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# Natural Probiotics and Nanomaterials: A New Functional Food

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## Abstract

Natural probiotics are functional foods with several biological properties and nutritional value inherent to their chemical composition and can play a potentially beneficial role in reducing the risk of chronic degenerative diseases. In order to improve the stability of these compounds, increase the encapsulating power, delay oxidation, increase their effectiveness, control their release and improve the bioavailability of their combination with nanomaterials is a potential tool in the food area enabling the development of new products with functional and nutraceutical characteristics. In addition, the study of nanomaterials in natural probiotics is rarely reported in the literature, being an area of paramount importance in the development of new functional foods. Therefore, in this chapter, a review of nanomaterials' use in natural probiotics will be addressed to specify their advantages and methodologies of preparation and characterization.

**Keywords:** natural probiotics, nanomaterials, new functional foods, nutraceutical characteristics, nanobiotechnology

## 1. Introduction

The consumer's interest in a healthier lifestyle has led to the development of foods that meet nutritional and health needs and that at the same time are attractive, tasty, and with good acceptance in the market. Products that support positive health effects or ingredients with these characteristics, claimed or proven, are called "emitted foods" [1].

The relationship between food and health is one of the keys to disease prevention and well-being promotion. As a result, industries have started to enrich foods with specific ingredients, differentiating them about the benefits offered to health compared to foods in their traditional forms [2–4].

In the present century, the scientific literature reports functional foods as allies in the treatment of obesity [5], prevention of cardiovascular diseases [1], plasma cholesterol balance [6], and cancer prevention [7]. Among functional foods, the

literature reports prebiotics (added with non-digestible fibers), fortified (with vitamins, omega-3), altered (removing harmful components), and probiotics [8].

According to Resolution No. 19, of April 30, 1999, on the claim of functional property of food, it is that related to the metabolic or physiological role that the nutrient or non-nutrient has in the growth, development, maintenance, and other normal functions of the human organism [9]. Among the functional compounds most investigated by science, we have probiotics, which according to RDC n° 241, of July 26, 2018, are defined as live microorganisms that confer benefits to the individual's health [10, 11].

The word probiotic has a Greek derivation in which it means “for the sake of life”, that term was first introduced by Lilly; Stillwel in 1965 to describe substances secreted by a microorganism, which stimulates the growth of another [12–14]. Fuller (1989) defined probiotics as a supplement composed of live microorganisms that benefit the host's health through the balance of the intestinal microbiota. The term probiotic can be complemented as a pure culture or composed of living microorganisms that supplied to man or animals benefit the host by stimulating the properties existing in the natural microbiota [15].

Probiotics, after ingested, must be able to survive the stress conditions present in the gastrointestinal tract, such as gastric juice, the presence of bile salts, and digestive enzymes, and maintain their viability and metabolic activity in the intestine to exert beneficial effects on the hosts. As for the technological challenges for the industrial production of cells, they must remain stable and viable at satisfactory levels throughout the product's validity period [16–18]. Based on this assumption, there is a recent and growing scientific interest in improving the stability, bioavailability, and shelf life of products with probiotic sources using nanotechnology as an enhancement technique, since nanostructured systems may be able to control stability, improve solubility, bioavailability, and controlling the release of bioactive compounds [19–21].

The reduction of materials to the nanoscale leads to new and exciting properties and the increase of the surface volume ratio, increasing its reactivity. This characteristic of nanoparticles has attracted commercial interest in the manufacture of nano-ingredients, supplements, and nutraceuticals. Several types of nanoparticles can be found in the literature, such as metallic, semiconductor, carbon-based, metallic, and polymeric oxides, which can be applied in various sectors, predominantly personal care, health care, and cosmetics. The benefits of nanotechnology in the food sector go through the entire food chain, starting from production to processing, transportation, security, storage, and delivery [22, 23]. Based on the above, we will cover in this chapter a review on the use of natural probiotics and nanomaterials, aiming to specify their advantages and methodologies of preparation and characterization.

## 2. Natural probiotics

Probiotics can be defined as food supplements that contain live microorganisms or microbial components that, when ingested in a certain number, have a beneficial effect on the health and well-being of the host [17].

Among these benefits include antimicrobial activity; control of pathogenic microorganisms [24]; lactose hydrolysis; modulation of constipation; antimutagenic and anticarcinogenic activity [25, 26]; reduction of blood cholesterol, improvement of patients with type 2 diabetes (insulin resistance) and obesity [27–29]; modulation of the immune system; improvement in inflammatory bowel disease; and suppression of *Helicobacter pylori* infection [30–32]. Some of these

benefits are already well established, such as constipation and lactose hydrolysis modulation, while other benefits have shown promising results in animal models, requiring further clinical studies [33].

Probiotics can be incorporated into a wide variety of food products, mainly in dairy products, such as milk, ice cream, yogurt, and cheese. Its application has also grown in other types of foods, such as soy milk, mayonnaise, pates, meats, baby food, confectionery, sweets, cakes, and chewing gum [34–37].

The selection of probiotic bacteria is based on the following criteria: gender, origin (which must be human), stability against stomach acid and bile salts, the ability to adhere to the intestinal mucosa, the ability to colonize, at least temporarily, the human gastrointestinal tract, the ability to produce antimicrobial compounds and metabolic activity in the intestine [38–40].

In order for the microorganism to be able to promote the aforementioned beneficial effects, a minimum intake of  $10^8$ – $10^9$  colony-forming units (UFC) per day is recommended [41]. In addition, the minimum concentration of live bacteria should not be less than 10 CFU/g of food since many cells die during passage through the gastrointestinal tract (TGI) [1, 42].

## 2.1 Types of probiotics

Specific probiotic strains give the benefits transmitted to health, and not by specific species or genus. However, that each strain is related to a specific benefit. In this way, no strain will provide all of the proposed benefits. For example, *Lactobacillus casei* lineage Shirota, in which evidence supports the view that its oral administration can assist in the digestion and absorption of nutrients and restore the normal balance of the intestinal microbiota [43]. Other relevant factors are the addition of mixtures of probiotic cultures instead of individual strains [44] and the number of viable cells of these microorganisms in the marketed product.

In a healthy adult intestine, the predominant microbiota is composed of health-promoting microorganisms (Table 1), mostly belonging to the genera *Lactobacillus* and *Bifidobacterium* [45]. Other lactic acid bacteria with probiotic properties are:

Lactobacilli	Bifidobacteria	Other bacteria	Fungus
<i>Lactobacillus acidophilus</i> sp	<i>Bifidobacterium bifidum</i>	<i>Enterococcus faecium</i>	<i>Saccharomyces boulardii</i>
<i>L. acidophilus</i> LA-1*	<i>B. lactis</i> Bb-12	<i>Enterococcus faecalis</i>	<i>Saccharomyces cerevisiae</i>
<i>L. casei</i> sp.*	<i>B. breve</i>	<i>Escherichia coli</i> Nissle 1917	
<i>L. rhamnosus</i> GG*	<i>B. infantis</i>	<i>Streptococcus salivarius</i> subsp. <i>Termophilus</i>	
<i>L. reuteri</i> *	<i>B. longum</i>	<i>Sporolactobacillus inulinus</i>	
<i>L. delbrueckii</i> subs.* <i>bulgaricus</i>			
<i>L. plantarum</i> sp			
<i>L. plantarum</i> 299 V			
<i>L. fermentum</i> KLD			
<i>L. johnsonii</i>			

\*Strains that have been used in the prevention and treatment of allergic diseases [45].

**Table 1.**  
 Main microorganisms used for their probiotic properties, in the form of drugs or added to foods.

*Ent. faecalis*, *Ent. faecium*, and *Sporolactobacillus inulinus*, while the microorganisms *Bacillus cereus*, *Escherichia coli* Nissle, *Propionibacterium freudenreichii*, and *Saccharomyces cerevisiae* have been cited as non-lactic microorganisms associated with probiotic activities mainly for pharmaceutical or animal use [32, 33, 46].

Some individuals may experience little of the side effects related to the ingestion of probiotics due to the death of pathogens in the intestinal environment since they release toxic cellular products, a reaction called a “die-off reaction”. In such cases, the use of probiotics should be persisted in order to improve symptoms. There is a slight increase in gas production, abdominal discomfort, and even diarrhea, which resolves over time [12].

## 2.2 Mechanism of action

Three possible mechanisms of action are attributed to probiotics: the suppression of the number of viable cells through the production of compounds with antimicrobial activity, competition for nutrients, and competition for adhesion sites. The second of these mechanisms would be the alteration of microbial metabolism by increasing or decreasing enzyme activity. The third would be to stimulate the host's immunity by increasing the levels of antibodies and increasing the activity of macrophages. The spectrum of activity of probiotics can be divided into nutritional, physiological, and antimicrobial effects [47, 48]. The direct modulation of the immune system may be secondary to the induction of anti-inflammatory cytokines or by the increase in the production of secretory IgA [45].

Despite the scientific evidence regarding the mechanisms of action of probiotics, there is still a lack in the literature on biochemical and molecular pathways that fully explain these effects, such as, for example, increasing the function of the intestinal barrier. Despite the scientific evidence regarding the mechanisms of action of probiotics, there is still a lack in the literature on biochemical and molecular pathways that fully explain these effects, such as, for example, increasing the function of the intestinal barrier [49].

Currently, the mechanisms of action of probiotics for anticarcinogenic effects have been studied. These are believed to occur through (1) inhibition of bacteria responsible for converting pre-carcinogenic substances (such as polycyclic aromatic hydrocarbons and nitrosamines) into carcinogens; (2) direct inhibition in the formation of tumor cells; and (3) the ability to bind and/or inactivate carcinogenic substances [25]. Several mechanisms of action have been suggested, including the stimulation of the host's immune response (by increasing phagocytic activity, IgA synthesis, and the activation of T and B lymphocytes), the binding and degradation of compounds with carcinogenic potential, qualitative changes and/or quantitative in the intestinal microbiota involved in the production of carcinogens and promoters (ex: bile acid degradation), production of antitumor or antimutagenic compounds in the colon (such as butyrate), alteration of the metabolic activity of the intestinal microbiota, alteration of the physical- colon chemicals with decreased pH and effects on host physiology [33, 50].

The use of probiotics represents a promising and rapidly growing area for the development of functional foods. Probiotic cultures are successfully applied to different food matrices. However, the development of non-dairy products represents a challenge for the industry, as each food matrix has unique characteristics, and it is necessary to optimize and standardize each type of product [51].

In this context, nanomaterials have been widely studied as a technique to improve the stability of these microorganisms and functional foods, protecting them from unfavorable environments, improving the uptake, absorption, and bioavailability of nutrients for the body (**Table 2**) [19].

Documented effects on humans and/or animals		Possible immunomodulation mechanism
<b>Local effects</b>		
Mucous barrier	Maintenance and repair of the intestinal barrier and intercellular junctions	Reduced permeability and decreased systemic absorption of allergens/antigens
Enterocytes	Increased production of TGF- $\beta$ and prostaglandin E2 responsible for promoting tolerance of antigen-presenting cells	Reduction of local inflammation and promotion of tolerance
Receivers enterocytes (toll-like)	Anti-inflammatory effects of probiotics mediated by toll-like receptors 9	Inhibition of allergic responses, type Th2: mechanism not yet clarified
Cells presenting antigens (dendritic cells)	Increased activity of dendritic cells in the intestine	Promotion of tolerogenic effect by dendritic cells
Auxiliary (or effector) T cells	Increased Th1-type response	Inhibition of Th2 response differentiation
Regulatory T cells	Production of Il-10 and TGF- $\beta$ associated with oral tolerance. Increased TGF- $\beta$ (Th3)	TGF- $\beta$ produced locally (including by enterocytes) promotes tolerogenic effect by dendritic cells, local IgA, and increased Treg activity
B cells and antibodies	Colonization: enlarged lymphoid tissue	Promotion of a tolerogenic environment
<b>Systemic effects</b>		
T cells	Increased Th1 differentiation	Secondary to the effects of T cells in the gastrointestinal tract
B/IgA cells	Increased production of IgA in other tissues (respiratory tract)	Secondary to the effects of B cells in the tract gastrointestinal

*Adapted from Souza et al. [45].*

**Table 2.**  
*Immune mechanisms of action associated with probiotics.*

### 3. Nanomaterials

Technological advances aimed at developing imaging equipment and techniques for characterization make it possible to develop and characterize systems on a nanoscale through scanning electron microscopy and transmission electron microscopy. Nanotechnology in the food area is designed to encapsulate, carry, and release bioactive ingredients to incorporate and modify the food structure. In addition, they make it possible to study the structures in detail, make it possible to understand their properties and facilitate their handling to obtain new, high-quality and safe foods [52].

Nanotechnology involves the characterization, fabrication and/or manipulation of structures, devices, or materials that have at least a dimension of about 1–100 nm in length [53, 54] and has emerged as one of the most promising scientific areas of research. Numerous companies are currently specialized in the manufacture of new forms of materials (nanometric size) with typical applications in medical therapy, diagnostics, energy production, molecular computing, and structural materials [55]. This technology in food introduces new opportunities for innovation in the food industry with immense speed. Thus, some of the applications result in the presence of nanoparticles or nanostructured materials in the food. This innovation can be applied to the macroscale characteristics of foods, such as texture, taste,

other sensory attributes, color intensity, processability, and stability during shelf life, leading to many new products. In addition, nanoencapsulation technology can also improve water solubility, thermal stability, and oral bioavailability of bioactive compounds [14, 56].

One of the biggest focuses of nanotechnology in the food industry is encapsulation systems and the controlled release of nutrients. The use of nanomaterials has shown improved properties for the encapsulation of probiotics. Due to their unique physical and chemical properties, nanostructured encapsulating materials show great promise of protecting microorganisms from acidic stomach conditions, increasing absorption and, therefore, allowing the successful release of probiotic cells trapped in the intestinal lumen with natural pH [57, 58].

The clinical efficacy of oral administration of probiotic bacteria is still diminished due to loss of viability during the gastrointestinal passage, resulting in poor intestinal distribution. Microencapsulation technology using nanomaterials is a successful strategy to solve this problem, maintaining the viability of probiotics, thus improving their effectiveness after oral administration [58]. In recent years, the production of probiotic and functional foods using nanotechnology represents one of the main current challenges [59].

The most basic nanomaterials used are nanoparticles. These can be presented in different forms, such as spherical nanoparticles (three nanometric dimensions); nanotubes and nanofibers (elongated structures with two dimensions on a nanoscale), and nanoplates (only have the nanometric thickness). Several examples of nanoparticles are cited in the literature, such as nano-clay, silver (Ag), titanium dioxide (TiO<sub>2</sub>), and zinc oxide (ZnO) nanoparticles [22].

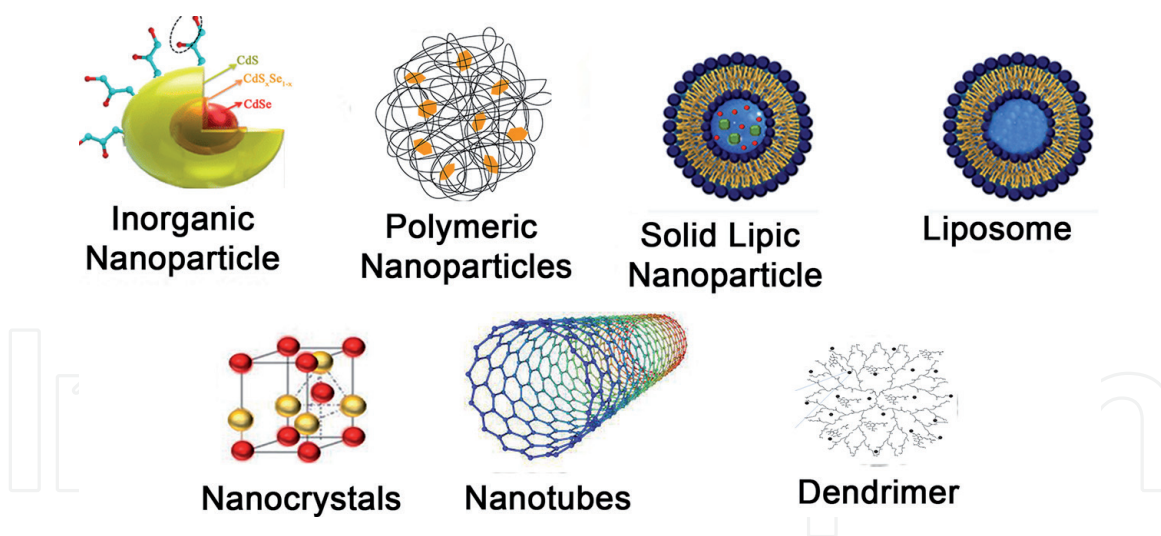
Different types of nanoformulations can be used, which requires the adequate formulation and timely processing conditions. Among them, polymeric nanoparticles, nanocomposites, solid lipid nanoparticles (NLS), liposomes, and nanoemulsions are suitable for food applications [57, 60].

### **3.1 Types of systems for encapsulation of bioactive compounds**

Nanoparticles (NPs) and nanostructured materials (NSMs) represent an active area of research with application in several domains. They are exciting nanoscale systems due to the ease with which they can be produced in different ways. NPs and NSMs arouse interest due to their adjustable physicochemical characteristics, such as melting point, wettability, electrical and thermal conductivity, catalytic activity, light absorption, and dispersion, resulting in improved performance compared to their mass counterparts [61]. NPs and nanosystems are broadly divided into several categories, depending on their morphology, size, and chemical properties [62]. Currently, some of the most studied nanostructured delivery systems are nanoemulsions, nanoliposomes, nanohydrogels, lipid nanoparticles, and coacervates with application in food (**Figure 1**) [63].

#### *3.1.1 Polymeric nanoparticles*

Polymeric nanoparticles are formed by a polymeric matrix (nanospheres) or a reservoir system in which the main content is hydrophobic or oily surrounded by a polymeric wall (nanocapsule) [64]. They are among the delivery systems for bioactive compounds most accepted and approved by GRAS [65]. In addition, they gained considerable attention in nanomedicine due to the potential for surface modification, pharmacokinetic control, suitability for targeted delivery of therapies [66], mechanical properties [67], and design flexibility. More specifically, size, surface morphology, chemistry and charge, porosity and diffusivity of the drug,



**Figure 1.**  
*Types of nanoparticles. Inorganic nanoparticles, polymeric nanoparticles, solid lipid nanoparticles, nanosomes, nanocrystals or quantum dots, carbon nanotubes, and dendrimers.*

and encapsulation efficiency are properties that push polymeric nanoparticles to the forefront of nanomedicine applications [68] and may behave similarly when incorporated into food.

The chemical and biocompatibility properties of polymeric nanoparticles have been studied extensively in recent years and allow these nanometric delivery systems formed by natural or synthetic polymers to be helpful in the controlled release of natural bioactive compounds, hormones, genes, and anticancer drugs with greater effectiveness than micrometric systems such as microparticles [69]. Due to a high surface contact area occur an intense interaction between the matrix in which they are inserted and the nanoparticles [70].

Currently, the most used polymers for the formation of the nanometric system are poly (lactic acid) (PLA), poly (glycolic acid) (PGA), poly (lactic-co-glycolic acid) - (PLGA), and polycaprolactone (PCL). Nanoparticles and microparticles can be obtained through different techniques that can be classified into four categories. Category 1: a traditional method based on the formation of an emulsion consisting of single emulsion, double emulsion, and multiple emulsions, followed by evaporation of the solvent. Category 2: methods based on nanoprecipitation, the rapid expansion of supercritical fluid in liquid, salting, and dialysis. Category 3: direct composition methods, such as fusion technique, spray-drying, supercritical fluid. Category 4: new approaches, including microfluidic and mold/mold-based techniques [65, 69].

The main active substances used for encapsulation by the methods of obtaining nanoparticles are isolated substances. However, some authors, such as Nascimento et al. [71] and Azevedo et al. [65], developed polymeric nanoparticles of Brazilian red propolis extract contributing to the development of nanostructured technologies for natural products.

### 3.1.2 Nanoliposomes

Nanoliposomes are defined as spherical lipid bilayer vesicles, resemble the lipid bilayer of cell membranes, and maintain nanometric or submicronic bands during storage and applications [72–74]. Its bilayer structure, formed by one-half of the lipid bilayer, contains a hydrophilic head and a lipophilic acyl chain. Thus, its amphipathic nature allows it to encapsulate hydrophilic and hydrophobic compounds individually or simultaneously due to its bifunctional physicochemical



properties and, consequently, it presents interaction with a wide range and variety of molecules [75]. Nanosystems are drug-carrying structures with potential for application in the medical field and food industry. However, they have low robustness regarding physical and thermal stability and pH variations, being considered significant challenges for their intended commercialization [76].

The most common method used for the production of nanoliposomes is to obtain a double emulsion followed by a microfluidization process at room temperature after the previous removal of the solvent. It is possible to produce nanoliposomes using low-cost natural ingredients (for example, soy, egg yolk, sunflower, milk), optimizing the cost-effectiveness of the final product [72]. The literature reports several clinical trials using nanoliposomes, and studies reveal that they are excellent candidates for various distribution systems, such as anticancer, antifungal and antibiotic drugs, administration of genetic drugs, and administration of anesthetics and anti-inflammatory drugs [77]. Similarly, it will have application in the food area, allowing the incorporation and simultaneous release of two or more bioactive compounds with different solubilities, as is the case of medium-chain liposomes and vitamin C, enhancing food functionality [74].

### *3.1.3 Solid lipid nanoparticles*

Lipid nanoparticles are similar to nanoemulsions in which the oil phase was totally or partially solidified [56]. It is a colloidal carrier system that makes it possible to encapsulate, protect and distribute functional lipophilic components, such as bioactive lipids and drugs [70]. The size and structure of the lipid nanoparticles are similar to nanoemulsions, with a size that usually ranges from 50 to 1000 nm. The lipid nucleus in nanoemulsions is liquid, but the lipid nucleus is in a solid-state [78].

Solid lipid nanoparticles can be classified as solid lipid nanoparticles (SLNs) and nanostructured lipid transporters (NLCs). In general, homogenization techniques of cold or hot high pressure and double emulsions are currently being used more to produce SLNs and NLCs to encapsulate bioactive oils [79]. The composition of SLNs is usually lipids such as triglycerides (tristearin), partial glycerides (glyceryl monostearate), fatty acids (stearic acid), sterols (cholesterol), and waxes (cetyl palmitate) [70].

There is a great difficulty associated with lipophilic bioactive agents in food matrices in the food industry, one of the main problems for manufacturers in the development of nutraceutical and functional foods [80]. Thus, SLNs and NLCs aim to assist as a nanoparticle carrier of bioactive compounds with a lipophilic character. SLNs are nanometric lipid matrices between 50 nm and 1  $\mu$ m in diameter, and these nanostructured systems are capable of effectively encapsulating active, sensitive molecules that must be protected from different environmental conditions, such as light, moisture, and oxidation. In addition, the solid matrix allows a controlled release and a high capacity to reach the target organ [79]. NLCs, whose matrix consists of a mixture of lipids with different physicochemical properties instead of just one type of lipid, were initially synthesized to avoid SLN problems with loading. They can form physical lipid mixtures through the mixture of solid and liquid lipids (oil), but without crystallization, presenting a more unstructured (entropic) matrix that allows the control of the molecular load [74, 79].

### *3.1.4 Nanohydrogels*

Nanohydrogels are defined as an infinite network of hydrophilic three-dimensional polymers swollen by water without losing their interconnected porous structure, expanding, and disintegrating [81–83]. For application in food, they must be composed

of non-toxic, biodegradable, and biocompatible biopolymers to deliver bioactive compounds in / or through the mucosa of the gastrointestinal tract. Nanohydrogels are soft materials widely used by the food and nutraceutical industries [83].

Generally, hydrogels are formed by chemical or physical cross-linking polymers. They are basically formed by three integral parts: monomer, initiator, and cross-linker [84]. Different techniques can be adapted to obtain the nanohydrogels such as mass polymerization, solution, and suspension, taking into account that the impurities, including unreacted monomer, initiators, crosslinkers, and unwanted products generated, need to be removed after their preparation [81].

Nanohydrogels formed by biopolymeric proteins or polysaccharides are the best alternatives for application in food since they can offer improved functional properties compared to native proteins. The size, structure, load, permeability, porosity, and stability to environmental and solution conditions are essential and fundamental characteristics for nanohydrogels and depend in general on the physicochemical properties of the biopolymers chosen to obtain the gel. [85]. The proper adjustment of these variables allows the functional compounds to be loaded and then released from the polymeric matrix [86]. The choice of the type of polymeric matrix must be adequate considering that hydrophilic compounds can be released from a protein matrix by diffusion, while lipophilic compounds are released mainly by enzymatic degradation of the protein matrix in the GI tract [21, 81, 85].

### *3.1.5 Nanoemulsion*

The definition of nanoemulsion consists of an excellent dispersion composed of an oily phase (triglycerides or hydrocarbons) and an aqueous phase (water or water with some electrolyte or polyol), which appears as spherical drops with a diameter less than 100 nm [70]. The nanoemulsion droplets most often have a core of lipophilic material, which one or more non-polar components may form. The surrounding contents of the nucleus are formed by the material of opposite polarity [81].

There is a wide variety of methods for making stable nanoemulsion. The nanoemulsion preparation is divided according to the energy level adopted in the system as the high and low energy method [87]. The main methods used to obtain a nanoemulsion include high-pressure homogenizers and ultrasound generators representing the high energy method, including microfluidization [88]. Low-energy emulsification methods are cost-effective, in which nanoemulsions with tiny droplets are prepared using low amounts of energy, which stand out the methods of spontaneous emulsification, reverse phase technique, membrane emulsification method, and solvent displacement method [89].

Nanoemulsions offer a wide range of applications due to their composition flexibility in several fields, including food, beverage, and pharmaceutical industries for product storage and delivery. Currently, it can be used to encapsulate lipophilic components, such as vitamins, substances that impart flavors, colors, preservatives, nutraceuticals, and medicines. In addition, it can be applied to preserve food and bioactive compounds, increasing bioavailability and shelf life. Another essential application aimed at the food industry is the possibility of masking unpleasant odors and flavors and protecting bioactive molecules from oxidation and hydrolysis by the action of air and water, respectively [89].

## **4. Conclusion**

Nanotechnology is a potential new technology in food, being one of the primary resources for development and innovation. Reducing the particle size of bioactive

compounds can improve bioavailability, release control, delivery targeting, and solubility. The choice of the preparation technique for the nanostructured systems depends on the characteristics of the bioactive compound, such as hydrophilic or lipophilic, solubility, stability, and the desired properties for the product, such as particle size and bioavailability, among others. Thus, it is possible to verify some of the nanoencapsulation techniques that can be used in bioactive compounds, and many undesirable characteristics can be circumvented with nanotechnology.

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## **Conflict of interest**

The authors declare no conflict of interest.

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