitro and UHT contaminated goat milk (Malheiros et al. [2016](#page-45-0)). The method used for encapsulation was the thin-flm hydration method. The encapsulation showed high efficiency of encapsulation (95%), low

polydispersion index, and excellent stability, and was able to delay bacterial growth in 5 days in UHT goat milk stored at 7 °C.

Among the existing bacteriocins, the most used is nisin, "Generally recognized as safe" (GRAS) by the US Food and Drug Administration (USFDA) and approved as a suitable additive for food applications by the European Food Safety Authority (ESFA). In Pinilla et al., nisin-loaded liposomes showed better results for the treatment against *L. monocytogenes* compared to free nisin. The encapsulation obtained by the thin-flm hydration method presented a bacteriostatic effect on *L. after* 30 min and reduced the expression of proteins that contribute to infection and resistance to nisin due to the stress imposed on the cells (Pinilla et al. [2021\)](#page-47-4).

Another nisin encapsulation was developed to evaluate the biopreservative effect of nanoparticles using poly-γ-glutamic acid (γ-PGA) and poly-L-lysine (PLL) using the self-assembled electrostatic method. Based on the microbial, chemical, color, and texture analysis, nanoparticles could effectively control the growth of *S. aureus* in pork meat and have no impact on the quality of the product samples (Cui et al. [2018\)](#page-41-3). Furthermore, the antimicrobial activity of nisin encapsulated in olive oilbased microemulsions enriched with essential oils was investigated against *S. aureus, L. monocytogenes,* and *B. cereus.* Rosemary, thyme, oregano, and dittany essential oil-contained microemulsions were formulated by the W/O microemulsion method. This technique increases the membrane's fexibility and facilitates the diffusion of nisin to the outer environment, enhancing the antimicrobial effect of the system (Chatzidaki et al. [2019](#page-40-5)).

Finally, nisin-loaded alginate nanoparticles were encapsulated by ionic gelation and further complexed with chitosan. Nanoparticles were prepared by ionic gelation of alginate upon dropwise addition of nisin, followed by complexation with dropwise addition of chitosan, under stirring. The nanocapsules were tested for antimicrobial activity against *L. monocytogenes* culture and as biopreservative agents of refrigerated, vacuum-packaged lean beef meat. The encapsulated nanoparticles not only delayed the growth of *L. monocytogenes* but also displayed sustained activity over time, both in vitro and in vivo assays (Zimet et al. [2018](#page-51-2)).

Other bacteriocins can be used as an antimicrobial agent in the food industry. CAMT2, a bacteriocin with antilisterial activity, was encapsulated in phosphatidylcholine nanovesicles by the reverse-phase evaporation method, which guaranteed an encapsulation efficiency of about 70% (Jiao et al. [2020\)](#page-43-3). Liposome-encapsulated pediocin (extracted from *Pediococcus pentosaceus*) also showed an improved antibacterial effect compared with free purifed pediocin (Suganthi et al. [2021\)](#page-49-1). Enterocin Gr17 is a bacteriocin that presents inhibitory effects on many pathogens found in food and has the potential for application as a natural food additive, such as liquid smoked fsh. However, bacteriocins with a high content of hydrophobic amino acids, such as Enterocin, easily bind to charged or hydrophobic macromolecules in food products. As salmon has a fat content of about 8% to 14%, the antimicrobial activity of bacteriocins is impaired. Thus, Duan et al. proposed the encapsulation of bacteriocins and EOs in a nanoemulsion, which can be used as a delivery system to protect the effects of bioactive compounds. In addition, the controlled release of the biomolecule allows for a longer duration of action of the active

substances, maintaining their antimicrobial activity for a more extended period. The nanoemulsion system incorporating Enterocin Gr17 and cinnamaldehyde essential oil was able to signifcantly inhibit microbial growth and maintain better color and texture sensory profles during the storage of smoked salmon at 4 °C. From a microbiological, physical–chemical, and sensory point of view, the treatment can extend the product's shelf life to 42 days (Duan et al. [2023](#page-41-4)).

Sakacin-A is a bacteriocin produced by *Lactobacillus sakei*, which plays an antimicrobial effect, especially against *Listeria* sp. In Mapelli et al., sakacin-A was adsorbed on cellulose nanofbers without chemical modifcations to obtain an antimicrobial material. Cellulose nanofbers have been widely used to improve mechanical and barrier properties when applied to packaging materials, which can help extend shelf life and enhance food quality (Mapelli et al. [2019\)](#page-45-1).

In the same line of work, Lacticin is a noncytotoxic bacteriocin produced by *Lactococcus lactis*, comprised of two peptides that work synergistically to kill even antimicrobial-resistant bacteria such as *Staphylococcus aureus* and *Clostridioides diffcile*. In addition, solid lipid nanoparticles (SLNs) are an excellent alternative transport system to traditional lipid-based methods, as they are biocompatible and biodegradable and can increase the stability, solubility, and, subsequently, the bioavailability of a variety of drugs. In Ryan et al., seeking to optimize the encapsulation method and increase the molecule's stability, the two peptides of Lacticin were encapsulated individually in SLNs using a modifcated nanoprecipitation method. The nanomaterial showed a higher encapsulation efficiency, cytotoxic activity, and protection from proteases in the duodenum (Kaur and Kaur [2021\)](#page-43-5).

The biopreservative effect on food packaging can also be done by bacterial enzymes, organic acids, or bacteria. Lysozyme is an enzyme naturally produced by bacteria capable of decomposing the cell wall of other species, acting as an antimicrobial peptide. Bugatti et al. proposed a preparation of bio-based membranes composed of Polyamide 11 from renewable sources and lysozyme encapsulated into halloysite nanotubes through the electrospinning process (Bugatti et al. [2018](#page-40-6)). On the other hand, lactoperoxidase (LPO), an enzyme included in the lactoperoxidase system (LPOS), has been widely used in food packaging due to its bactericidal or bacteriostatic properties, which inhibit microbial growth by transmitting sulfhydryl groups (SH) to microbial enzymes and other proteins. The research of Jasour et al. was based on an active packaging system for specifc microorganisms of the fsh industry. A chitosan solution was solubilized in acetic acid under stirring. Then, the solution was heated to add glycerol as a plasticizer. With the pH adjusted, LPOS was added to the chitosan solution. After antimicrobial, physical–chemical, and sensory tests, the authors could maintain the trout's quality and guarantee the storage time extension (Jasour et al. [2015\)](#page-43-4).

Lactic acid can also be used as an antimicrobial agent, especially when combined with other compounds with the same properties. A biocomposite flm made of chitosan, pectin, and starch was loaded with nisin, acid lactic, rosemary, and mint essential oils to prevent lipid oxidation and microbial spoilage of foods. After incorporating the compounds in a flm-forming solution, it was placed in glass plates and dried for a few hours. The flms were tested for antioxidant and antimicrobial

activity against *Bacillus subtilis, Listeria monocytogenes* and *Escherichia coli* and showed that the incorporation positively infuenced not only these properties but also the barrier and mechanical quality (Akhter et al. [2019](#page-39-4)). Since the bacterial antagonists are sensitive to environmental conditions such as competition, pH fuctuations, and temperature changes, the colonization process gets challenging, and they can rapidly disappear. Le et al. developed an alginate-gelatin compound that could protect *Lactobacillus plantarum* bacteria from these threats so it could survive and produce bacteriocins directly in refrigerated pork meat. The bacterial cells were extruded inside the nanomaterial and found to increase the percentage of antimicrobial activity. Bacteriocins activity was enhanced with high temperatures and was not affected by pH. The formulation also inhibited the growth of pathogenic bacteria in pork meat throughout the 12 h period (Pour et al. [2019](#page-47-5)). A similar study was carried out by encapsulating *Lactobacillus lactis* in a flm of corn starch and carboxymethylcellulose to improve nisin production and application on food packaging. The flms showed the best performance and the lowest water vapor transmission while preserving the antibacterial activity against *Staphylococcus aureus* for 8 days (Lan et al. [2021\)](#page-44-4).

The World Health Organization (WHO) defnes probiotics as live microorganisms that confer various health benefts to the host when administered adequately to the desired target site (Food and Agriculture Organization and World Health Organization and others [2006](#page-41-6)). These advantages encourage their wide use in sectors such as the agricultural, food, pharmaceutical, and cosmetics industries. The most frequent genera are *Lactobacillus, Bifdobacterium, Bacillus, Saccharomyces,* and *Escherichia coli*, which are particularly sensitive to the harsh conditions of many foods and the human intestine (Yao et al. [2020a\)](#page-51-4). Its preventive and therapeutic properties against infectious diseases, metabolic, anticancer, and antimutagenic activities come mainly from the production of nutrients and cofactors, competition with pathogens for nutrients or adhesion sites, and stimulation of the host's immune response (George Kerry et al. [2018](#page-42-4)). Although the potential for probiotics in treating or even preventing gastrointestinal diseases is high, their clinical effcacy still needs to improve due to conficting results in clinical trials for many diseases. This is partly due to the lack of viability of probiotics using traditional manufacturing and packaging methods (Centurion et al. [2021](#page-40-1)). Several factors signifcantly affect the viability and survival rate of probiotics during processing, storage, and consumption, such as harsh conditions within the upper human GI. such as the presence of antimicrobial lysozyme in the mouth, low pH conditions in the stomach, bile salts and digestive enzymes in the small intestine, and other complex factors, including osmotic pressure and oxidative stress throughout the gastrointestinal tract (Techo et al. [2019\)](#page-50-2). Thus, probiotics must survive the hostile gastric environment, remain metabolically active, and be released in suffcient quantities and controlled at the site of action in the lower gastrointestinal (GI) tract to confer benefcial health effects (Razavi et al. [2021](#page-48-8)).

Nanoencapsulation of probiotics has been proposed as an effective solution to improve survival, resistance, and targeted release of sensitive microorganisms in the GI tract, as it can trap small amounts of bioactive compounds and/or microorganisms in small nanostructured compounds (Centurion et al. [2021](#page-40-1)). In essence, the goal of nanoencapsulation is to create a microenvironment that protects bacteria from exposure to external factors (such as low gastric pH) during digestion and subsequently reduces cell injury or death before their release to the target site (Pateiro et al. [2021](#page-46-1)). There are many reports on nanoencapsulation of probiotic bacteria resulting in highly increased viability of probiotics during storage and administration. Due to unique physical and chemical properties, nanostructured materials show great promise for protecting microorganisms from the acidic conditions of the stomach and therefore allow the successful release of trapped probiotic cells into the intestinal lumen at natural pH (Centurion et al. [2021;](#page-40-1) Sharma et al. [2019\)](#page-49-3).

In the food industry, the so-called "functional foods" have gained importance since consumers have been looking for foods with properties that go beyond nutrition. A functional food can be defned as one that provides benefcial effects to the human body in addition to its basic nutritional properties, as is the case with probiotic foods (Atraki and Azizkhani [2021\)](#page-39-7). The dietary supplemented probiotics market is predicted to grow at a compound annual growth rate of 7% compound annual growth rate through 2027 ([2022\)](#page-47-6). Accordingly, studies have been conducted to increase the viability of these products both in the production stages and through the GI passage. Azfaal et al. used sodium alginate and carrageenan to encapsulate Lactobacillus acidophilus in ice cream to evaluate the viability of probiotic bacteria under simulated GI conditions. The nanocapsules could provide protection and enhanced survival of probiotics in food, guaranteeing the health recommended level for the best benefts (Afzaal et al. [2019\)](#page-39-5). In Yilmaz et al., the authors used the electrospinning method to produce alginate-based nanoparticles with *Lactobacillus paracasei* KS-199. The viability in simulated GI conditions was improved, as well as the survival in kefr and the protection against thermal degradation (Yilmaz et al. [2020\)](#page-51-3). Similarly, cells of *Lactobacillus gasseri* were encapsulated in sodium alginate capsules using ionic gelation and emulsifcation methods. The encapsulated probiotics retained 100% of their viability, compared with the free cells, and enhanced the viability in stored apple juice for 21 days (Romero-Chapol et al. [2022\)](#page-48-7).

As already mentioned, probiotics are beneficial in different sectors of production. In the pharmaceutical industry, probiotics are used as supplements and can be added to other health products, such as infant milk formulas. Probiotic agents are becoming a vital part of the tools against GI problems, especially in formula-fed infants (Putta et al. [2018\)](#page-47-7). Algaithi et al. fortifed camel milk infant formula with *Lactobacillus reuteri* encapsulated in sodium alginate and galactooligosaccharides via spray drying. The nanocapsules were evaluated for stability in simulated infant GI, storage conditions, physicochemical properties, and *L. reuteri* viability and proved to be an excellent delivery system of probiotics for kids (Algaithi et al. [2022\)](#page-39-6).

In addition, probiotics have benefcial effects on the skin, not only when taken orally but also when applied topically. Oral consumption of probiotics improves the overall metabolic content of the human body by inhibiting harmful intestinal microfora. Similarly, inhibiting harmful microbial growth by topical application of probiotics alters the epithelial microbiome by lowering surface pH and generating an

amino acid layer, preserving skin moisture (Puebla-Barragan and Reid [2021\)](#page-47-8). Moreover, probiotics produce valuable metabolites with antioxidant and tyrosinase inhibitory activity, which induce skin-lightening effects. Therefore, these extracts could be exploited as multifunctional natural preservatives in the cosmetics industry. In this scenario, *Lactobacillus curvatus* cells were encapsulated in liposomes using the O/W emulsion technique. Characterization and cytotoxic tests indicated great stability, permeability, and functionality in lotion emulsion and inhibitory effect against *Candida albicans* and *Aspergillus niger* (Kim et al. [2021\)](#page-44-5).

Lastly, the agricultural sector is one of developing countries' most important economic sectors. Using soil microorganisms as biofertilizers for different agricultural products has grown daily. When interacting with plants, these bacteria stimulate plant growth and health through mechanisms such as nitrogen fxation and phytohormone production. In addition, antagonistic bacteria play an essential role in the biocontrol of pathogens by producing substances that inhibit the growth of other microorganisms. However, the fundamental challenge to the success of biocontrol is the survival of the antagonist bacteria and the provision of the necessary conditions for the production of inhibitors in the correct amount and the correct place (de Souza Vandenberghe et al. [2017\)](#page-41-7). Two crucial plant growth-promoting bacteria were encapsulated in silica and carbon nanotubes to work as a delivery system in pistachio micropropagation. In a comparison of the free bacteria, the nanoformulation with *Pseudomonas fuorescens* and *Bacillus subtilis* successfully enhanced root length and proliferation and phytohormone production by rhizobacteria after three days (Moradipour et al. [2019\)](#page-46-2).

3.2 Nanoencapsulation of Biomolecules from Yeasts

Yeasts are eukaryotic, unicellular microorganisms classifed as members of the fungal kingdom. With their unicellular growth habit, yeasts can be contrasted with molds, which develop hyphae. Fungal species that can assume both forms (depending on temperature or other conditions) are called dimorphic fungi (Coradello and Tirelli [2021](#page-40-7)). The benefcial physiological properties of yeast have led to its use in biotechnology. The yeast species *Saccharomyces cerevisiae* converts carbohydrates into carbon dioxide and alcohol through fermentation. The products of this reaction have been used in baking and making alcoholic beverages for thousands of years. *S. cerevisiae* is also an important model organism in modern cell biology research and is one of the most studied eukaryotic microorganisms. In addition, yeast is used as an ingredient in foods for its umami favor, which contains free glutamic acid (Hittinger et al. [2018\)](#page-43-6). Other yeast species, such as *Candida albicans,* are opportunistic pathogens and can cause human infections. Some yeasts may fnd potential applications in the feld of bioremediation. Yeasts such as *Yarrowia lipolytica* are known to degrade hydrocarbon contaminants such as alkanes, fatty acids, fats, and oils and have been investigated for their potential as a heavy metal biosorbent (Saeed et al. [2022\)](#page-48-0). Yeasts are also used as probiotic supplements to maintain and restore the natural microbiota of the gastrointestinal tract (Tamang and Lama [2022\)](#page-49-4). In this way, for all these applications, it is vital to improve yeast bioavailability and reduce its degradation during storage to develop effcient nutraceutical additives and biocontrol agents based on fungi cells or their products. The results presented here are summarized in Table [2](#page-18-0).

Like bacteria, yeasts can also face the same threats to survive during food processing and passage through the human GI system. Some studies with this purpose include *Sacharomycopsis fibugera* encapsulated in electrospun wheat bran fber and exopolysaccharide combined with polyvinylpyrrolidone (Ragavan and Das [2020\)](#page-47-9); *Pichia barkeri, Yarrowia lipolytica, Wickerhamomyces anomalus,* and *Saccharomyces cerevisiae* in sodium alginate nanoparticles or combined with chitosan or starch (Suvarna et al. [2018\)](#page-49-5); *Saccharomyces boulardii* extruded in calcium alginate nanocarriers (Morales-Amparano et al. [2019](#page-46-3)); and *Kluyveromyces lactis* in a gelatin hydrogel complexed with graphene oxide and glutaraldehyde (Patarroyo et al. [2021\)](#page-46-4).

Alginate is an anionic polysaccharide mainly found in the cell wall of brown algae, which includes two copolymers, guluronic acid, and mannuronic acid. The use of alginate hydrogels for biomolecule delivery is being widely used (Angra et al. [2021](#page-39-8)). Alginate-encapsulated dextranase exhibited maximum stability and activity in toothpaste for three months. Dextranase, generally extracted from *Chaetomium gracile* or *Penicillium* spp., is GRAS in cosmetics and drug formulations for oral care products because it has excellent antibioflm activity. Alginate beads were able to protect dextranase activity from harsh conditions, offer a higher release in toothpaste during brushing, and improve stability after long-term storage (Juntarachot et al. [2020](#page-43-7)). In Nguyen et al., sodium alginate and β-lactoglobulin were used to formulate a nanocapsule loaded with *Saccharomyces cerevisiae* by the layer-by-layer method. This technique facilitates the survival of the yeast since it avoids signifcant chemical changes in the cell caused by dehydration at the high processing temperatures of some food products (Nguyen et al. [2020\)](#page-46-5).

Saccharomyces cerevisiae is a unicellular eukaryotic organism that belongs to the Fungi kingdom. It is the yeast used in the production of bread and beer, in addition to being used for the production of fuel alcohol. In the case of fermented alcoholic beverages, *Saccharomyces cerevisiae* converts sugar into ethyl alcohol and can also contribute to the formation of secondary constituents responsible for favor, as is the case with beer, rum, and whiskey (Vanderwaeren et al. [2022](#page-50-3)). This way, microencapsulated *S. cerevisiae* was used to promote a continuous fermentation process on a green beer. Chitosan-calcium alginate double-layer microcapsules reached 91% of encapsulation efficiency. They maintained physicochemical parameters such as pH, color, alcohol content, and bitterness, demonstrating an excellent approach for beer production (Benucci et al. [2021\)](#page-39-9). Similarly, the same formulation was made to produce a sparkling wine, which displayed increased pressure and oxygen consumption compared to the free yeast. After six months, few differences in sensory properties were observed, similar to those produced with free cells (Benucci et al. [2019](#page-39-10)).

Yeast	Encapsulation system	Encapsulation method	Application	References
Saccharomycopsis fibuligera	Wheat bran fiber or exopolysaccharide + polyvinylpyrrolidone (PVP)	Electrospinning	Probiotic delivery	Ragavan and Das (2020)
Pichia barkeri; Yarrowia lipolytica; Wickerhamomyces anomalus: Saccharomyces cerevisiae;	Sodium alginateSodium alginate $+$ chitosanSodium alginate + starch	ExtrusionExtrusion + emulsification	Probiotic delivery	Suvarna et al. (2018)
Unknown yeasts	Polyacrylamide nanofiber	Electrospinning	Immobilized efficiency	Fan et al. (2021)
Penicillium roquefortii	Alginate	Encapsulator	Dextranase production in toothpaste	Juntarachot et al. (2020)
Trichoderma harzianum	Nanocellulose and/or Carboxymethylcellulose		Biocontrol activity	Brondi et al. (2022)
Yarrowia lipolytica	Organogel	Photopolymerization	Stability and physicochemical properties	Zhang et al. (2022)
Unknown yeast	Silica	Boron hydroxide click reaction	Cell protection and stability	Geng et al. (2019)
Saccharomyces cerevisiae	B-lactoblobulin Sodium alginate	Layer by Layer	Cell protection and stability	Nguyen et al. (2020)
Saccharomyces cerevisiae	Chitosan-calcium alginate	Microencapsulation	Production of sparkling wine	Benucci et al. (2019)
Saccharomyces boulardii	Calcium alginate	Extrusion	Probiotic delivery	Morales- Amparano et al. (2019)
Saccharomyces pastorianus	Calcium alginate	Encapsulator	Biosorption performance	Rusu et al., (2022) ; Webster et al. (2022)
IM7-displaying yeast cells	Calcium alginate	Crosslink reaction	Protein purification	Yin et al. (2022)
Saccharomyces cerevisiae	Chitosan-calcium alginate	Encapsulator	Optimization of bear production	Benucci et al. (2021)
Meyerozyma caribbica	Resistant maltodextrin	Electrospraying	Biocontrol activity	Aguirre- Güitrón et al. (2022)
Kluyveromyces lactis	Gelatin hydrogel + graphene oxide + glutaraldehyde	$\overline{}$	Probiotics and bioreactor packings	Patarroyo et al. (2021)

Table 2 Nanoencapsulation of fungi products and its application

Despite their relevance as a eukaryotic model organism for medical and biotechnological applications, the potential use of antagonistic yeasts as biocontrol agents still needs to be explored. However, in addition to powerful antifungal activities, yeasts also show intense antagonistic activity, culture ability, formability, applicability, and stress resistance and are therefore promising for developing biological plant protection agents. In addition, because they are extensively studied organisms, it is possible to take advantage of the molecular tools and the infnity of data developed for these organisms for basic and application-oriented studies in biocontrol yeasts (Freimoser et al. [2019](#page-41-9)). Aguirre-Guitrón et al. encapsulated *Meyerozyma caribbica* cells in whey protein processed with resistant maltodextrin to act as a biocontrol nanomaterial against *Colletotrichum gloeosporioides*. The electrospraying process showed effciency in increasing cell viability and stability in storage at 4 °C, as well as a great antagonist effect (Aguirre-Güitrón et al. [2022\)](#page-39-11).

Still, in the environmental feld, persistent organic pollutants in different environmental matrices are a primary concern worldwide. Over the past two decades, pharmaceuticals have been detected in surface water, seawater, groundwater, drinking water, and effuent from sewage treatment plants. To avoid the negative impact of pharmaceutical products, it is necessary to develop technology for their complete removal from wastewater before being discarded in the environment. Although these conventional procedures have exciting characteristics such as effciency, sustainability, and cost, they cannot eliminate pharmaceuticals from water (Brazesh et al. [2021\)](#page-40-9). Biosorption using natural polymers as support for biomass can represent an alternative to these methods because they are effcient, cheap, nontoxic, and readily available. The ability of microorganisms to remove pharmaceutical products from aqueous solutions has been studied and shows limited effciency due to their separation from effuents after treatment. In this sense, researchers developed a calcium-alginate matrix encapsulating *Saccharomyces pastorianus* cells, used as a biosorption microorganism for ethacridine lactate contamination. The authors tested the infuence of the main parameters on the biosorption process, and the best removal effciency obtained for ethacridine lactate was over 85%, demonstrating to be a great low-cost material for environmental treatment (Webster et al. [2022\)](#page-50-4).

Yeast cells can also act as encapsulating agents, particularly for fat- and watersoluble compounds. It is a simple and highly economical process. The species most used for encapsulation are *S. cerevisiae, Torulopsis lipoferrina, Saccharomyces bayanus, Endomyces vernalis,* and lactic yeasts such as *Candida utilis* and *Kluyveromyces fragilis* (Coradello and Tirelli [2021](#page-40-7)). In general, two parts of yeast cells can be used for encapsulation: the cell wall and the plasma membrane. The cell wall maintains the cell's shape, protects the cytoplasm from cell lysis, and surrounds certain enzymes that prevent unwanted activities. The plasma membrane is composed of two phospholipid chains with excessive steroids and neutral lipids. It has a liposome-like structure and makes the yeast cell suitable for use as a coating in encapsulation. These materials have been applied to encapsulate poorly soluble actives, such as favoring agents, antioxidants, and biocides, resulting in increased water dispersibility, thermo/oxidative stability, mechanical protection, and release control (Dadkhodazade et al. [2021\)](#page-41-10).

3.3 Nanoencapsulation of Bacteriophages

Bacteriophages are viruses that exclusively infect bacterial cells that have been known for over a century. They are harmless to all organisms, including humans, except their target bacterial hosts and are the leading ones responsible for infections in bacteria. They interact with bacterial cells that express specifc surface membrane receptors. However, suppose a bacterial cell does not have the specifc receptor for a bacteriophage on its surface. In that case, the bacteriophage cannot infect it, which is why it is a mechanism of very high specifcity. When a phage infects a bacterial cell, it replicates, and the new virions are released into the extracellular milieu and can infect other cells (Furfaro et al. [2018](#page-41-11)).

As a consequence, phages control bacterial population growth and, at the same time, contribute to moving genes from one cell to another. Their genomes encode proteins useful for biotechnological applications, including food safety, diagnostics, antibiotic therapy of infections caused by antimicrobial-resistant bacterial strains, DNA delivery vehicles, and many other relevant technologies. The study of bacteriophage particles provides information about the evolution of genomes, adaptive bacterial evolution, and the way DNA is expressed and copied, and potentially sheds light on the development of new biotechnological products (Harada et al. [2018](#page-42-6)).

However, these products need careful formulation development, and an assessment of the chemical and physical stresses bacteriophages may encounter during processing and storage. Phage inactivation and long-term reduction after storage are highly undesirable. Delivery of high levels of phages and their controlled release to the treatment site affects pharmacokinetics and treatment effciency. Phages are composed primarily of proteins and are therefore susceptible to factors known to denature proteins, such as exposure to organic solvents, high temperature, pH variations, ionic strength, and interfacial effects (Malik [2021\)](#page-45-4). Incorporating bacteriophages in therapeutic formulations usually involves encapsulation within a stabilizing substance. Through this approach, various antimicrobial materials can be produced, offering effective delivery to the site of infection and, consequently, better patient outcomes. Table [3](#page-21-0) summarizes all the results presented in this section (Rosner and Clark [2021\)](#page-48-10).

In Rahimzadeh et al., bacteriophages isolated from three different bacteria strains (*Salmonella enterica, Shigella fexneri,* and *Escherichia coli*) constituted a phage cocktail to be encapsulated in chitosan nanoparticles using the ion gelation method. The study aimed to use this novel nanocapsule to treat bacterial diarrhea in rats. The authors demonstrated that the encapsulation protected the phage from enzymes and stomach acid, thus effectively transporting it to the site of action. It prevented the rats from weight loss when submitted to gastrointestinal infection (Rahimzadeh et al. [2021\)](#page-47-10). In another study, PEV2 (*Podovirus*) and PEV40 (*Myovirus*), two types of *Pseudomonas* phages, were encapsulated in a liposome matrix of soy phosphatidylcholine and cholesterol by two different techniques. The authors compared the microfuidic and conventional thin flm hydration methods, followed by extrusion. PEV2-derived nanoparticles showed the smallest, and liposomes got the highest

	Encapsulation	Encapsulation	Bacteria		
Bacteriophage	system	method	target	Application	References
Phage cocktail	Chitosan	Ionic gelation	S. enterica, S. flexneri and $E.$ coli	Biocontrol agent	Rahimzadeh et al. (2021)
T ₃	W/O emulsion	Encapsulator	E. coli	Animal feed and biocontrol	Richards and Malik (2021)
Podovirus PEV ₂ Myovirus PEV ₄₀	Liposome	Thin-film hydration + extrusion and microfluidic	P. aeruginosa	Encapsulation analysis	Leung et al. (2018)
UFV-AREG1	Alginate and alginate $+$ chitosan. carrageenan or whey protein	Extrusion	E. coli	Encapsulation analysis	Silva Batalha et al. (2021)
ISP	Hydrogel	Emulsion	S. aureus	Bacteriophage therapy in rabbits	Onsea et al. (2021)
Paer4, Paer14, Paer ₂ , W2005A	Hydrogel	Crosslink reaction	P. aeruginosa	Bacteriophage therapy in mice	Wroe et al. (2020)
phiIPLA- RODI	Liposome	Encapsulator	S. aureus	Food industry	Menéndez et al. (2018); Webster et al. (2022)
S ₁	Alginate	Ionotropic gelation	Salmonella spp.	Bacteriophage therapy in chicken	Gomez- Garcia et al. (2021)
pAh-6C	PLGA/alginate	W/O/W double- emulsion	A. hydrophila	Bacteriophage therapy	Kim et al. (2022)
Podoviridae T7	PolyHIPE	W/O/W triple emulsion	E. coli	Bacteriophage therapy	Kopač et al. (2021)
ZCEC ₅	Chitosan- alginate	Extrusion	E. coli	Animal feed and biocontrol	Abdelsattar et al. (2019)

Table 3 Nanoencapsulation of bacteriophage and its application

encapsulation efficiency $(59\%$ and 50% , respectively) and minimal titer reduction with the microfuidic formulation (Leung et al. [2018](#page-44-7)). Gomez-Garcia et al. used ionotropic gelation to formulate alginate nanoparticles loaded with lytic bacteriophage S1 for *Salmonella enterica*. The phages were encapsulated at a rate of 70% and were protected from pH changes in the chicken (*Gallus gallus domesticus*) gastrointestinal system for phage therapy for 3 h (Gomez-Garcia et al. [2021\)](#page-42-7). Phageloaded alginate matrices were used in PLGA microspheres for application in bacteriophage therapy against resistant pathogens. The encapsulation ensured a higher bacteriophage concentration than the PLGA matrix alone and showed a diminished immune response. The controlled release of the infective particles was extended for 60 days in vitro and 28 days in vivo after the W/O/W double emulsion (Kim et al. [2022\)](#page-44-8). The same technique was used to formulate nanocellulose-based hydrogels with lytic T7 bacteriophage isolated from *E. coli*. The encapsulation acted as a mechanical protection and a fast phage delivery system, providing improved patient compliance and reducing drug administration frequency. Finally, Abdelsattar et al. formulated *E. coli* phage ZCEC5 chitosan-alginate nanoparticles by extrusion for the delivery system in oral administration to farm animals (Kopač et al. [2021\)](#page-44-9). The capsules demonstrated an effcient protective effect against pH changes and sustained particle release and lysis activity for an extended period, proving to be an excellent application in the animal feed industry (Abdelsattar et al. [2019\)](#page-38-1).

4 Nanoencapsulation of Biomolecules from Plants

The secondary metabolism of plants is directly related to the primary metabolism, which is responsible for plant growth and development, energy production, and production of small molecules that directly affect secondary metabolism, photosynthesis, citric acid pathway, and other pathways (Xu et al. [2021](#page-51-7)). Although most plant-derived bioactive compounds are produced as part of their secondary metabolism, they can also be found in leaves, fruits, stems, and roots. Bioactive compounds from the secondary metabolism of plants have been used for thousands of years due to their medicinal properties. Although its exact mechanism of action was unknown, its positive effects in promoting health and combating disease were noted. Bioactive compounds have a major disadvantage since they are susceptible to degradation when exposed to various factors such as oxygen, humidity, light, and heat, which compromise their bioactivity (Zambrano-Zaragoza et al. [2017](#page-51-8)). In this context, nano/microencapsulation techniques present an alternative to extend the useful life of compounds obtained through plant extracts (Hosseini and Jafari [2020](#page-43-8); Rahaiee et al. [2020\)](#page-47-11). Although encapsulation is advantageous for maintaining the integrity of the BCs, its functional characteristics will directly depend on the choice of materials used in the encapsulation, which need to be able to prevent the degradation of the BCs and, at the same time, maintain their properties without modifying them.

Bioactive compounds originating from plants are grouped, in general, into terpenes, nitrogen-containing compounds, and phenolic compounds (Montiel-Sánchez et al. [2021](#page-45-6); Maccelli et al. [2020](#page-45-7)). The methods and materials used in nano/microencapsulation, as well as the applications, characterizations, and limitations of the technique for each compound, will be discussed below.

Terpenes are one of the largest classes of inorganic compounds produced by plants. They are responsible for the taste, fragrance, and pigment of plants, being the major constituents of essential oils (Hosseini and Jafari [2020;](#page-43-8) Diniz et al. [2021\)](#page-41-12). Terpenes are widely known for their anti-infammatory effects and anticancer, insecticidal, fungicidal, bactericidal, and antiviral properties. However, their low water solubility and high susceptibility to oxidation limit their use (Diniz et al. [2021;](#page-41-12) Tunç and Koca [2019\)](#page-50-6).

The terpenes present in black pepper essential oil are volatile, and their properties can be reduced under certain conditions. Encapsulation can protect the EO and preserve its terpenes. Complex coacervation was chosen due to several advantages. Bastos et al. analyzed the composition of black pepper essential oil, determining the most suitable conditions for forming the complex between gelatin and sodium alginate. The primary terpene identifed in black pepper essential oil was β-caryophyllene, followed by limonene and sabinene. The ratio of 6:1 (gelatin/sodium alginate) at pH 4.0 was the ideal condition found by the authors for encapsulation. The encapsulation efficiency ranged from 49.13% to 82.36%, and the chemical composition of the encapsulated EO was identifed by gas chromatography. GC analysis indicated good core protection with the materials used. These biopolymers can serve as a potential delivery system for black pepper EO (Heckert Bastos et al. [2020\)](#page-42-8).

Terpenes can be effciently encapsulated within YPs by passive diffusion through porous cell walls, as YPDs are hollow, porous microspheres, a by-product of some yeast extract manufacturing processes. The frst generation YP terpene materials were developed with a <2:1 terpene:YP weight ratio. Soto et al. reported methods to increase terpene carrying capacity in YPs up to a 5:1 terpene:YP weight ratio. A mixture of geraniol, eugenol, and thymol (GET) previously used to develop YP-GET 1.1:1 was used as a model terpene composition to prepare hyperloaded YP-GET. Hypercharged YP terpenes extend payload release kinetics by up to three times compared to commercially available terpene YP formulations. The hypercharged YP-terpene compositions were optimized to achieve high terpene storage encapsulation stability from −20 °C to 54 °C. The development of hypercharged YP terpenes has a wide range of potential agricultural and pharmaceutical applications with terpenes and other compatible active substances that can beneft from a delivery system with high payload capacity combined with increased payload stability and sustained release properties (Soto et al. [2022](#page-49-7)).

Tackenberg et al. study aim to improve the understanding of a counter rotating twin screw extrusion process. Orange terpenes as model favor, maltodextrin, and sucrose as matrix materials, were encapsulated by extrusion, amorphous and partly crystalline samples were obtained. The loss of crystalline sucrose was linked to a dissolution process of the sugar in the available water amount. Melting of the excipients did not arise, resulting in a plasticization extrusion process. Maximally 67% of the favor was retained (corresponding to a 4.1% product favor load). The favor loss correlated with insufficient mixing during the process and flavor evaporation after extrusion. Based on these results, recommendations for an improved encapsulation process are given (Tackenberg et al. [2015\)](#page-49-8).

The encapsulation of essential oils from fruit juices and the effect of delivery on the antimicrobial activity of terpenes were investigated by Donsi et al. to increase the antimicrobial capacity and increase the quality of the fnal product. A terpene mixture and d-limonene were encapsulated into nanoemulsions based on foodgrade ingredients. The sunfower oil or essential oil-in-water nanoemulsions were prepared using a high-pressure homogenization (HPH) technique. Three different microorganisms were tested: *Lactobacillus delbrueckii*, *Saccharomyces cerevisiae*, and *Escherichia coli*. The increase in antimicrobial activity depended on the

formulation and average diameter of the delivery systems as well as on the microorganisms class. The nanocapsules with the greatest antimicrobial capacity were pear and orange juices inoculated with *L. delbrueckii*. Due to the higher antimicrobial activity of the nanoencapsulated compounds, lower antimicrobial concentrations are required for a bactericidal action under accelerated aging at 32 °C, with a minimal alteration of the organoleptic properties of the juice (Donsì et al. [2011](#page-41-13)).

Propolis extract is a bioactive compound with several properties and potent pharmacological effcacy. Casein-maltodextrin nanocomplexes loading propolis was successfully synthesized by Soleimanifard et al. The characterization of the resulting nanocomplexes was carried out by monitoring the average size, polydispersity index (PDI), zeta potential, encapsulation efficiency (EE), Fourier transform infrared spectroscopy (FT-IR), X-ray diffraction (XRD), color properties, and morphology. FT-IR and XRD analysis showed that propolis extract had been located adequately inside nanoparticles. Most of the particles were between 500 and 3800 nm: particle size higher than 1000 nm could be due to particle aggregation or an increase in the amount of larger molecules of sodium casein. The results of DLS and EE showed that smaller nanoparticles with less polydispersity index and less coral material/encapsulant amount had better size distribution and stability. The authors demonstrated that the encapsulated propolis extract could be used in several applications by the pharmaceutical industry (Soleimanifard et al. [2021](#page-49-9)).

The use of essential oils from *Origanum glandulosum* Desf. has been used as an alternative to antibiotics due to the various bioactive compounds present. To overcome the drawbacks of using oils as the hydrophobicity and negative interaction with the environmental conditions, in addition to increasing their activity, encapsulation for the oil was performed using high-speed homogenization (HSH) into nanocapsules and high-pressure homogenization (HPH) into nanoemulsion (Bouaouina et al. [2022](#page-40-10)). The antimicrobial activity of the essential oil, nanoemulsion, and nanoencapsulation were tested against *E. coli, S. aureus*, and *A. baumannii*. Nine components were identifed in the essential oil, thymol (48.52%), carvacrol (16.13%), p-cymene (27.56%), and γ-terpinene (5.59%) were the predominates. A considerable change in composition was observed in oil nanocapsules concerning the essential oil. The mean particle size of the nanoemulsion was 54.24 nm, while that of nanocapsules was 120.60 nm. The antimicrobial activity assays demonstrated that the nanocapsules were more effective than the nanoemulsion, but both showed lower effectiveness in relation to the essential oil. The nanoencapsulation using intensive-energy techniques is responsible for the changes in aroma profles discussed above and, consequently, for the antibacterial activities. Both formulations have shown relatively signifcant action against bioflm state at subinhibitory concentrations, where nanoemulsion was more potent than nanocapsules due to the higher thymol concentration.

Betalains are the leading group of compounds containing hydrogen atoms found in fruits. They are water-soluble and responsible for the fruits' color, which varies between red and yellow (Montiel-Sánchez et al. [2021\)](#page-45-6). To develop new products and applications, recent studies have been carried out to explore new alternatives for optimizing bioactive compounds. The antioxidant capacity, elimination of free

radicals and reactive oxygen species, inhibition of lipid peroxidation, and antiinfammatory and antimicrobial activities are among the biological properties of interest (Coy-Barrera [2020;](#page-40-11) Yao et al. [2020b\)](#page-51-9).

To encapsulate caffeine, a highlighted compound of the alkaloid family, the ideal conditions for manufacturing chitosan-coated liposomes were investigated. The morphological properties of the developed nanochitosan were investigated by FESEM, TEM, and AFM analyses. FT-IR analysis was also conducted to evaluate the possible interaction between chitosan and caffeine (Seyedabadi et al. [2021\)](#page-49-10). Nanoliposomes were synthesized by dissolving in deionized water and heating at 50 °C. 0.09 g lecithin, 0.01 g cholesterol, and 0.02 g Tween® 80 were dissolved in absolute ethanol using a magnetic stirrer and dropped into the aqueous solution containing caffeine. The prepared solution was sonicated for 20 min (1 s on and 1 s off) at different ultrasonication power levels. Morphology evaluation revealed the formation of uniform spherical nanoliposomes with a size of 100 nm. Furthermore, the zeta potential of the sample was 31.9 mV after adding chitosan and −25.5 mV before chitosan addition. The FTIR analysis demonstrated that the inclusion of caffeine took place at the polar sites of phosphatidylcholine of the nanostructures, which are present on the internal surface of nanoliposomes. The increase in sonication power resulted in increased stability of chitosomes due to the size reduction of the developed chitosomes and homogenous distribution of the nanoparticles in the solution. It was demonstrated that chitosan nanoliposomes efficiently encapsulate caffeine and could be used in the pharmaceutical and food sectors.

The effects of microencapsulation of phenolic components obtained from grape pomace extract were investigated by Tolun et al. (Tolun et al. [2020](#page-50-7)). The polyphenols obtained from grape pomace were encapsulated using the spray drying technique. After manufacturing the microcapsules, they were stored in two different humidity conditions (33% and 52%) for 75 days. Analyzes were performed every 15 days to measure total phenolic content, antioxidant activity, and individual phenolic compounds. The combination of maltodextrin and gum arabic as a microencapsulating material resulted in improved stability of the polyphenols when compared to the microcapsules obtained only with maltodextrin. The most promising results were obtained with encapsulation with maltodextrin DE4-7 prepared by adding gum arabic to the material in a ratio of 8:2. Stable polyphenols microencapsulated have great importance for several areas like the food industry to contribute to human health.

Curcumin is a phenolic component found in the turmeric plant, the best known of which is *C. longa*, native to India and tropical Asia. In addition to being widely used as a food spice, turmeric is also known for its orange and yellow dye. Due to its pharmaceutical properties, it has been used for thousands of years to treat common ailments such as arthritis, wounds, acne, digestive tract problems, and infections. Such functional properties have been mainly related to the unique active compounds called curcuminoids in the rhizome, the most important of which is curcumin (Maheshwari et al. [2006](#page-45-8)).

5 Nanoencapsulation of Biomolecules from Marine and Freshwater Organisms

Of the total water available on the planet, 97% is in the seas and oceans, and only 3% is fresh water. Of that small percentage, just over 2% is in glaciers, so less than 1% is available for consumption. The seas and oceans make up approximately 70% of the coverage of the entire planet Earth, which is the largest natural habitat on Earth (Grosberg et al. [2012](#page-42-9)). Marine and freshwater ecosystems represent a high and complex biological and chemical diversity, being a potential carrier of biomolecules of interest for developing new technologies, especially in the health feld (Irfan and Alatawi [2019](#page-43-9)). Exploring the potential of aquatic habitats began with the cultivation of algae, sponges, and corals, later expanding to the study of cyanobacteria and fungi and, at the latest, to large organisms such as fsh, crustaceans, and some aquatic mammals (Fig. [2\)](#page-26-0). Through these pioneering studies, it was observed that these organisms are unique sources of unique bioactive compounds. Among these biomolecules, polyunsaturated fatty acids (PUFAs), polysaccharides, minerals and vitamins, enzymes, and bioactive peptides stand out (Nova et al. [2020\)](#page-46-7). Currently, these compounds are being widely studied, as in many cases, they have various molecular targets, which may be potential biomolecules for developing essential drugs.

5.1 Nanoencapsulation of Biomolecules from Algae

Algae are critical aquatic organisms in both marine and freshwater ecosystems. An infnity of algae is cataloged and subdivided into macro- or microalgae, differing by their cellular structure and the number of cells that form them. Macroalgae are

Aquatic Environmental

Fig. 2 Aquatic environment and its primary sources of biomolecules

eukaryotic and multicellular organisms that do not have the specialized structures and forms of reproduction of true plants (Menaa et al. [2020](#page-45-9)). The different photosynthetic pigments can distinguish them in their cells (green, brown, and red). Microalgae are unicellular/multicellular eukaryotic algae (green algae) or prokaryotic (cyanobacteria), the main base of the aquatic trophic chain. They are rich in bioactive compounds such as carbohydrates, proteins, minerals, polyunsaturated fatty acids, fatty acids, amines, amides, antioxidants, and pigments such as carotenoids, chlorophylls, carotene, xanthophylls, and phycobilins (Harwood [2019](#page-42-10)). The production of these compounds is totally infuenced by the algae species and by the cultivation conditions (availability of nutrients, temperature, pH, salinity, luminosity, etc.) (Menaa et al. [2021\)](#page-45-10). This way, metabolites and extracts from algae are potential biomolecules for their encapsulation and subsequent use with drugs, stabilizers, and food supplements. The results presented below are summarized in Table [4](#page-28-0).

Commercial algal oils rich in PUFAs, such as docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), have been widely encapsulated to improve their stability. Wang et al. encapsulated commercial algal oils in different proportions through the microfuidization method, using stearic acid as a solid lipid and poloxamer 188 as a surfactant (Wang et al. [2014](#page-50-8)). The EE obtained in all proportions was greater than 88%, with a maximum loading capacity of approximately 18%, stable between 4 °C and 25 °C and at acidic pH. In another work, Prieto et al. analyzed the effect of whey protein purity on the encapsulation of commercial seaweed oils using the electrospraying method assisted by pressurized gas (Prieto et al. [2022](#page-47-12)). It was found that the whey protein's purity interferes with the encapsulated material's morphology, which is refected in its oxidative stability. Chen et al. investigated nanoemulsions obtained by ultrasound using commercial seaweed oil with phytosterols stabilized by quillaja saponin to reduce the number of oxidized compounds from the oil that cause unpleasant odors to food (Chen et al. [2016](#page-40-12)). An excellent reconstruction of these nanoemulsions was observed 30 days after being dried and sprayed. In addition, compared to pure oil, there was a decrease in oxidized compounds that cause unpleasant odors, revealing that the nanoemulsion decreases the oxidation of this oil.

As previously mentioned, algae have a very rich composition of biomolecules, making their extract possess unique nutritional and pharmacological properties. The in vitro phytochemical release profles of *Jania rubens* extract encapsulated with chitosan were analyzed using the ionic gelation method (Maghraby et al. [2022](#page-45-11)). A high EE was obtained for these nanoparticles (99.7%), observing that the release of the extract occurred in a controlled manner, maintaining its high antioxidant power. In another work, the same authors observed that the same extract encapsulated with chitosan and tripolyphosphate by the ionic gelation method considerably reduces the rancidity of vegetable oils, making this extract a natural alternative to the use of synthetic antioxidants (Maghraby et al. [2021\)](#page-45-12). *Sargassum boveanum* extracts were encapsulated in lecithin using the Mozafari method and used as antimicrobial agents in mayonnaise formulations (Savaghebi et al. [2021](#page-48-12)). It was observed that the encapsulated extract delayed the lipid oxidation of mayonnaise and considerably reduced

(continued)

the number of microbial colonies without deteriorating the product's sensory properties, extending its shelf life by up to 4 months. Chitosan nanoparticles loaded with *Ulva ohnoi* extract were obtained through the ionotropic gelation method, producing active nanocarriers to be used against *S. senegalensis* macrophages (Fernández-Díaz et al. [2017\)](#page-41-14). A drug delivery system was created using magnetic nanoparticles and gum arabic as carriers of *Dunaliella salina* extract (Zamani et al. [2019](#page-51-10)). For this system, an EE greater than 91% was observed with a minimum load capacity greater than 79%. This system's antioxidant and cytotoxic analysis was evaluated in MCF-7 and HeLa cells, indicating a high antioxidant and anticancer effect, time-dependent, and dose-dependent. Polypeptides extracted from *Chlorella pyrenoidosa* with antitumor action (HepG2 human liver cancer cells) were encapsulated through complex coacervation and ionotropic gelation methods using chitosan as encapsulating polymer (Wang and Zhang [2013\)](#page-50-9). The encapsulation effciency (EE) achieved was 74.5% and 30.1% for the complex coacervation and ionotropic gelation method, respectively, despite the polypeptide content being approximately equal (12.7% and 12.3%, respectively). Both encapsulations showed good preservation of gastric enzymatic degradation against in vitro release tests of these polypeptides.

Specifc biomolecules from algae are also of extreme interest when it comes to pharmacological control and development (Takaichi [2013](#page-49-12)) Carotenoids are important liposoluble pigments in bacteria, algae, fungi, and vegetables responsible for the orange, yellow, and red colors (Christaki et al. [2013\)](#page-40-13). Photosynthetic organisms participate as coadjuvants in the photosynthesis process and help to protect against possible damage caused by light (Allen et al. [1964](#page-39-12)). The main applications of carotenoids involve their antioxidant and anti-infammatory properties. Chaari et al. extracted carotenoids from *Archaea halophilic,* encapsulating them in oil-in-water dispersions to increase their water solubility and use them as a functional food (Chaari et al. [2018\)](#page-40-0). The encapsulation processes used were high-pressure homogenization and spontaneous microemulsion, using limonene with oil phase, Triton X-100/Tween-80 mixtures as emulsifers, and water/glycerol solutions. The oxidizing activity of this compound was evaluated by radical scavenging using Electron Paramagnetic Resonance Spectroscopy (EPR) in the stable free radical scavenging Tempol.

Among the carotenoids, a biomolecule of interest is astaxanthin (ATX). ATX is one of the few carotenoids that does not convert to Vitamin A in the human body, a powerful antioxidant (Dose et al. [2016\)](#page-41-15). Due to its antioxidant properties, it has numerous health benefts, especially in the immune system (Anarjan et al. [2010\)](#page-39-13). The primary sources of ATX come from marine organisms such as algae, krill, and shrimp. Salatti-Dorado et al. encapsulated ATX from *Haematococcus pluvialis* using a combination of molecular solvents and the hot homogenization method (Salatti-Dorado et al. [2019\)](#page-48-13). The EE obtained from this process was at least 58%, with a loading capacity of 19%, depending on the surfactant and solid phase used. The oxidizing capacity of this new type of encapsulation was superior to Trolox e and α -Tocopherol standards, in addition to protecting human endothelia from attack by reactive oxygen species (ROS). Similar studies used a combination of DNA and chitosan for the effcient encapsulation of ATX (Wang et al. [2017\)](#page-50-11). These particles showed good cytoprotective effects against oxidative cell damage and high effciency in eliminating ROS, being quickly endocytosed by Caco-2 cells.

The infuence of the aqueous phase to encapsulate ATX nanoemulsions was evaluated by Khalid et al. using the high-pressure homogenization method (Khalid et al. [2017\)](#page-43-10). Lecithin and sodium caseinate were used as aqueous phases, showing that when lecithin is used, stability at different pHs and temperatures is greater, in addition to its bioavailability. In another work, the infuence of the encapsulated polymer was evaluated against EE and its thermal degradation (Tachaprutinun et al. [2009\)](#page-49-11). Poly(ethylene oxide)-4-methoxycinnamoylphthaloylchitosan (PCPLC), poly(vinylalcohol-co-vinyl-4-methoxycinnamate) (PB4), and ethylcellulose (EC) were used as encapsulating agents, showing that only PCPLC presents satisfactory EE (98%) and loaning capacity (40%). The supercritical antisolvent (SAS) process was effcient when encapsulating ATX using poly (l-lactic acid) (PLLA), with EE of 91.5% and stability gain during storage (Liu et al. [2019b\)](#page-44-10). A process similar to SAS is used for the encapsulation of ATX using poly(lactic-co-glycolic acid) (PLGA) nanoparticles coated with chitosan oligosaccharides, and EE greater than 85% and loading capacity greater than 15% are obtained (Liu et al. [2019a](#page-44-11)). Potato protein also proves to be an effcient way to increase the oral bioavailability of ATX when encapsulated, presenting a nonallergenic and vegan way to encapsulate lipophilic bioactive (Abuhassira-Cohen et al. [2020\)](#page-38-2). The process of enhanced dispersion in solution by supercritical fuids (SEDS) also appears as an effcient alternative for the encapsulation of ATX (Kaga et al. [2018](#page-43-11); Tirado et al. [2019\)](#page-50-10). The pressure and temperature parameters used in SEDS are fundamental since they impact the bioavailability and antioxidant activity of the fnal material.

Another marine carotenoid from brown algae and diatoms is fucoxanthin (FXT) (Nomura et al. [1997](#page-46-11)). FXT is one of the most abundant carotenoids and is characterized as an orange pigment (Shimoda et al. [2010](#page-49-13)). The main potential healthpromoting effects of FXT are associated with its antioxidant, anti-infammatory, antitumor, antiobesity, and antidiabetic effects (Peng et al. [2011](#page-47-14)). This way, encapsulating it to protect its properties and increase its bioavailability and storage time is essential for this compound to be used as a nutraceutical additive or in developing new drugs. Indrawati et al. encapsulated carotenoids (mostly FXT) from *Sargassum* sp., using maltodextrin and Tween-80 as encapsulating agents (Indrawati et al. [2015\)](#page-43-12). EE values greater than 88% were obtained, showing that the Freeze-Drying method effectively encapsulates these carotenoids. Koo et al. encapsulated FXT extracted from *Phaeodactylum tricornutum* using alginate, casein, and chitosan through the ionic gelation method, achieving EE greater than 78% (Koo et al. [2023\)](#page-44-13). It was observed that during simulated gastrointestinal digestion, there was a controlled release of FXT and improvement in the permeability of Caco-2/TC7 cells. In addition, an increase in FTX metabolites was observed by analyzing the plasma of mice after oral ingestion. Chitosan nanogels with glycolipids also proved effective in increasing FXT bioavailability and PPARγ expression (Ravi and Baskaran [2017\)](#page-48-14).

There is currently a growing interest in nutraceutical supplements derived from *Spirulina*. *Spirulina* is a microalgae known to be a superfood, consisting of carbohydrates, lipids, and between 50% and 70% proteins (Stejskal et al. [2020\)](#page-49-14). In addition, it is rich in fatty acids, vitamins, and some pigments such as phycobiliproteins (Fernández-Rojas et al. [2014\)](#page-41-16). C-phycocyanin has been widely studied among phycobiliproteins due to its antioxidant, antitumor, anti-infammatory, and COX-2 enzyme inhibitor properties (Eriksen [2008](#page-41-17)). C-phycocyanin is an important blue pigment, and with carotenoids, it is one of the most studied pigments from algae. Encapsulation of *Spirulina* protein extract with chitosan nanoparticles was carried out by Karimzadeh et al., reaching a maximum EE of 67% and a loading capacity of 14% (Karimzadeh et al. [2023](#page-43-13)). These particles reduced microbial contamination in the adequate storage of fsh. Liposomes with phycobiliproteins extracted from *Gracilaria gracilis* were obtained using soy lecithin as an encapsulating agent (Haghdoost et al. [2022\)](#page-42-11). The results of this work show that the EE obtained was approximately 84%, providing less lipid oxidation and microbial deterioration in the storage of carp burgers. Liposomes of rice and soy lecithins with *Spirulina* phenolic extracts were obtained (with EE greater than 88%) and evaluated against the controlled release of these extracts in a dynamic gastrointestinal system, getting satisfactory results (Machado et al. [2019\)](#page-45-13).

Ultrafne fbers with antioxidant activity from *Spirulina sp.* LEB 18 was obtained from the electrospinning method, aiming to obtain smart packages (Moreira et al. [2019\)](#page-46-8).

In another work, polyvinyl alcohol (PVA) and polyethylene oxide (PEO) fbers containing phycocyanin were obtained using electrospinning (Moreira et al. [2018\)](#page-46-9). The fbers were used as pH sensors to be used in smart packaging since phycocyanin is a natural visible pH sensor. Fast-dissolving fbers were also obtained by electrospinning using gelatin and *Spirulina* protein extract, evaluating the DPPH and ABTS radical scavenging activity (Mosayebi et al. [2022](#page-46-10)). The electrospray technique is also effective for obtaining particles with encapsulated phycocyanin. Schmatz et al. used PVA to encapsulate commercial phycocyanin through the electrospraying technique with EE above 75%, in addition to high thermal resistance and maintenance of its antioxidant activity (Schmatz et al. [2020](#page-49-0)).

Spirulina encapsulated materials have shown high potential to be used in developing new antitumor drugs. Wen et al. observed a signifcant antiproliferative effect on human colon cancer cells HCT116, indicating that electrospinning fbers based on polysaccharides, prebiotics, and phycocyanin have the potential to be used as antitumor agents (Wen et al. [2020](#page-50-12)). The antitumor activity of the Y2 polypeptide extracted from *Spirulina platensis* and encapsulated with chitosan was evaluated in human breast cancer MCF-7 cells and liver cancer HepG2 (Zhang and Zhang [2013\)](#page-51-11). The EE obtained for this material was 49% with a loading capacity of 15%, with an IC_{50} for both cancer cells of 61 mg/mL.

Fucoidan is a term used to defne heterofucan-type polysaccharides that contain less than 90% L-fucose from brown algae (Yoo et al. [2019\)](#page-51-12). This polysaccharide has been extensively studied due to its varied pharmacological activities such as anticoagulant, antiviral, antitumor, antithrombotic, etc. (Luthuli et al. [2019](#page-44-14)). Qadir et al. obtained fucoidan nanoliposomes encapsulated with lecithin to investigate its antitumor and immunomodulatory activity (Qadir et al. [2008](#page-47-13)). It was found that after the particles were uptake by the cells, the antitumor activity increased by up to 10%

compared to free fucoidan. In another work, fucoidan was encapsulated with protamine, showing an inhibitory effect against metastatic breast cancer cells (Lu et al. [2017\)](#page-44-12).

5.2 Nanoencapsulation of Biomolecules from Fishes and Krill

In the last decade, several works have been developed on the effects of diets supplemented with fsh oil as a source of omega-3 PUFAs in preventing several diseases, especially for the treatment of cardiovascular diseases (Sargent [1997\)](#page-48-15). The fatty acids that form omega 3 are DHA and EPA, have numerous critical pharmaceutical properties, such as antiarrhythmic, antithrombotic, antiatherosclerotic, antiinfammatory, help to reduce blood pressure and decrease the concentration of triglycerides, etc. (Yetiv [1988](#page-51-13); Harris [2004\)](#page-42-12). In this way, improving its bioavailability and reducing its degradation during storage are extremely important for the development of nutraceutical additives and drugs based on fsh oil. The results presented below are summarized in Table [5](#page-35-0).

One of the most used techniques for fsh oil encapsulation is electrospinning since it has high reproducibility and high EE rates. Zein is a polymer widely used to encapsulate fsh oil by electrospinning. Yang et al. encapsulated fsh oil and ferulic acid with zein using a small portion of glycerol in the encapsulating phase, obtaining EE of 94% and a loading capacity of 20% (Yang et al. [2017\)](#page-51-14). In this case, ferulic acid decreased the oxidation of fsh oil without changing its bioavailability. Alcoholic zein solutions can also reduce fsh oil oxidation and increase the EE of electrospinning fbers (Moomand and Lim [2014](#page-46-12)). Torres-Giner et al. produced DHA capsules encapsulated with zein, achieving better results against its degradation in confned environments (sealed packaging situation) (Torres-Giner et al. [2010\)](#page-50-13). Cod liver oil nanofbers encapsulated with PVA were obtained from this technique with EE greater than 92% and a loading capacity of 11%. However, PVA encapsulation did not protect the fsh oil from oxidation, with a higher content of oxidized products than pure oil (García-Moreno et al. [2016](#page-41-18)). Kafrin and mixtures of whey protein with carbohydrates also proved to be good encapsulating biovectors of fsh oil by electrospinning, with an EE above 94% (García-Moreno et al. [2018;](#page-42-13) Cetinkaya et al. [2021\)](#page-40-14).

Drying techniques can also produce stable fsh oil encapsulations. Berjrapha et al. compared the stability of fish oil in poly- ϵ -caprolactone (PLCPL) nanocapsules obtained by the emulsion-diffusion method, followed by vacuum freeze drying (VFD) and conventional freeze drying (CFD) (Bejrapha et al. [2010\)](#page-39-14). The sample obtained by CFD showed higher EE, in addition to having greater oxidative stability, since VFD can compromise the PLCPL membrane. Sage polyphenols appear as an alternative for stabilizers of the encapsulating agent's sodium caseinate and gum arabic in the encapsulation of sardine fsh oil by spray-drying, presenting the fnal nanoencapsulated product with high EE, high thermal resistance, and low oxidation (Binsi et al. [2017\)](#page-40-15). Peptides from *Sardina pilchardus* and *Trachurus mediterraneus*

Source	Encapsulated compound	Encapsulation method	Encapsulation system	Application	References
Commercial cod liver oil	\overline{a}	Electrospraying	Polyvinylalcohol	Stability	García-Moreno et al. (2016)
Commercial fish oil	÷,	Electrospraying	Zein	Stability	Yang et al. (2017)
Commercial fish oil	\equiv	Electrospraying	Zein	Stability	Moomand and Lim (2014)
Commercial fish oil	\overline{a}	Electrospraying	Kafirin	Stability	Cetinkaya et al. (2021)
Commercial fish oil	DHA	Electrospraying	Zein prolamin	Stability	Torres-Giner et al. (2010)
Commercial cod liver oil	\equiv	Electrospraying	Whey protein and carbohydrates	Stability	García-Moreno et al. (2018)
Menhaden	Fish oil	Freeze drying	Polycaprolactone	Stability	Bejrapha et al. (2010)
Sardinella longiceps	Fish oil	Spray drying	Sodium caseinate and gum arabic	Stability	Binsi et al. (2017)
Commercial fish oil	Omega-3	Gas-Saturated Solutions-dried	Modified starch	Stability	Melgosa et al. (2019)
Commercial fish oil	Omega-3	Microfluidization	Sunflower oil	Stability	Komaiko et al. (2016)
Commercial fish oil	DHA	Emulsification- solvent evaporation	Casein	Stability	Zimet et al. (2011)
Commercial fish oil	Omega-3	Low energy spontaneous emulsification	Tween 80	Stability	Walker et al. (2015)
Sparus aurata	Sea bream scales collagen	Emulsification- solvent evaporation	Soybean lecithin	Stability	Mosquera et al. (2014)
Menhaden	Fish oil	Nanoelmulsion	Palmitic acid and quercetin	Stability	Azizi et al. (2019)
Commercial fish oil	$\overline{}$	High intensity ultrasound method.	Tween 80 and span 80	Stability	Nejadmansouri et al. (2016)
Commercial fish oil	Omega-3	Hot high- pressure homogenization	Tristearin and lecithin	Stability	Salminen et al. (2013)
Engraulis encrasicolus L.	Omega-3 fatty acids	Controlled crystallization and kneading method	B-cyclodextrin	Stability	Ünlüsayin et al. (2016)

Table 5 Type of fish/krill, target biomolecule(s), encapsulation technique, encapsulating agent, size, morphology, and application of nanoencapsulated bioactives from fshes and krill

(continued)

Source	Encapsulated compound	Encapsulation method	Encapsulation system	Application	References
Clupeonella cultriventris caspia	Peptídeos	Electrospraying	Chitosan and polyvinylalcohol	Antioxidant	Hosseini et al. (2019)
Sardina pilchardus and Trachurus mediterraneus	Fish oil	Spray-drying	Glucose syrup	Antioxidant	Morales- Medina et al. (2016)
Oncorhynchus mykiss	Peptides	Liposome	Chitosan	Antioxidant	Ramezanzade et al. (2017)
Oncorhynchus mykiss	Fish oil	Liposome	1,2-dipalmitoyl- sn-glycero-3-- phosphocholine	Antioxidant	Hosseini et al. (2017)
Commercial fish oil	$\overline{}$	Liposome	Soy lecithin and sunflower oil	Food additive	Ghorbanzade et al. (2017)
	EPA and DHA	Ionic gelation	Sodium caseinate and gum arabic	Food additive	Ilyasoglu and EI(2014)
Commercial fish oil	$\overline{}$	Freeze drying	Gum arabic and tween 80	Food additive	Moghadam et al. (2019)
Commercial krill oil	L,	Nanostructured lipid carriers	Palm stearin acid and lecithin	Stability	Zhu et al. (2015)
Euphausia superba	$\overline{}$	Emulsion- electrostatic interaction method	Chitosan and tripolyphosphate	Food additive	Haider et al. (2017)
Curcumin	$\overline{}$	Reflux followed by thin drug-lipid film hydration method	Commercial krill oil	Antitumor	Ibrahim et al. (2018)

Table 5 (continued)

were also successfully encapsulated by spray-drying using different polymers with high cytocompatibility (Hosseini et al. [2019](#page-43-14); Morales-Medina et al. [2016](#page-46-15)). Obtaining omega 3 nanocapsules with modifed starch by the Gas-Saturated Solutions (PGSS) drying method showed EE above 95% and lowered susceptibility to oxidation (Melgosa et al. [2019](#page-45-14)).

Nanoemulsions obtained from microfuidization of fsh oil in water using sunflower phospholipids as an emulsifier were obtained by Komaiko et al., and they have a series of advantages over artifcial emulsifers, such as high oxidative stability and being hypoallergenic (Komaiko et al. [2016\)](#page-44-15). Surfactant concentration becomes a critical point in optimizing the encapsulation of fsh oil in emulsions, refecting on the emulsions' morphology, size, EE, and stability (Walker et al. [2015\)](#page-50-14). Zimet et al. took advantage of the self-assembly properties of casein to encapsulate DHA with high colloidal stability and bioactive conservation (Zimet et al. [2011\)](#page-51-15).

The emulsifcation-solvent evaporation obtained peptide nanoliposomes from sea bream scales encapsulated with soy lecithin (Mosquera et al. [2014](#page-46-13)). The

maintenance of antioxidant activity and ACE inhibitory activity remained constant for 8 days at low temperatures, preserving the biological activities of the extracted peptides. Oncorhynchus mykiss peptides were encapsulated in nanoliposomes with EE above 46%, depending on the polymer concentration used for encapsulation (Hosseini et al. [2017](#page-43-15); Ramezanzade et al. [2017\)](#page-47-15). To improve the properties of the encapsulated fsh oil, quercetin is an antioxidant additive that may help slow down the oxidation of fsh oil (Azizi et al. [2019\)](#page-39-15). Other important factors to be considered when designing new encapsulations are the composition of the encapsulating agent, the surfactant's nature, and the fsh oil's composition, which can abruptly alter the stability of nanoemulsions (Salminen et al. [2013](#page-48-16); Nejadmansouri et al. [2016;](#page-46-14) Ünlüsayin et al. [2016](#page-50-15)).

Due to their excellent nutraceutical properties, fsh oils are potent additives for fortifying foods. Ghorbanzade et al. used fsh oil nanoliposomes with lecithin and soybean oil to fortify yogurt (Ghorbanzade et al. [2017\)](#page-42-14). After 21 days of storage, the authors reported that liposome-fortifed yogurt had higher EPA and DHA content. Gum arabic as an encapsulating agent and tween 80 as an emulsifer were also used to encapsulate fsh oil by freeze drying to observe the viability of *Lactobacillus plantarum* in probiotic fermented milk (Moghadam et al. [2019](#page-45-15)). The results showed EE of 87% with increased probiotic bacterial viability and high content of EPA and DHA, which can be used as a food fortifer. Ilyasoglu et al. also used gum arabic to encapsulate EPA and DHA by ionic gelation to fortify fruit juice, achieving good nutritional values (Ilyasoglu and El [2014](#page-43-16)).

As an alternative source of omega 3 to fish, krill oil is gaining prominence, as 30% to 65% of the fatty acids in krill oil are in the form of phospholipids (Kolakowska et al. [1994\)](#page-44-16). This factor alters the bioavailability of krill oil, providing it with better bioavailability (Schuchardt et al. [2011\)](#page-49-15). Nanostructured lipid carriers containing high krill oil content were prepared using palm stearin as solid lipid and lecithin as the surfactant, with EE of 97% and a maximum loading capacity of 12%, aiming at their application in functional foods (Zhu et al. [2015](#page-51-16)). Haider et al. used chitosan and tripolyphosphate to encapsulate krill oil from *Euphausia superba*, obtaining a maximum EE of 59%, a loading capacity of 25%, and a high oxidant capacity (Haider et al. [2017\)](#page-42-15). Liposomes with krill oil enriched with curcumin also showed good EE and loading capacity values used with antitumor agents against A549 lung cancer cells (Ibrahim et al. [2018\)](#page-43-17).

6 Conclusions

Natural products generated from fungal, bacteria, plant, marine, and animal sources have a wide variety of applications with a high global impact. To preserve and maintain the natural molecules properties generated from these sources, it is necessary to apply robust techniques. Due to the diversity of properties that bioactive compounds present, different techniques can be used to guarantee protection and maintenance of the biomolecule, the performance of the selected matrix, and mainly the

bioavailability to exert the required action. Encapsulation is a viable alternative to protect active compounds against the deterioration of environmental conditions, maintaining their natural compounds. Many encapsulation methods can be used, whether physical or chemical, and their use is intrinsically linked to their application. Among them, we can highlight electrospinning methods and micelles' formation with several applications.

Each technique for forming encapsulated materials (nano or microencapsulation) depends on a series of factors that are related to the material to be protected, since the matrix-forming substance and the conditions in which the techniques will be applied are also fundamental in the process. In food, nanoencapsulation application can prolong postharvest shelf life. This process when applied to functional food ingredients can help increase their water solubility and/or dispersibility in foods and beverages, improving their bioavailability. Encapsulation imparts several benefts including improved thermal and chemical stability, preserves or masks favor, taste, or aroma, controlled and targeted release, and enhanced bioavailability. The biopreservative effect on food packaging can also be done by bacterial enzymes, organic acids, or bacteria.

Nanoencapsulation is a technique based on enclosing a bioactive compound in liquid, solid or gaseous states within a matrix or inert material for preserving the coated substance (food or favor molecules/ingredients). Nanoencapsulation strategies, including the possibility to deliver controlled natural compounds, synthetic molecules, or other actives (viruses) for the treatment of different human disease could revolutionize conventional medical and food science, pharmaceutical and food industry.

Acknowledgments M.A. was supported by the Margarita Salas postdoctoral contract MGS/2021/21 (UP2021-021) fnanced by the European Union-NextGenerationEU. F.F.A. The authors would also like to thank the fnancial aid from the Fundação de Amparo à Pesquisa do Estado de São Paulo—FAPESP (2013/07296-2, 2016/13423-5, 2018/13408-1, 2020/06732-7, 2023/00857-0, and 2022/06293-9), the Financiadora de Estudos e Projetos—FINEP, the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior—CAPES (Financial Code 001), and the Conselho Nacional de Desenvolvimento Científco e Tecnológico—CNPq (Grant no. 130268/2022-4 and 148751/2022-9).

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