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Nano-encapsulation of probiotics: Need and critical considerations to design new non-dairy probiotic products

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ABSTRACT

People worldwide need to improve their health or intention to control diseases more naturally through ingredients incorporated into foods and beverages, called functional foods. Functional foods include probiotic organisms and bioactive compounds incorporated into dairy and fermentable foods, whose consumption is recommended for various vulnerable groups linked to the need to stay healthy. The increase in the production of functional foods containing live probiotics has led to the development of new products, particularly of the non-dairy type, to counteract the disadvantages of dairy products, such as low digestibility, allergies, intolerance, increased cholesterol, and saturated fatty acids, or the consumer's food preference, which impacts the consumer's health. In this sense, the continuous development of non-dairy matrices through the application of nanotechnological strategies has had a significant impact on food science, providing nano-encapsulation systems for the transport, storage, and release of probiotic organisms, preserving their properties to exert their beneficial effects without affecting the sensory characteristics of the product.

This paper addresses recent advances in non-dairy matrices for probiotic strains, pointing out their advantages and limitations according to the characteristics of the matrix and the encapsulation techniques used. Considerations are presented to design non-dairy matrices based on nano-systems that allow obtaining quality products with nutritional value and high bioavailability, that increase viability, protect from factors such as pH and temperature, improve stability for bioactive compounds, and decrease adverse interactions between food components while acting as controlled release systems. Additionally, toxicological aspects and the need to continue toxicity testing for nano-systems intended to be used as non-dairy matrices in food, even when dealing with raw materials recognized as non-toxic, are pointed out, considering that each nanosystem presents different properties. As research and the use of non-dairy matrices for probiotics progresses, it may contribute to mitigating environmental damage.

1. Introduction

Society has embraced healthier lifestyles, emphasizing physical activity and good eating habits as a new mantra. Modern diets are geared towards disease prevention and enhancing physical and mental well-being, surpassing mere nutritional fulfillment. Consequently, there has been a significant rise in the integration of health-promoting ingredients into foods and beverages, giving rise to what is known as 'functional foods' (Jafari & McClements, 2017; Mishra, Behera, Biswabandita, & Ray, 2018). Functional food was first introduced in Japan in the mid-1980s, denoting processed food containing ingredients that offer health benefits and are nutritious (Mishra, Behera, Biswabandita, & Ray, 2018). Terms such as nutraceuticals, therapeutic foods, superfoods, and medicinal foods also encompass the domain of functional foods (Prado et al., 2008). The appeal of functional foods and bioactive components lies in their potential to act as tools for preventing, reducing, or

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sometimes even curing various diseases. Furthermore, they are typically suitable for consumption by vulnerable groups of people (Bao et al., 2019; Durazzo et al., 2020).

Functional foods can be categorized into foods: (1) containing naturally occurring bioactive substances like dietary fiber, (2) enriched with bioactive substances such as probiotics, and (3) incorporating food ingredients introduced to conventional foods, like prebiotics (Mishra Pandey & Mishra, 2015). Therefore, functional foods encompass bioactive ingredients like probiotics, prebiotics, fiber, vitamins, and minerals. These are commonly consumed as fermented beverages, milk products, fruits, cereals, sports drinks, baby foods, and more (Cassani, Gomez-Zavaglia, & Simal-Gandara, 2020; Mishra, Behera, Biswabandita, & Ray, 2018).

Food development containing probiotics represents the fastest-growing segment in functional food production, as evidenced by the rising publications number, patents, intellectual property rights, and the abundance of products available in the market (Aspri et al., 2020). Due to the health-promoting benefits linked to the consumption of live probiotics, there is a substantial demand for both dairy and non-dairy products (Valero-Cases et al., 2020). Dairy products are suitable matrixes for probiotic delivery, but lactose intolerance and other medical conditions, such as vegetarianism, emerging veganism, allergenicity, and dairy products' high fat and cholesterol content, are considered disadvantages (Rasika et al., 2021; Küçükgöz et al., 2022). This has led to the development of non-dairy matrices for probiotics.

However, incorporating probiotics into functional foods must surmount several challenges associated with various factors categorized into (1) inherent to the probiotic strain, (2) related to the manufacturing process, (3) influenced by storage conditions, and finally, (4) proper to the administration route (see Fig. 1) (Tolve et al., 2016; Aspri et al., 2020; Hosseini & Jafari, 2020; Valero-Cases et al., 2020).

The strategies to overcome these challenges include using acid and bile-resistant probiotic strains, fermentation, incorporating micronutrients and probiotic substrates, employing refrigerated storage, ensuring oxygen impermeability, utilizing aseptic packaging, and employing encapsulation techniques. Recently, micro- and nanoencapsulation have been used to shield probiotic strains against detrimental factors such as pH, light, water, oxygen, moisture, heat, etc., thereby enhancing the survival rate and improving bioavailability, functionality, and nutritional value. Microbial nanoencapsulation strategies become promising options to enhance their survival because they allow the creation of optimal microenvironments for their stability and survival. Additionally, encapsulation helps mask unfavorable flavors and odors while adding targeted release properties as well as enhances the physico-chemical and organoleptic properties of food-based probiotic products (Augustin & Hemar, 2009; Fung et al., 2011; Tolve et al., 2016; Hosseini & Jafari, 2020; Kaur et al., 2022; Sun et al., 2023). In the encapsulation technique, probiotics are packed (core material) or dispersed into another compound (coating, membrane, shell, or wall material) to shield them from environmental conditions (Tolve et al., 2016; Ahmad et al., 2019; Hosseini & Jafari, 2020).

The final challenge lies in designing a suitable delivery system utilizing the appropriate material and encapsulation technique that does not compromise probiotic strain bioavailability and activity while also considering the sensory attributes of food products, including taste, appearance, and texture. In this regard, nanotechnology presents a promising opportunity wherein nanostructured materials facilitate the creation of innovative food products with enhanced physicochemical properties (stability, appearance), sensory aspects (taste, texture), and nutritional value (gastrointestinal absorption rate) (Jafari & McClements, 2017). This review offers a comprehensive overview of nanosystems, illustrating how they could drive the development of novel non-dairy probiotic products. Additionally, we briefly discuss criteria for probiotic strain selection, various types of nano-carrier systems,

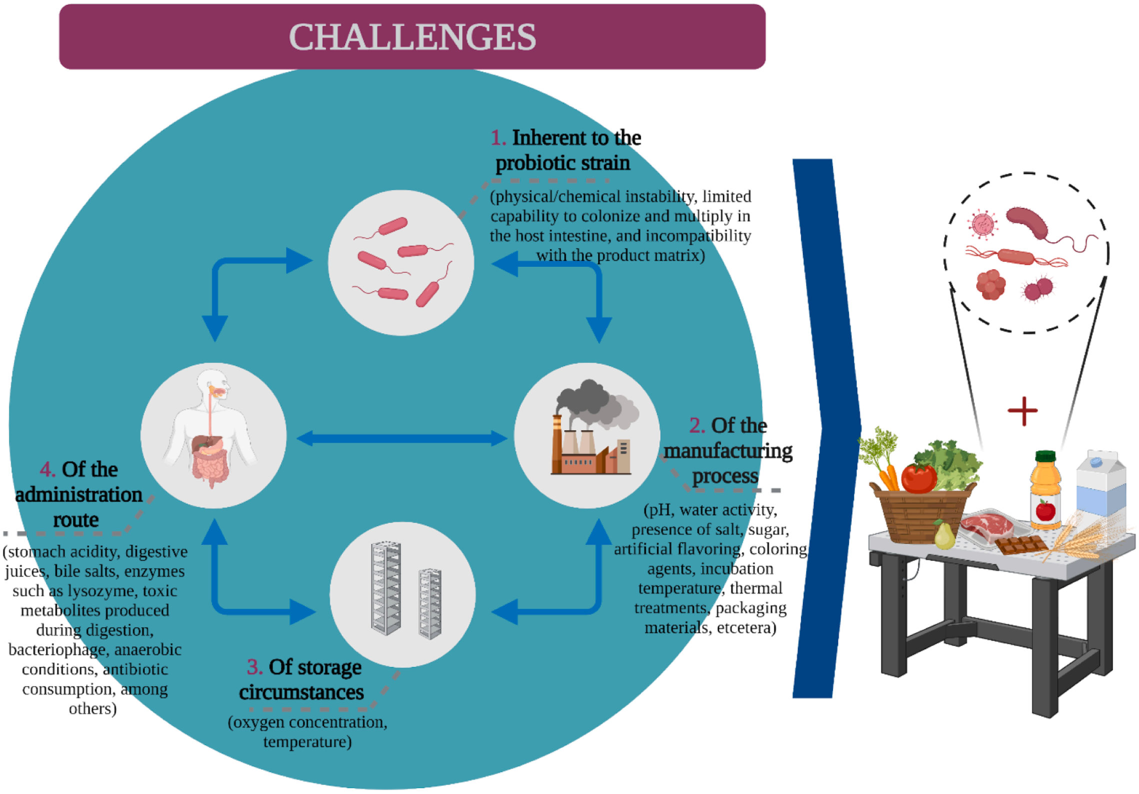

Fig. 1. Challenges to Overcome in the Addition of Probiotics to Functional Foods. The success of new probiotic products depends on the capability of the developed product to overcome challenges: 1. inherent to the probiotic strain; 2. related to the manufacturing process; 3. influenced by storage conditions; 4. associated with the administration route.

appropriate encapsulation techniques, and the toxicological and safety considerations associated with nano-systems.

2. Description of probiotics

Centuries ago, Hippocrates stated, ‘All diseases begin in the gut’. Scientific evidence supports this hypothesis today, suggesting that intestinal microbiota balance contributes to good health (Valero-Cases et al., 2020). The human gastrointestinal microbiota is host to approximately 10^{14} microorganisms, even more significant than the number of cells in our body. The vast majority of bacteria in healthy individuals belong to the phyla *Bacteroides* and *Firmicutes* (~70–90 %), while a smaller percentage comprises *Actinobacteria* and *Proteobacteria* (Hameed et al., 2021).

Each individual possesses a highly variable intestinal microbiota at the species level (Martinez et al., 2015). It is well-known that the gut microbiota must maintain a balanced composition between symbiotic microorganisms recognized as health promoters and pathobionts or potentially pathogenic microorganisms (Martinez et al., 2015). This balance has given rise to terms like ‘eubiosis’ and ‘dysbiosis’ to reference this microbiota’s quantitative and qualitative composition (Pinart et al., 2022). Eubiosis describes the mutually beneficial state between microbes and the host, while dysbiosis refers to the situation in which one or more harmful microorganisms are dominant over that beneficial (Do Espírito Santo et al., 2011; Hameed et al., 2021). Gut dysbiosis is linked to the development of gastrointestinal diseases, metabolic syndrome, cancer, celiac disease, and muscle disorders, among other pathologies (Power et al., 2014; Ghebretatios et al., 2021).

Factors such as age, antibiotic treatment, exposure to toxins, family size, hygiene level, and diet impact the balance between beneficial and pathogenic microorganisms (Gawkowski & Chikindas, 2013). The functions of the microbiota encompass the production of vitamins (especially B and K), butyrate, acetate, and propionate; degradation of xenobiotics; participation in the formation of the intestinal wall; interactions with the mucosal immune system; and providing colonization resistance against pathogens (Martinez et al., 2015).

Today, it is widely acknowledged that the metabolic activity of gut microbiota significantly impacts an individual’s health. The reported health benefits resulting from probiotic consumption include the prevention of a range of diseases such as constipation, diarrhea, and skin illnesses; synthesis of vitamins; reduction of blood cholesterol levels; decrease in the incidence and duration of respiratory infections; improvement in gut microbiota balance and blood pressure indices; positive effects on metabolic function and the immune system; as well as anti-carcinogenic and antibacterial activity (Sarao & Arora, 2017; Cassani et al., 2020).

In line with Hippocrates’ principle of ‘Let food be your medicine and medicine be your food,’ there is growing interest in disease prevention through a healthy lifestyle and nutrition (Witkamp & van Norren, 2018). The demand for healthy products that cater to more than just nutritional needs is increasing daily, as evidenced by the extensive number of published research and the plethora of products available in the market. In this regard, functional food products containing probiotics represent a relevant option because they can be utilized to prevent or delay health issues (Cassani et al., 2020).

The term ‘probiotic’ is widely used in nutrition, referring to ‘live microorganisms that, when administered in adequate amounts, confer health benefits on the host’ (FAO/WHO, 2001; de Oliveira Ribeiro et al., 2020). Over the years, lactic acid-producing bacteria have been the most commonly used species of microorganisms as probiotics (*Lactobacillus*, *Streptococcus*, *Enterococcus*, *Lactococcus*, *Bifidobacteria*). *Bacillus spp*., *Fungi spp*., and some yeast species are also considered probiotics. Currently, only a few microorganisms, such as *Lactobacillus spp*., *Lactococcus spp*., and *Bifidobacterium spp*., have achieved a GRAS status (generally recognized as safe) or belong to species with a qualified presumption of safety (QPS) designation by the European Food Safety Authority (EFSA). These can be marketed and applied in the food industry (Sarao & Arora, 2017; Cunningham et al., 2021).

The significant mechanisms of action responsive to the biological activity of probiotic strains can be categorized into luminal, mucosal, and submucosal mechanisms. Luminal mechanisms encompass improving intestinal microbial balance by stimulating the growth of beneficial microorganisms and inhibiting the growthpathogens growth. Probiotics also adhere to the gut, occupying the living space of pathogens. Mucosal mechanisms include the induction of mucin secretion and enhancement of the epithelial barrier. Submucosal mechanisms involve the modulation of insulin-sensitive tissues and immune systems, and the synthesis of antimicrobial substances (Sarao & Arora, 2017; Davoodvandi et al., 2021).

For a strain to be considered a probiotic, it must possess the following characteristics: a) be isolated from the same host and mucosal environment where it will be administered, b) be correctly identified both phenotypically and genotypically, c) demonstrate its mechanism of action through *in vitro* and *in vivo* experiments, d) must not be pathogenic (must be tolerated by the immune system and not provoke antibody formation), e) demonstrate its efficacy and beneficial effects in clinical trials, f) survive during long periods of storage, and finally, g) be able to survive through the gastrointestinal tract in adequate amounts and metabolic active state to reach its possible site of action and exert its functions in the host (Sarao & Arora, 2017; Durazzo et al., 2020).

The number of colony-forming units (CFU) *per* gram (g) or milliliter (mL) in food-based probiotic products varies among countries. The USA sets the acceptance threshold at a minimum of 10^7 CFU/g or mL, whereas Canada requires 10^9 CFU/g or mL. However, to ensure the health effects of probiotics, the most crucial factor is maintaining the minimum therapeutic level of live probiotic microorganisms, namely at least 10^6 CFU/g or mL per day. Although, the required dose could be lower depending on the strain (Pimentel et al., 2020).

An ideal probiotic strain must have properties related to its survival and function in the host, resulting in beneficial effects for human health (Fig. 2). It must also have characteristics attractive for its use at the industrial level (Prado et al., 2008) (Fig. 2).

An ideal probiotic strain, to be considered for the development of new probiotic products, should possess characteristics such as resistance to acid and bile, attachment to human epithelial cells, colonization in the intestine, production of antimicrobial substances resulting in beneficial effects for human health, cost-effectiveness, viability during processing and storage, easy incorporation into products, and resistance to the physicochemical processing of foods for use in probiotics.

3. Criteria for probiotic strain selection

For achieving efficiency and success in designing new probiotic products, two criteria guide the selection of strains. First, their ability to confer health benefits on the host, and second, their ‘stress-resistance phenotype’, which refers to the strain’s ability to remain viable during processing and storage, and to survive the gastrointestinal tract. However, in food-based products, probiotics are not pure but are incorporated into carrier systems, such as food matrices (Terpou et al., 2019). Two approaches enable the incorporation of probiotics into food-based products: direct growth within the final product and drying or encapsulation.

The first approach involves fermentation and the direct growth of microorganisms in the food matrix. The resulting food-based probiotic products can be classified into dairy products (e.g., yogurt, cheese, milk, fermented milk, cream, and ice cream) and non-dairy products (e.g., fruit and vegetable juices, fruits, cereals, chocolate, bread, meat, and meat products). The second approach refers to adding microorganisms to the product (Flach et al., 2017). In the first case, when choosing a probiotic strain, consideration must be given to the potential relationship between traditional starter cultures and the probiotic strain; cultures should not compete during product processing and should remain

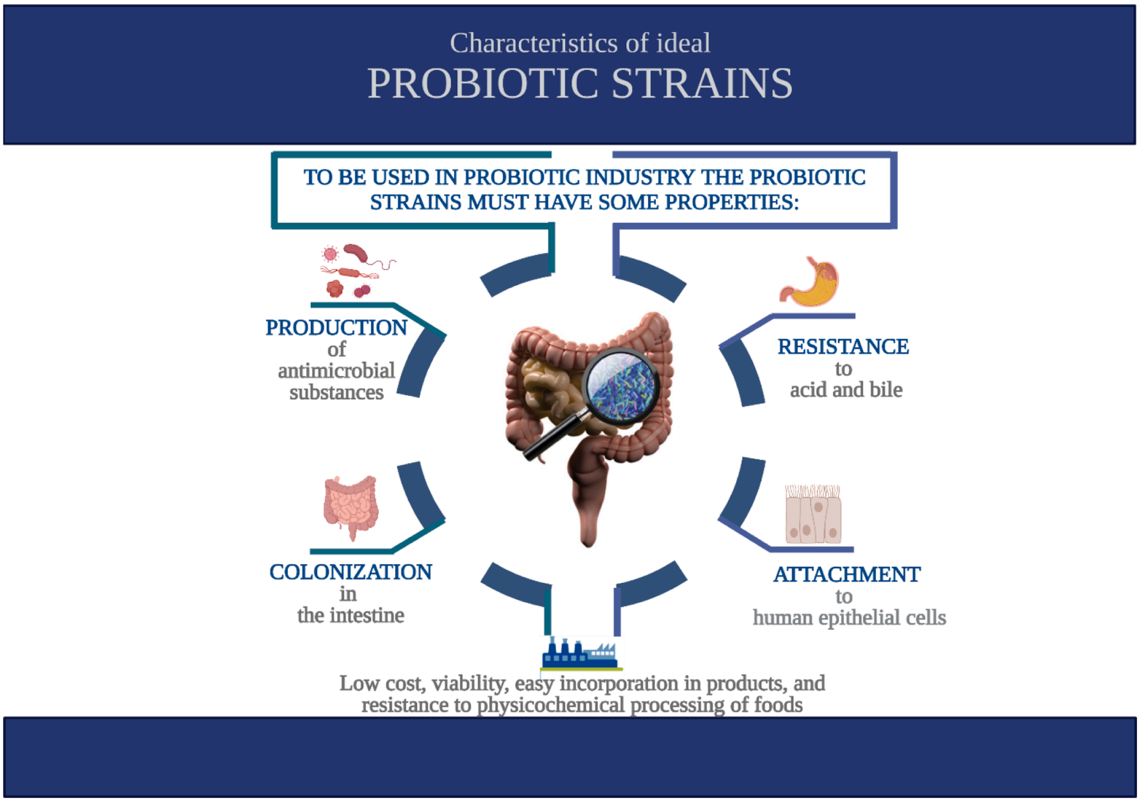

Fig. 2. Characteristics of an Ideal Probiotic Strain. An ideal probiotic strain, to be considered for the development of new probiotic products, should possess characteristics such as resistance to acid and bile, attachment to human epithelial cells, colonization in the intestine, production of antimicrobial substances resulting in beneficial effects for human health, cost-effectiveness, viability during processing and storage, easy incorporation into products, and resistance to the physicochemical processing of foods for use in probiotics.

stable during storage. In the second case, the selection of the probiotic strain to be added must be based on safety and the technological performance of the probiotic strain (Champagne et al., 2005).

Furthermore, other criteria for strain selection should also be considered, such as the potential physicochemical interactions between food matrix components and cell functionality. It is well-known that the probiotic strain can negatively influence the product, altering sensory quality, and the food matrix can reduce physiology, efficacy, and cell viability (Matouskova, Hoova, Rysavka, & Marova, 2021).

4. Probiotic classical transport systems

4.1. Dairy matrices

Dairy matrices serve as the primary vehicle for probiotic delivery worldwide, possibly because most probiotic strains are compatible with milk components. Milk fat and its buffering action can protect against harsh gastrointestinal conditions. Moreover, the storage conditions of most dairy products, such as low temperatures, make them an ideal medium for developing probiotic products. Fermented foods are well-recognized as a natural source of probiotics, and fermented milk products, including yogurt, fermented milk, and cheese, are the most well-known probiotic foods. During fermentation, microorganisms utilize organic compounds as energy sources under anaerobic conditions, producing metabolites such as lactate, acetate, and short-chain fatty acids that promote physiological functions beneficial to human health. Furthermore, the fermentation process amplifies the probiotic populations, ensuring adequate cell counts to exert health benefits in the host (Ranadheera et al., 2017; Fenster et al., 2019; Marco et al., 2021).

Although the earliest records of the intake of bacterial drinks date back over 2000 years, there is a growing modern interest in consuming fermented products driven by scientific knowledge of their health benefits (Sarao & Arora, 2017). Many people worldwide associate gut health with consuming dairy products (Fenster et al., 2019). Due to this association, dairy products are the most consumed among other fermented products, with a market exceeding six billion people worldwide (Lopamundra & Kumar Panda, 2018).

The microbial strains used in fermented foods encompass 195 bacterial species and 69 yeast species (Cassani et al., 2020). The most common microorganisms essential for fermented foods include lactic acid bacteria, acetic acid bacteria, bacilli, other bacteria, yeasts, and filamentous fungi. These microorganisms act as starter cultures but do not necessarily possess probiotic properties (Marco et al., 2021).

Despite the advantages offered by dairy matrices, inevitable drawbacks are associated with the consumption of dairy products, such as lactose intolerance, milk protein allergies, and the high content of cholesterol and saturated fatty acids. Additionally, cultural considerations, such as strict veganism, and religious beliefs, limit these matrices' use (Vijaya Kumar et al., 2015; Ranadheera et al., 2017; Salmerón, 2017).

4.2. Non-Dairy matrices

Due to the drawbacks associated with using dairy matrices for probiotic delivery, such as lactose intolerance, high cholesterol content, and considerations related to vegetarianism, the demand for new probiotic foods is increasing. This demand drives the development of non-dairy-based probiotic products in the food industry. Meeting these needs and providing products with high nutritive value are just some advantages of consuming non-dairy probiotic products. Non-dairy probiotic products are available in beverages and fermented foods, primarily based on fruits, vegetables, or cereals (Bansal et al., 2016). The most commonly used probiotic strains in non-dairy foods are *Bifidobacterium* (*B. animalis*, *B. longum*, *B. lactis*, *B. bifidum*, *B. infantis*, *B. breve*), *Lactobacillus* (*L. acidophilus*), *Lacticaseibacillus* (*L. casei*, *L. rhamnosus*), *Lactiplantibacillus* (*L. plantarum*), *Ligilactobacillus* (*L. salivarius*), and *Limosilactobacillus* (*L. fermentum*, *L. reuteri*). Certain *Bacillus* and *Streptococcus* species and the yeast *Saccharomyces cerevisiae* are also used as probiotics in non-dairy foods (Pimentel et al., 2020).

Non-dairy probiotic foods have not recently developed; preparations based on cereals, fruits, vegetables, meat, etc., have been traditionally crafted for centuries worldwide. Presently, a diverse array of non-dairy probiotic products is readily available. However, their development presents a unique set of challenges, primarily concerning the maintenance of probiotic viability in non-refrigerated products, susceptibility of microbes to high temperatures, pH levels, oxygen and water content, acidity, light exposure, low nutrient availability, presence of competing microorganisms and antimicrobial compounds, and the type of matrix (Gawkowski & Chikindas, 2013). Factors related to the food matrix modify the final product's sensory attributes (smell, texture, or taste) of the final product and the probiotic resistance to acid and bile, consequently impacting their efficacy (Casarotti et al., 2015; Aspri et al., 2020). Various technologies, such as nanotechnology, could help overcome these challenges. Nanoencapsulation of probiotic cells makes it possible to minimize the environmental stresses that reduce probiotic viability and affect food quality.

4.2.1. Fruit and vegetable juices

Fruit and vegetable beverages with probiotics are among the most successful non-dairy products in the market because they are well-accepted by all age groups due to their appealing taste profiles. Moreover, they are perceived as healthy and refreshing (Rivera-Espinoza & Gallardo-Navarro, 2010; Žuntar et al., 2020). Juices are considered an ideal medium for the delivery of probiotics primarily due to their content of healthy ingredients. Additionally, they contain nutrients and sugars that support the growth of microorganisms. Furthermore, juices spend less time in the stomach than other foods, thereby exposing probiotics to acidic conditions from the stomach for a shorter period (Kandylis et al., 2016).

However, some challenges related to incorporating probiotic strains into this product. Unsuitable aroma and flavor, often perceived as 'dairy', 'acidic', 'medicinal', 'salty', 'artificial', 'astringent', 'bitter', 'earthy', and 'dirty', are some reported drawbacks (Aspri et al., 2020). Another challenge is the low pH value (2.5–3.7) in juices. The microorganisms in these beverages face an acidic environment that could reduce their viability, survival, and stability in fruit juices. These aspects vary depending on the strain (Prado et al., 2008; Rivera-Espinoza & Gallardo-Navarro, 2010; Bansal et al., 2016; Min et al., 2018). For example, *Bifidobacteria* tend to resist and survive less than *Lactobacillus* in fruit juices with a pH of 3.7 to 4.3 (Aspri et al., 2020).

As mentioned before, nanotechnology could help overcome these drawbacks through encapsulation techniques. These techniques can mask unsuitable odors/flavors and protect microorganisms from low pH values by applying a protective coating (Dima et al., 2020).

4.2.1.1. Fruits and vegetables. Fruits and vegetables are considered healthy due to their rich content of compounds such as antioxidants, vitamins, fiber, and others, making them desirable candidates for consumption (Lillo-Pérez, Guerra-Valle, Orellana-Palma, & Petzold, 2021). Scientific literature indicates that the growth and viability of probiotics in fruits and vegetables depend on the strain used (Rivera-Espinoza & Gallardo-Navarro, 2010). The success of probiotic products in fruit-based matrices relies on the interactions between the probiotic strain and the food components (Terpou et al., 2019). Strains resistant to acidic environments are recommended to incorporate probiotics in these matrices. Additionally, if possible, physical barriers like nanoencapsulation should be employed to enhance the viability of microorganisms under harsh acidic conditions (Rivera-Espinoza & Gallardo-Navarro, 2010).

4.2.1.2. Cereals. Cereal grains have garnered significant attention as potential non-dairy carriers to transport probiotics (Mani-López et al., 2023). This interest is primarily due to their association with a reduced risk of chronic diseases, their easy accessibility to the population, and their recognition as a rich source of fiber, carbohydrates, protein, vitamins, and minerals. These components can act synergistically with probiotics, potentially acting as prebiotics, protecting them from adverse conditions, and selectively stimulating the growth of *Lactobacillus* and *Bifidobacteria* in the human colon (Rivera-Espinoza & Gallardo-Navarro, 2010). Cereal grains such as oats, maize, soy, sorghum, wheat, millet, rice, etc., are being utilized for this purpose (Vijaya Kumar et al., 2015).

The nutritional value of cereals is generally lower than milk, possibly due to their low protein content, deficiency in essential amino acids like lysine, and limited starch availability, among other factors. However, these limitations can be effectively addressed through the fermentation of cereals (Vijaya Kumar et al., 2015). Fermentation processes can enhance the quality of protein, reduce carbohydrate levels, and offer additional benefits, including improved availability of B vitamins and the facilitation of mineral releases, such as manganese, iron, calcium, and zinc (Rivera-Espinoza & Gallardo-Navarro, 2010; Flach et al., 2017). The practice of fermenting cereal grains is not recent; for centuries, Asian and African countries have employed fermentation to produce beverages and other food products. Recently, this practice has gained recognition and importance in Western countries. Nevertheless, using cereals as a matrix for probiotic foods presents challenges, primarily related to their ability to support probiotic growth and maintain viability during storage, particularly at room temperature. The flavor and aroma of fermented cereals undergo significant changes after product processing and during storage, constituting one of the major drawbacks that need to be addressed in fermented cereal probiotic beverages (Lopamundra & Kumar Panda, 2018; Morales-de la Peña, Miranda-Mejía, & Martín-Belloso, 2023).

Nanoencapsulation can mask unpleasant odors, colors, and tastes or prevent their occurrence by protecting reactive substances from the environment, enhancing the market acceptance of these products (Tolve et al., 2016).

4.2.1.3. Meat and meat products. Probiotic meat products are less common than non-dairy probiotic foods in the market. However, they represent a particular matrix type, as studies have shown that probiotic strains added to these products demonstrate bile tolerance. Additionally, meat products contain bioactive compounds with health benefits, and their processing typically does not require high temperatures that could decrease probiotic viability. Products such as sausages can create protective environments for microorganisms against harsh stomach conditions and bile salts. Moreover, meat products like loin, ham, and sausages undergo maturation, supporting probiotics growth within the matrix (Lopamundra & Kumar Panda, 2018; Min, Bunt, Mason, & Hussain, 2018).

Adding probiotics to meat and meat products poses significant drawbacks, including salt, nitrites, nitrates, and fermentative microorganisms that could reduce cell counts. The process complexity for adding probiotics, production of substances such as lactic and acetic acid, alcohols, ketones, aldehydes, and bacteriocins, as well as factors like water activity, low content of natural sugars, and oxidation of lipids and proteins, are also disadvantages. These factors can cause a color loss, reducing the quality and acceptance forfinal product (Aspri, Papademas, & Tsaltas, 2020; Lopamundra & Kumar Panda, 2018). Among the numerous strategies developed to protect probiotics during the meat processing process and improve their viability (Cavalheiro, Ruiz-Capillas, & Herrero, 2015; Šipailienė & Petraitytė, 2018), encapsulation emerges as a promising approach to achieve high survival rates of probiotics, shielding the strains from adverse conditions (Li et al., 2011; Dimitrellou et al., 2016), while also preserving the quality of meat products (Munekata, Pateiro, & Tomasevic, 2022).

5. Nano-systems generalities

Trillions of nano-systems are present in our bodies, performing biological functions. Nanotechnology draws inspiration from nature, utilizing it as a source of insight to create systems at the nanometric scale (1–100 nm (nm) or < 1000 nm in the pharmacological and food industry) that can actively function in our bodies. Due to their remarkable properties and versatility, pharmaceutical disciplines leverage materials at the nanoscale to overcome many drawbacks related to drugs, such as poor water solubility and low bioavailability, enabling targeted/controlled release. It has led to the emergence of nanopharmaceuticals (Jafari & McClements, 2017). Nanopharmaceuticals focus on incorporating therapeutic molecules into nanoparticle delivery systems (Durazzo et al., 2020) to address the challenges mentioned (Assadpour & Mahdi Jafari, 2019). The evolution of techniques to create new food-grade delivery systems began in the pharmaceutical field. Today, through Nanoscience and the application of Nanotechnology techniques in food industry, it has become possible to design new probiotic products with improved food quality, enhanced nutrient bioavailability, and disease prevention properties (Tolve et al., 2016; Salmerón, 2017).

The transition from microscale to nanoscale alters particle features and their physicochemical properties. This shift constitutes the scientific significance of nanotechnology. Systems at the nanometric scale increase the surface-to-volume ratio, making them biologically more active than larger particles of same material (Assadpour & Mahdi Jafari, 2019; Lopamundra & Kumar Panda, 2018). The application of nanotechnology in food sector is already a reality and includes delivery systems for bioactive compounds, the use of nano-sized food ingredients and additives, and food packaging. The general term used to describe these products is 'nano-foods' (Dasgupta, Ranjan, & Mundekkad, 2015; Jafari & McClements, 2017; Paul, 2015).

6. Critical consideration aspects to promote the creation of nano-systems to develop new Non-Dairy probiotic products

A nano-system for delivering probiotics consists of two essential components: a carrier (which should have at least one dimension at the nanometric scale) and a probiotic strain. The probiotic strain is the bioactive component capable of exerting beneficial health effects in the host. The carrier, typically a nanomaterial or nanoparticle, is a transporter that encapsulates the microorganism and ensures its viability and activity upon reaching the targeted site. Each component's specific characteristics and interactions define the properties and behavior of the nano-system (Barboza Duarte, Oliveira Nascimento Mergulhão, & da Costa Silva, 2021).

Nano-systems are highly suitable for delivering bioactive molecules due to their physicochemical characteristics and significant impact on these active compound's stability and bioavailability. It is particularly evident regarding thermal characteristics, p*K*a, and overall functionality. From a food industry perspective, the design of nano-systems should prioritize the protection of sensitive food ingredients, such as probiotics, while also providing controlled release properties, enhancing nutritional content, extending shelf life and product stability, and masking taste, odor, or color without altering the overall taste, aroma, or flavor of foods. From a pharmaceutical standpoint, the focus should be on improving efficacy, stability, and specificity and minimizing adverse effects (Dhapte & Pokharkar, 2019; Delfanian & Sahari, 2020).

Through nano-systems, it is possible to enhance the survival rate of probiotics and improve product quality, primarily due to reduction in particle size. This reduction leads to improved water dispersibility, better protection against chemical or biochemical degradation, and prevention of adverse interactions between food components (Jafari & McClements, 2017). Moreover, materials at the nanometric scale improve timing and targeted delivery. However, encapsulating probiotic strains into nano-systems presents significant challenges. The first challenge is related to size from probiotic cell (diameter between 1 and 5 µm), and the second challenge is to ensure probiotic strains remain live and viable, arriving in the intestine in adequate amounts and an active metabolic state to exert their health benefits in the host (Sarao & Arora, 2017).

Two key factors are critical to successfully overcoming these challenges: the appropriate selection of material and encapsulation technique. Other relevant aspects include the probiotic strain, material concentration, particle size, and charge. The choice of these parameters will be contingent upon the type of food formulation and its specific physicochemical conditions (Delfanian & Sahari, 2020).

Indeed, as previously discussed, selecting the appropriate probiotic strain requires careful consideration of several aspects. These include the strain's ability to impart health benefits, its resistance to environmental stressors, potential interactions with starter cultures in fermented products, interactions with the food matrix, technological performance, and safety concerns (Sarao & Arora, 2017).

Particle size and electric charge are critical when designing nano-systems for food-based probiotic products. They can significantly influence the stability of the system in colloidal suspensions, the release rate of probiotics, and their absorption through the intestine. Particle size plays a vital role in the biological fate of the system. Sizes lower than 10 nm are rapidly cleared, while sizes lower than 30 nm can easily cross intestinal junctions, although this could pose a health risk. The optimal size range for a delivery nano-system is generally considered to be around 10–250 nm (Dhapte & Pokharkar, 2019; Dima et al., 2020). Regarding electric charge, positively charged nano-systems are preferred as they can more easily traverse the lipophilic bilayer of enterocytes (Dima et al., 2020).

Before selecting a nano-encapsulating material or technique, it is crucial to consider the type of food matrix and its physicochemical conditions. Non-dairy food matrices like juices, vegetables, and meat contain various nutrients such as carbohydrates, fats, proteins, vitamins, and minerals, which can influence the behavior of probiotic-loaded nano-systems. Each nutrient can adsorb onto the nanoparticle surface, altering its properties like size, charge, and hydrophobicity, consequently affecting bioavailability and transport through the intestine and mucus layer (Dima et al., 2020). Additionally, the physicochemical conditions of the food, such as pH and temperature, play a significant role in the release mechanism of the nano-system. Understanding the release mechanism, whether it is sustained or delayed, and the triggering conditions are essential for a successful design (Augustin & Hemar, 2009; Delfanian & Sahari, 2020). Once the food matrix is understood, appropriate encapsulation materials and synthesis methods can be considered.

6.1. *Criteria for selection of Nano-Encapsulating materials*

In the food sector, encapsulation techniques have been utilized for a considerable time, stemming from biotechnology. Immobilized cell culture techniques have been employed in biotechnological industries to optimize production processes and efficiently separate fermentative microorganisms from their metabolites (Nedovic et al., 2011). Physical or chemical encapsulation involves enclosing substances within matrices that can release their content under specific, controlled conditions (Mahdi Jafari, 2017). When this process is performed at a nanometric scale, it is termed nanoencapsulation. Like regular encapsulation, nanoencapsulation aims to protect substances against adverse environmental conditions and enable their controlled release at the target site (Terpou et al., 2019).

Various materials have been utilized in the food industry to encapsulate probiotic strains. Regardless of their nature (natural or synthetic), nanoencapsulating materials must be food-grade and selected from a range of Generally Recognized as Safe (GRAS) materials. Commonly used materials include food biopolymers (proteins, carbohydrates), fats, copolymers (protein-carbohydrates), minerals, and surfactants (Augustin & Hemar, 2009; Jafari & McClements, 2017). Critical criteria for material selection for nanoencapsulation of probiotics include: a) physicochemical and biological characteristics (color, odor, taste, non-toxicity, chemical inertness, absence of impurity or residue release), b) functional properties (solubility, viscosity, gelling ability, electric charge, etc.), c) sources (synthetic, natural, or hybrid), and d) cost considerations (Tolve et al., 2016; Dima et al., 2020).

The selection of the encapsulating material is a crucial step that requires careful consideration and planning in the design of the nanosystem. This choice significantly impacts bioaccessibility and biodisponibility, as well as the physicochemical characteristics of the synthesized nanomaterials. These characteristics play a pivotal role in determining the biological fate of nanomaterials (Dhapte & Pokharkar, 2019; Dima et al., 2020).

6.2. *Nanoencapsulation techniques*

Two primary methodologies are employed in the realm of nanoscale material synthesis for food sector: top-down (physical methods) and bottom-up (chemical methods). In the top-down approach, the bulk material is broken down or fragmented to nanometer scales from its larger form. Conversely, in the bottom-up approach, structures are meticulously constructed, atom by atom, and molecules coalesce to form structures at the nanometer scale (Dhand et al., 2015).

Nanoencapsulation techniques can be classified into physical, chemical, and biological processes. Physical methods are environmentally friendly as they do not require solvents and produce uniform-sized materials, but they tend to be costlier. These methods involve applying thermal or electrical energy to cause abrasion, mechanical pressure, high-energy radiation, melting, evaporation, or condensation to reduce bulk materials to nanoparticles. Standard physical methods include high energy ball milling, spray pyrolysis, laser pyrolysis, laser ablation, and electrospraying (Dhand et al., 2015; Tolve et al., 2016). Chemical methods, on the other hand, operate under controlled heat and pressure conditions to produce nanomaterials from solutions with specific quantities of ions. Examples of chemical methods include chemical vapor deposition, colloidal dispersion, microemulsion, epitaxial growth, hydrothermal route, sol-gel, polymer route, and other precipitation processes (Tulinski & Jurczyk, 2017). Lastly, biological methods use parts of plants and microorganisms to produce nanoscale materials. These methods are eco-friendly, simple, and often involve a single step (Kolahalam et al., 2019).

The properties of synthesized materials can vary based on the chosen synthesis method. Selecting the appropriate nanoencapsulation technique requires careful consideration of the chemical and physical properties of both the core and coating materials, the desired particle size, the complexity of the technology, the processing time, cost, commercial viability, and environmental impact (Dima, Assadpour, Dima, & Jafari, 2020; Jafari & McClements, 2017; Tolve, Galgano, & Caruso, 2016).

7. Nano-system based strategies used in the design of non-dairy probiotic matrices

Incorporating probiotics into food products and delivering them to the intestine at a minimum therapeutic concentration while maintaining their metabolic activity to exert their functions in the host and avoiding undesirable interactions with the food matrix presents a significant challenge. Strategies such as Nanoencapsulation can address this challenge. Nano-systems have the potential to enhance bioavailability, adhesion to the intestinal mucosa, interactions with enzymes, and other metabolic factors without altering the sensory characteristics of food products (Reque & Brandelli, 2021). This section discusses applying the most commonly nano-encapsulating systems used in the food sector and the main advantages when applied to different probiotic strains to address the challenges above, as outlined in Table 1.

7.1. *Nanofibers*

Nanofibers are cylindrical structures with a diameter below 1000 nm and an aspect ratio greater than 50 (Wilson, 2010). This class of materials can be tailored to specific applications, such as the encapsulation of probiotic strains. The electrospinning technique enables the fabrication of nanofibers using a wide variety of biopolymers, with a preference for biodegradable and biocompatible polymers for nanoencapsulation of probiotics (Fung et al., 2011; Ceylan et al., 2018). The electrospinning process involves creating long, thin polymeric structures by extruding a solution from a syringe through the distortion of an electric field. The probiotic strain can be incorporated into the solution, typically composed of polyvinyl alcohol alone or combined with other polymers (Torp et al., 2022).

One critical characteristic of nanofibers is their substantial surface area, imparting several benefits, including high immobilization efficiency, enhanced protection, and minimal sensory effects when applied in food systems as carrier materials (Jayani et al., 2020). Table 1 summarizes the impact of probiotic strains nanoencapsulation, focusing on improving cell survival under adverse conditions.

Numerous studies have highlighted the potential of nanoencapsulation in bolstering the survival of probiotic strains and shielding them from challenging environmental conditions such as oxygen exposure and harsh gastric and intestinal transit. Nanoencapsulation of probiotics using nanofibers has demonstrated a significant increase in survival rates, both at elevated temperatures (35 °C) (Jayani et al., 2020) and lower temperatures (4 °C) during storage for durations ranging from 3 to 24 weeks (Škrlec et al., 2019; Xu et al., 2022). Moreover, nanofibers have shown promise in enhancing strain delivery, leading to increased colonization in the jejunum and cecum (Ajalloueian et al., 2022). This beneficial effect can be attributed to the protective role of nanofibers against the harsh conditions encountered in the gastric and intestinal environments (Mojaveri et al., 2020; Yilmaz et al., 2020; Ajalloueian et al., 2022).

In addition, nanoencapsulation using nanofiber systems enhances the thermal protection of the strains, consequently improving their viability within food systems (Yilmaz et al., 2020). Notably, integrating nanofibers in non-dairy matrices has been shown to reduce bacterial growth (Ceylan et al., 2018). Consequently, the nanoencapsulation of probiotics within nanofibers supports the shielding of probiotic strains against adverse conditions, resulting in an increased survival rate and enhanced colonization of beneficial bacterial strains within the small intestine. This approach augments the stability of probiotic products and extends their storage period. Moreover, when applied in dairy and non-dairy food systems, it enhances cell viability and imparts properties of industrial interest, such as improved thermal stability and antimicrobial activity.

7.2. *Lipid-Based nano-systems*

This class of carrier systems is among the most promising in nanoencapsulation. It includes nanoliposomes, nanoemulsions, solid lipid nanoparticles, and nanostructured lipid carriers. Lipid-based systems offer numerous advantages, including targetability, encapsulating water-soluble and lipid-soluble molecules, and the potential for industrial-scale production using various materials (Khorasani et al., 2018). Below, we describe two main lipid-based carrier systems, nanoliposomes, and nanoemulsions, and discuss their applications in developing non-dairy probiotic-based products.

Table 1
Effects of nanoencapsulation of probiotic strains as a strategy to increase the survival of cells under adverse conditions by distinct nano-system.

Probiotic strain	Wall material	Nanoencapsulation system/ technique	Food system	Results	Ref
Lactobacillus acidophilus 016	Bacterial cellulose	Nanofibers/ electrospinning	–	Immobilization of strain onto nanofibers without any damage; 71 % of immobilized strains survive up to 24 days at 35 °C.	(Jayani et al., 2020)
Bifidobacterium animalis Bb12	Chitosan/poly vinyl alcohol/inulin	Nanofibers/ electrospinning	–	Higher survivability of cells enclosed in chitosan/poly vinyl alcohol/inulin nanofibers in comparison to free cells even under simulated gastric and intestinal fluids.	(Mojaveri et al., 2020)
Lactobacillus paracasei KS-199	Alginate	Nanofibers/ electrospinning	Kefir	Nanoencapsulation enhanced thermal protection, enhanced survival of the strain in simulated gastric juice, and improved its viability/survival in kefir.	(Yilmaz et al., 2020)
Lactobacillus plantarum ATCC 8014	Polyethylene oxide/ lyoprotectant	Nanofibers/ electrospinning	–	High loading capability (up to 7.6 × 10^8 colony-forming unit/mg). The presence of lyoprotectant in the nanofibers promoted the survival of the cells. The probiotic strain was stable over 24 weeks at low temperature.	(Škrlec et al., 2019)
Lactobacillus plantarum	Poly vinyl alcohol/FOS	Nanofibers/ electrospinning	–	The survivability of cells encapsulated was significantly enhanced (6 log CFU/mL) under moist heat treatment (70 °C).	(Feng et al., 2018)
Lactobacillus rhamnosus	Polyvinyl alcohol/sodium alginate	Nanofibers/ electrospinning	Fish fillet	Delayed the total mesophilic aerobic bacteria and psychrophilic bacteria growth up to 38 %.	(Ceylan et al., 2018)
Lactobacillus rhamnosus	Polyvinyl alcohol/Pectin	Nanofibers/ electrospinning	–	Survival rate of 84.63 % after period storage of 21 days at 4 °C.	(Xu et al., 2022)
Lactobacillus rhamnosus GG	Poly.lactic-co-glycolic acid	Nanofibers/ electrospinning	–	Enhance the delivery of the strain, protect them from the intestinal transit, increasing their colonization in the jejunum and cecum.	(Ajalloueian et al., 2022)
Escherichia coli strain Nissle 1917	Polyvinylalcohol/ cellulose acetate	Nanofibers/dual-nozzle electrospinning	–	Free cells lost their viability within 100 min, whereas encapsulated cells survived with a final count of 3.9 log CFU/mL (from an initial count of 7.8 log CFU/mL	(Çanga & Dudak, 2021)
Lactobacillus acidophilus	Gelatin-chitosan polyelectrolytes coated	Nanoliposomes/ Extrusion	–	Improved its viability when exposed to simulated gastrointestinal environments.	(Adeel, Afzaal, & Saeed, 2023)
Lactobacillus rhamnosus	Chitosa-gelatin polyelectrolytes coated	Nanoliposomes/ Extrusion method	–	The entrapment of *L. rhamnosus* cells in the lipid-based nanovesicles significantly improved the resistance of the loaded cells to simulated gastrointestinal conditions compared to unencapsulated cells.	(Fakhreddin et al., 2022)
Lactobacillus acidophilus and *Bifidobacterium bifidum*	Alginate	Nanoemulsions/ Internal gelation method	Grape juice	The survivability of the bacteria in the encapsulated form was significantly higher than that in the free cells after a storage period of 60 days.	(Mokhtari et al., 2019)
Lactobacillus salivarius spp. *salivarius*	Alginate	Nanoemulsions/High pressure homogenization	Apple juice	Higer amount of viable cells that in those non-encapsulated after the process of dried at 40 °C during 24 h. and gastrointestinal simulation.	(Ester et al., 2019)
Lactobacillus plantarum and Staphylococcus xylosus	Alginate-starch	Nanoemulsion/ Emulsion method	Dry fermented sausage	Probiotic strains were protected by encapsulation against heat treatment at 70 °C during 20 min and against fermentation process with lower reduction rates.	(Bilenler et al., 2017)
Lactobacillus plantarum, Lactobacillus fermentum, Lactobacillus casei, Lysinibacillus sphaericus and Saccharomyces boulardii	Alginate coated chitosan	Nanoemulsion/-	Tomato and carrot juices	High reductions of viable probiotic cells were found in non-encapsulated cells (1.6–5.2 logs) in both vegetable juices, which were significantly higher than encapsulated strains (0.8–2.7 logs). *Lysinibacillus sphaericus* showed a better survivability in both tomato and carrot juices reduced 1.3–1.8 logs.	(Sivudu et al., 2016)
Lactobacillus rhamnosus GG	Alginate and chitosan with inulin	Polymeric system/ Extrusion	Apple juice	Encapsulation of *Lactobacillus rhamnosus* experienced a greater survival rate which was 4.5 times higher than that of free bacteria at day 90. Additionally, improved bacterial viability since 27.7 % of the bacterial population survived the gastrointestinal model.	(Gandomi et al., 2016)
Lactobacillus plantarum	Aalginate-soy protein isolate	Polymeric Hydrogel beads/Gelation method	Mango juice	The encapsulated probiotic bacteria were found alive even after treatment at 72 ◦C for 90 s and very lowpH (pH 2 and 3) and also in mango juice under pasteurization process.	(Praepanitchai et al., 2019)
Lactobacillus acidophilus LA-5 *and L. casei* 431	Calcium alginate and Hi-maize resistant starch and coated of chitosan	Polymeric Microcapsules/ Emulsion technique	Bread	*Lactobacillus casei* 431 was more resistant to high temperature than *Lactobacillus acidophilus* LA-5. A significant increase in probiotic survival was observed when the protective coating of chitosan was used in addition to calcium alginate and Hi-maize resistant starch.	(Seyedain-Ardabili et al., 2016)

(*continued on next page*)

Table 1 (*continued*)

Probiotic strain	Wall material	Nanoencapsulation system/ technique	Food system	Results	Ref
Aerococcus viridans UAM21, *Enterococcus faecium* UAM10a, *Lactobacillus plantarum* UAM17, *and Pediococcus pentosaceus* UAM11	Acacia gum	Polymeric microcapsules/Spray drying	Meat batters	The addition of encapsulated bacteria did not have any effect on flavor and texture of bread. Spray-dried probiotic strains enhanced initial count with a concomitant Enterobacteria reduction.	(Pérez-Chabela et al., 2013)

7.2.1. Nanoliposomes

Nanoliposomes are spherical vesicles composed of single or multiple phospholipid bilayer membranes. They consist of biodegradable and biocompatible agents capable of encapsulating and delivering various bioactive compounds, including probiotics (Naskar & Kim, 2021). Nanoliposomes hold significant promise in the probiotic market due to their high encapsulation efficiency, allowing for the encapsulation of both hydrophilic and hydrophobic compounds either individually or simultaneously, thereby providing a synergistic effect, targetability and the possibility of being produced at industrial scales using natural ingredients (Khorasani et al., 2018). Another distinctive advantage of employing nanoliposomes in the food sector is their capability to evade our sensory perception, enabling the incorporation of bioactive agents without affecting the sensory attributes of products (Rasti et al., 2017). Several methods can be used to prepare nanoliposomes. However, the most common method for their production involves obtaining a double emulsion followed by the removal of the solvent and a microfluidization process (Barboza Duarte, Oliveira Nascimento Mergulhão, & da Costa Silva, 2021). Encapsulation of bioactive compounds, including probiotics, can be achieved by entrapping cells during the vesicle formation process (passive encapsulation) or loading into performed vesicles (active loading) (Khorasani et al., 2018). Nanoliposomes have been investigated for their potential to encapsulate, stabilize, and deliver various types of probiotic cells. For instance, *Lactobacillus acidophilus* encapsulated by nanoliposomes significantly improved the viability of the cells when exposed to simulated gastrointestinal environments (Adeel, Afzaal, & Saeed, 2023). Similarly, Fakhreddin employed polyelectrolytes-stabilized liposomes to encapsulate *Lactobacillus rhamnosus* to improve survivability under adverse conditions; it showed the lowest viability loss under simulated gastric and intestinal fluid conditions (Fakhreddin et al., 2022).

7.2.2. Nanoemulsions

Emulsions have been extensively used in the food industry to enhance bioactive molecules' solubility, bioactivity, and stability (Alemzadeh, Hajiabbas, & Pakzad, 2020; Rodrigues, Cedran, Bicas, & Sato, 2020). Emulsion systems are colloidal dispersions typically composed of two or more immiscible liquids, commonly water and oil. These systems have two phases: a continuous phase (the surrounding liquid) and a dispersed phase (droplets) and are categorized based on the relative arrangement of the phases, namely oil-in-water (O/W) and water-in-oil (W/O) emulsions (Gu et al., 2022). O/W emulsions involve dispersing the probiotics in an oil phase, where water and a hydrophilic emulsifier facilitate the formation of probiotic-loaded oil droplets. Conversely, in W/O emulsions, probiotics are generally dispersed in the water phase, with the addition of a hydrophobic emulsifier and the oil phase resulting in the formation of probiotic-loaded water droplets. These are referred to as simple emulsions. By incorporating an additional phase, double emulsions such as oil-in-water-in-oil (O/W/O) or water-in-oil-in-water (W/O/W) can be obtained (Alemzadeh et al., 2020). However, aqueous-based systems (W/O or W/O/W) with dispersed probiotics are often preferred due to the hydrophilic nature of probiotic strains (Wang et al., 2020).

Emulsions can be prepared through chemical and physical processes, and often a combination of these methods is employed (Haji et al., 2022). These systems play a crucial role in protecting probiotic strains from gastrointestinal conditions, thus enhancing their viability and activity (Wang et al., 2020; Frakolaki et al., 2021; Quintana et al., 2021). Additionally, nanoemulsions offer higher thermodynamic stability (Reque & Brandelli, 2021) and are considered a more suitable technique for industrial-scale applications (Abdul Khalil, 2020; Koh, Lim, & Tan, 2022).

In a previous study by Mishra Pandey & Mishra, 2015, simple emulsion systems were utilized to encapsulate *Lactobacillus plantarum 299v* and metronidazole. Gum (guar and xanthan) was used in the aqueous phase, and soil flower oil in the lipidic phase. This approach demonstrated an improvement in the survival rate of probiotics during storage. In another study, Vaishanavi & Preetha, 2020 exhibited the stability and survivability of *Lactobacillus delbrueckii* subsp. b*ulgaricus* has over 40 days of storage, and is loaded in nanoemulsions-based systems containing soy protein isolate, Tween 80, and gum Arabic. Additionally, Holkem et al., 2016 demonstrated that encapsulating *Bifidobacterium* BB-12 using alginate and calcium carbonate can protect the probiotic when exposed to simulated gastric and enteric juices. This encapsulation method improved the survival and stability of the bacteria during a 60-day storage period at 25 °C.

Double emulsion-based encapsulation systems have also proven effective in encapsulating *Lactobacillus rhamnosus* LC705. This system showcased its efficiency in safeguarding encapsulated probiotics under osmotic stress in a sucrose hypertonic solution (Huerta-Vera et al., 2017). Furthermore, Wang et al., 2020 reported that encapsulation of *Lactobacillus acidophilus* AS 1.2686 using double emulsions can enhance the viability of probiotic cells during a 14-day storage period and under simulated digestion, demonstrating that 84 % of the cells remained viable.

Using emulsion-encapsulated probiotics has been previously demonstrated to be effective in their incorporation into various food matrices. Research has shown successful application in diverse foods such as grapes (Mokhtari et al., 2019), apples (Ester et al., 2019), ice cream (Zanjani et al., 2018), dry fermented sausage (Bilenler et al., 2017), as well as tomato and carrot juices (Sivudu et al., 2016). Incorporating probiotics through emulsion techniques offers a viable solution to enhance the viability of probiotics within food matrices, even when exposed to adverse conditions.

7.3. Polymeric systems

Polymeric systems play a crucial role in developing probiotic-based products in food and non-food compounds (Asgari et al., 2020). A polymeric-based transporter involves employing polymers to create a vesicular system, where probiotic cells are encapsulated within a reservoir protected by a shell. The core of this system may consist of hydrophilic or lipophilic substances (Manickam & Ashokkumar, 2014). There is a diverse range of polymers utilized for the encapsulation of probiotics, broadly categorized into naturally derived polymers such as pectin, chitosan, guar gum, gellan gum, dextran, cyclodextrin, alginates, xanthan gum, whey protein, and inulin, among others. Additionally, synthetic polymers like Eudragit L 100, Eudragit S 100, Hydroxypropyl ethylcellulose phthalate 50 or 55, and cellulose acetate trimellitate are also used (Vandamme et al., 2016; Asgari et al., 2020). When selecting a polymer for probiotic encapsulation, factors such as neutrality to the probiotic strain, processability, biodegradability, and biocompatibility are crucial considerations (Asgari et al., 2020). In the context of polymeric systems, probiotic cell suspensions are mixed with the chosen polymer (Mokhtari et al., 2019; Nami et al., 2020).

The encapsulation of probiotic cells within polymeric systems has been shown to significantly enhance the survival and viability of probiotic products during storage. Additionally, when utilized as stabilizers, polymeric systems can augment viscosity and prevent syneresis in lactic products (Huq et al., 2013). Numerous studies have provided evidence that strains such as *L. acidophilus*, *Bifidobacterium* spp., *Lactobacillus lactis*, *Lactobacillus bulgaricus*, and *Lactobacillus rhamnosus* can effectively withstand and survive harsh acidic conditions when protected within biopolymeric systems. To optimize probiotic resistance and increase cell survival through polymeric-based encapsulation techniques, factors such as the probiotic strain, capsule size, and the number of membrane coatings must be carefully considered (Hansen et al., 2002; Huq et al., 2013; Asgari et al., 2020).

Polymeric encapsulation systems are promising for delivering probiotic cells in non-dairy food products. For instance, Sivudu and Cols., 2016 encapsulated various probiotic strains, including *Saccharomyces boulardii*, *Lactobacillus plantarum*, *Lysinibacillus sphaericus*, *Lactobacillus fermentum*, and *Lactobacillus casei*, within alginate-coated chitosan beads incorporated into tomato and carrot juices. The encapsulation showed higher viability of cells compared to free cells when stored at 4 °C. Similar positive results were reported by Gandomi and Cols., 2016 and Praepanitchai and Cols., 2019, who encapsulated *Lactobacillus rhamnosus GG* and *Lactobacillus plantarum*, respectively, using mixtures of sodium alginate, inulin, chitosan, and soy protein isolate, and incorporated them into apple and mango juices. The incorporation of probiotic cells in these beads proved beneficial for cell survival. The application of polymeric encapsulation systems extends to bakeries (Seyedain-Ardabili et al., 2016) and meat products (Pérez-Chabela et al., 2013), where these systems demonstrate the potential to ensure the survival of probiotic cells before, during, and after processing of these food matrices.

8. Toxicological aspects of nano-systems for Food-Derived probiotic products

Nanotechnology has the potential to revolutionize the food industry by employing nanotechnology techniques to create delivery systems for bioactive compounds such as vitamins, minerals, prebiotics, and probiotics. However, conducting thorough assessments of the health and toxicological factors associated with incorporating nano-systems into non-dairy food matrices is essential. This evaluation is necessary to ensure their consumption does not pose health risks.

Nano-systems designed using organic, biocompatible inorganic, or hybrid materials are preferable due to their lower toxic effects (Dhapte & Pokharkar, 2019). However, even when selecting materials known to be non-toxic or recognized as GRAS, it is crucial to consider various factors, including composition, particle size, surface charge, shape, aggregation, concentration, oxidation state, exposure time, individual susceptibility, type of surface coating, and breakdown products (Onyeaka, Passaretti, Miri, & Al-Sharify, 2022; Singh, Manshian, & Jenkins, 2009; Khan, Saeed, & Khan, 2019). Therefore, a case-by-case review is recommended before their utilization in food processing. In the context of packaging, the ability to migrate, diffuse, dissolve, and disperse in food has been identified as a factor related to toxicity (Onyeaka et al., 2022).

Once nanomaterials encounter the organism, regardless of the route of exposure (nasal, dermal, or oral), several direct or indirect mechanisms can cause cellular damage. Nanomaterials administered non-intravenously are distributed systemically, whereas those administered intravenously exhibit a faster elimination rate and tend to bioaccumulate in the liver and spleen due to these organs' rich phagocytic mononuclear system (Kaphle et al., 2018; Liu et al., 2022). Furthermore, nanomaterials can cross the blood–brain, blood-testicular, and placental barriers once in circulation, potentially affecting these tissues. Metabolism, degradation, and elimination of nanomaterials primarily occur at the hepatic and renal levels, although the lung and intestine are also targets for toxicity. Particularly in these organs, inflammation, granulomas, fibrosis, and dysfunction have been observed (Peng et al., 2020; Liu et al., 2022).

At the cellular level, nanomaterials can adhere to membranes and undergo endocytosis through caltrin- or caveolin-dependent or -independent pathways (Valsami-Jones & Lynch, 2015). Upon entry, they become trapped in vesicles for transport to endosomes and ultimately fuse with lysosomes, where they can undergo enzymatic modification or be expelled into the extracellular space. In caveolin-mediated transport, nanomaterials are confined to the Golgi apparatus and endoplasmic reticulum via caveolae (Donahue et al., 2019; Liu et al., 2022). Additionally, nanomaterials can be transported via micropinocytosis or phagocytosis (Valsami-Jones & Lynch, 2015). Nanomaterials that diffuse and evade lysosomes can be distributed across all cellular organelles.

These nanomaterials can induce diverse cytotoxic effects mediated by mitochondrial dysfunction, increased ROS production, an imbalance in the oxidation–reduction system, thus damaging macromolecular components such as lipids, proteins, nucleotides, and even altering chromatin conformation through interaction with histone proteins, which play a role in cancer development (Crisponi, Nurchi, & Lachowicz, 2017; Demir, 2020; Khanna, Ong, Bay, & Baeg, 2015; Liu, Zhu, & Gu, 2022; Navya & Daima, 2016; Ranjan, Dasgupta, Singh, & Gandhi, 2019). These effects lead to membrane disruption and increased permeability, reticulum stress induction, cytoskeleton alteration, proliferation signaling pathway blockade, genotoxicity, and activation of apoptosis cascades. Ultimately, this damage manifests through an NF-kB-mediated inflammatory process, cell cycle arrest, and cell death via different pathways (Liu et al., 2022).

Therefore, considering the potential safety risks associated with using nano-systems in food-derived probiotic products, a thorough understanding of nanomaterials' properties and possible toxicity is imperative to ensure food safety. Further evaluations and studies are needed to comprehensively identify the molecular mechanisms through which nanometric systems interact with food and living organisms. The advantages offered by nanometric systems may be limited if they pose clear health risks to the living beings they come into contact.

While numerous *in vitro* and *in vivo* toxicological evaluations have been conducted, the outcomes often present contradictions, underscoring the necessity for further assessments. A well-planned design is imperative, rooted in a comprehensive understanding of the physicochemical properties of nanomaterials and their biological interactions. Extensive evaluations using both *in vitro* and *in vivo* experimental models are crucial to identify potential toxicological responses, ensure biocompatibility, and minimize potential adverse health risks before the widespread application of nanomaterials in food-derived probiotic products.

The toxicological study of nanomaterials should go hand in hand with their regulation in the food industry. Presently, the Food and Drug Administration (FDA) regulates the use of nanoparticles in food packaging and requires manufacturers to notify the use of nanoparticles as additives through the Food Contact Notification (FCN) system. In Europe, the European Commission (EC) guidelines, specifically EC 1935/2004, regulate the use of nanoparticles in food packaging to ensure consumer health is not compromised (Sodano, 2018; Onyeaka et al., 2022).

However, regulatory processes must still be considered when using nanomaterials. In addition to being used in food packaging, nanomaterials can also be added as ingredients to food or nutraceuticals, potentially impacting the population's health. Legislation should comprehensively address the absorption routes of nanomaterials in all stages of the food chain, their potential synergy with other ingredients or microorganisms present in the food, and their impact on the environment during processing or disposal.

The study of the use and effects of consuming or coming into contact with nanomaterials, both in the short and long term, is a significant concern for the scientific and governmental community. Understanding emerging technologies is essential for establishing regulations and implementing intervention policies that safeguard the health of those working with these materials and the consumers at large.

9. Conclusions

The consumption of probiotics has increased due to the population's knowledge of their beneficial effects on intestinal health and gastrointestinal pathological processes. The bacterial genera *Lactobacillus* and *Bifidobacterium* are the most used in the design of functional foods, which are usually present in dairy products, cereals, beverages, and fermented foods. However, the market for functional foods based on probiotics has been evolving because today's consumer demands a greater variety of this type of product for their lifestyle or various health conditions that they may present.

Therefore, nanotechnology can be an opportunity due to the possibility of nanoencapsulation of these microorganisms in non-dairy matrices, thus offering a promising and innovative alternative to preserve viability, stability, resistance to external environmental factors and stress conditions, and enhanced bioavailability. Furthermore, they demonstrate significant potential as carrier systems for the storage, transport, and controlled release of probiotics without the drawbacks associated with dairy and fermentable matrices.

However, the encapsulation process in nano-systems must consider variables such as the selection of the probiotic organism, the nanocarrier material type, and the encapsulation techniques for the probiotic organism. Likewise, the choice of non-toxic materials must be considered, thus preventing their consumption from compromising health or the environment.

Ethical Statement.

This literature review does not involve direct research on human or animal subjects, and therefore, ethical approval was not required for the present study. The information presented in this work is based on a comprehensive review and synthesis of existing literature.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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