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ENCAPSULATED PROBIOTIC MICROORGANISMS IN FUNCTIONAL FOOD PRODUCTS

Alterations in the composition of the gut microbiota are associated with a wide range of pathologies, including not only inflammatory diseases of the gastrointestinal tract, but also diabetes, obesity, cancer, and diseases of the cardiovascular and central nervous systems. With an imbalance of the microbiota (dysbiosis), there is increased intestinal permeability and a violation of local or systemic immune responses. One of the possible ways to improve intestinal microbiota is the use of dietary supplements and functional food products enriched with highly effective encapsulated probiotic microorganisms, as well as prebiotic compounds. Such products contribute to the restoration of normal intestinal microflora and its integrity, and also indirectly affect the positive outcome in the treatment of many pathological conditions mediated by an imbalance in the intestinal microbiota. Maintaining the activity of probiotics in food carriers or functional food products designed for the prevention and complex therapy of various pathological conditions is important both for the normalization of the intestinal microflora and the health of the body as a whole. In this context, encapsulation is an effective approach to maintain the viability and stability of probiotics under adverse conditions in the gastrointestinal tract and also an effective way to protect from processing conditions, temperature, and transportation. The development of functional nutrition products enriched with highly effective encapsulated probiotic microorganisms is a priority for new research in the field of prevention and treatment in microbiota-targeted therapy. The use of such products is based on the conception of 3p — pathophysiology-based individualized use of probiotics and prebiotics in various pathological conditions mediated by a violation of the qualitative and/or quantitative composition of the intestinal microbiota: implementing a predictive, preventive, and personalized medical approach.

Keywords: *functional foods, probiotics, encapsulation, microbiota-targeted therapy.*

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Encapsulated probiotics applied in food is an upward trend in which «classic probiotic foods» (yogurt, cheese, butter, chocolate, etc.) are dominated, supplemented by «novel probiotic foods» (tea, peanut butter, and various dry-based foods). Encapsulation provides a strong impetus for the food application of probiotics [1—10].

Encapsulation is one of the most effective strategies for preserving the viability of probiotics [9]. An ideal encapsulation technology would protect probiotics from degradation during storage and in the upper gastrointestinal tract (GIT) and then release them into the colon. In addition, it should be economically feasible to produce microcapsules on an industrial scale. Microcapsules should not adversely affect the desired quality characteristics of foodstuffs and should be made from ingredients that are not restricted for use in various pathological conditions of consumers (for example, lactase deficiency and gluten intolerance). Calcium alginate microgels are one of the most commonly used microcapsules for the encapsulation, protection, and delivery of probiotics because they meet many of these requirements. Alginate is not digested in the upper GIT (mouth, stomach, and small intestine), but is fermented in the lower GIT (colon) by enzymes secreted by colon bacteria. The digestion/fermentation characteristics of alginate mean it can be used to assemble microgels that encapsulate and retain probiotics in the upper GIT but then release them in the lower GIT [9, 11].

As the probiotic market continues to grow at a rapid pace, the development of new strains is becoming increasingly valuable, but regulatory compliance for new probiotic strains can present challenges. New strains generally require regulatory approval to legally enter the market, even if some strains of the species have regulatory approval or history of use. Probiotic strains, as the basis of functional food products, dietary supplements, or probiotic preparations, must comply with regulatory requirements, namely: generally recognized as safe (GRAS) or new dietary ingredient notifications (NDIN) [12, 13].

There is an urgent need to develop new and effective food products containing probiotic microorganisms. The probiotic functional food market is dominated by dairy products (i.e., yogurt, cheese, and fermented milk products). However, dairy products are generally not stored for long periods of time and require low-temperature storage, which limits the convenience of delivering probiotics through food systems. Some studies have shown the possibility of including probiotics in shelf-stable products such as chocolate, peanut butter, and cereals [2], but the commercialization stage of these products remains difficult. In addition, lactose and milk protein limit the use of dairy products by people with lactose intolerance or allergies. Most commercial probiotic products and supplements are not available for custom probiotic strains and dosages, making them less effective for individuals and groups with special needs. Accordingly, there is a growing demand for effective and innovative functional foods enriched with viable probiotic microorganisms. The viability of the probiotic microorganism is an important aspect to consider in long-term storage.

Encapsulation technologies for probiotic microorganisms have shown excellent results in maintaining the high viability of probiotics in foods. Encapsulation is a process of capturing a cell of a probiotic microorganism inside a matrix or membrane, in the microenvironment of which living cells can maintain their viability under the influence of adverse environmental factors during production and storage, as well as in the digestive system. The most commonly used carrier materials for the encapsulation of probiotic bacteria are polysaccharides (alginate, gum, chitosan, and κ-carrageenan), protein (gelatin, whey protein, and milk protein), and fats. Alginate, a polysaccharide derived from algae, attracts special attention as a carrier of probiotic microorganisms during encapsulation, since it is biocompatible, biodegradable, and low toxic. Many studies have shown the effective possibility of encapsulating probiotics in alginate-gelatin

microcapsules (maintaining the concentration of viable probiotic bacteria after 4 months of storage at 8 °C), a gelatin coating of alginate microspheres (protects probiotic bacteria from the unfavorable environment of GIT) [1—13].

The study by Kuo C-C et al [1] demonstrated an integrated method of encapsulation, 3D printing, and freeze-drying for the manufacture of storage-stable food products and probiotic carriers. Namely, an alginate-gelatin hydrogel carrier of *Bifidobacterium lactis* and *Lactobacillus acidophilus* (singly or in combination) is assumed, with a cell viability of 10^6 CFU/g and above after using an integrated approach.

Classical and modern technologies of probiotic microorganisms' encapsulation. A number of studies have shown that the viability of probiotics can be improved by encapsulating them in microgels or other types of microcapsules [1—16].

Probiotic microorganisms' delivery systems to improve their viability can be developed in a variety of ways. First, they can form a physical barrier that protects probiotics from any problematic environmental components (stomach acids, bile salts, digestive enzymes). Second, they can be designed to co-encapsulate probiotics with specific nutrients (easily digestible carbohydrates, dietary fiber, proteins, lipids, and minerals) that help probiotic microorganisms survive [17]. Third, they may contain additives that provide a favorable local climate (antacids to control local pH) for probiotic microorganisms [18]. Finally, the microparticles may contain, in addition to probiotic microorganisms, products secreted by them that contribute to their survival. For example, some probiotic microorganisms secrete bile salt hydrolyzing enzymes that protect probiotics from bile salts in the small intestine.

Other additives are used, for example, regulating the level of oxygen or osmotic stress inside the microparticles. Table 1 presents various probiotic microorganisms' delivery systems and their functional features.

Currently, various techniques for the encapsulation of probiotic microorganisms are used. However, it is imperative that when choosing a technique, it should be taken into account that the process should not be aggressive provide sufficient viability of the encapsulated cells and have mechanical stability compatible with the purpose of application.

Several probiotic cell encapsulation methods are currently available, such as extrusion, emulsion, spray drying, spray cooling, fluid bed, freeze drying, spray freeze drying, coacervation, electrospray, etc. [3, 33].

The traditional methods for encapsulating probiotic microorganisms are as follows.

Spray drying is a widely used method in the food industry. It features low cost, fast processing, and high productivity. The method consists in spraying a solution containing an encapsulated agent in a high-temperature gas, instantly forming a powder. The combined use of different polymers can improve the survival of encapsulated cells. To overcome the negative effects of high temperatures during spray drying and improve the resistance and stability of encapsulated cells, prebiotics, soluble fibers, gums, and mucus are added to the sealing material as a thermal protection [34].

Emulsion method is used in the pharmaceutical and food industries to improve the solubility, physiological activity, and stability of compounds of interest. Emulsified systems can be used to encapsulate probiotics, improving the protection of encapsulated cells during storage as well as under the harsh conditions of the GIT [3, 35, 36].

The particle size of the encapsulated probiotic is extremely important for the organoleptic characterization of food products. For example, internal ionic gelation using alginate-whey protein mixtures to encapsulate *L. acidophilus* PTCC 1643 produces particles ranging in size from 33 to 180 μm, which is suitable for food applications. This method is used to improve the

Table 1. **Encapsulation of probiotic microorganisms in various delivery systems and their functional features**

Probiotic strain	Matrix composition	Delivery system	Functional features
Lactococcus lactis subsp. *cremoris* LM0230	alginate	simple microgel	Increasing resistance to aggressive environmental conditions [19]
B. animalis subsp. *lactis* BB-12	alginate	simple microgel	Increased viability in GIT and during storage; controlled release [20]
L. rhamnosus GG	pectin	simple microgel	Increasing viability in GIT [19]
L. plantarum KLDS 1.0344	potato starch	simple microgel	Reduced effects of chronic lead toxicity in mice [21]
L. plantarum CECT 220 and *L. casei* CECT 475	soy protein	simple microparticles	Increased stability during storage and passage of GIT [17]
B. longum subsp. *longum* and *B. longum* subsp. *infantis*	alginate, chitosan	core-shell microgels	Increased viability in GIT; colon-targeted [19]
B. bifidum	zein (corn protein), alginate	core-shell microgels	Increased viability in GIT; high resistance to gastric juice [22]
L. rhamnosus GG	polyalginate, polyelectrolyte	core-shell microgels	Increasing vitality and functional metabolism during passage of GIT [23]
L. acidophilus La-14	whey protein, alginate	core-shell microgels	Increased vitality in GIT [24]
L. salivarus Li01	gelatin, alginate	biopolymer microgel complex	Increased viability during long-term storage, high temperatures, and aggressive GIT conditions [16]
L. casei 01	starch, alginate	biopolymer microgel complex	Increased viability in GIT; the main materials are potential prebiotics; loading more probiotic cells [19]
L. acidophilus	gelatin, gum arabic, transglutaminase	biopolymer microgel complex	Higher encapsulation efficiency; increased stability and viability in GIT [25]
Pediococcus pentosaceus Li05	alginate, gelatin, MgO nanoparticles	gastro-resistant microgels	Pore filling; antacid [18]
B. pseudocatenulatum G7	alginate, $CaCO_3$, $Mg(OH)_2$	gastro-resistant microgels	Antacid [26]
L. rhamnosus	alginate, CNCs, lecithin	gastro-resistant microgels	Increased viability in GIT [27]
L. rhamnosus GG	alginate, EDTA	pH-sensitive microgels	Controlled release [28]
L. fermentum L7	oligosaccharides, alginate	nutrient-enhanced microgels	Cell protection at low temperatures and passage of GIT [29]
L. casei	alginate, sea buckthorn	nutrient-enhanced microgels	Protection of probiotics from elevated temperatures and during the passage of GIT [30]
Bacillus coagulans	alginate, chitosan	layer-by-layer direct layering on the cell surface	Protection during the passage of GIT, improving their adhesion and growth in the intestine [19]
Escherichia coli K12 MG1655	alginate	electrospinning of alginate-based nanofibers filled with bacteria	Protection during the passage of GIT, control of diffusion of the probiotic from the material [31]
B. animalis subsp. *lactis* DSM 33443	ethyl cellulose	electrospray	Increased viability and shelf life [32]

survival of encapsulated bacteria in the aggressive conditions of GIT [35].

Extrusion is used to encapsulate bacterial cells. Simple, easy to use, and low cost, in addition to being a relatively gentle process that ensures high viability of the encapsulated cells. This method involves the use of hydrocolloid solutions containing microbial cultures, which are extruded through a nozzle into a crosslinking solution, providing an instant transition of the hydrocolloid solution to a gel, which ends with the formation of beads. The resulting gel is usually stable in acidic media but degrades in alkaline media. The disadvantages of this method are that it is slow (making it difficult to apply on a large scale), ineffective for obtaining microspheres smaller than 500 μm, and requires the use of hydrocolloid solutions with low and medium viscosity.

For the encapsulation of bacterial cells by extrusion, various polysaccharides can be used such as alginate, chitosan, and gums.

Complex coacervation is one of the traditional encapsulation types for probiotics [37—39].

Spray chilling (spray cooling, spray freezing, spray congealing) in the encapsulation procedure consists in dispersing the encapsulated agent in a molten matrix formed by lipids, which is sprayed into a chamber where cold air is supplied, which allows the particles to solidify. The process conditions of this method represent an excellent alternative to encapsulating microbial cells, especially due to the low cost and industrial-scale application. In addition, it does not require the use of organic solvents and high temperatures, which favors the encapsulation of heat-sensitive agents. The use of lipid matrices can prolong cell viability during storage and provide a controlled release of encapsulated cells in GIT. In addition, spray cooling requires special carrier materials, which must be hydrophobic in nature and melt at temperatures lower than those that are detrimental to the encapsulated cells. *L. acidophilus* and *B. animalis* subsp. *lactis* have been effectively encapsulated by spray cooling. The resulting particles were added to savory cereal bars and did not interfere with sensory sensations. The encapsulated microorganisms had high viability when stored in the refrigerator for at least 90 days. Similarly, particles containing *Saccharomyces boulardii*, *L. acidophilus,* and *B. bifidum* have been obtained by spray cooling for cake production [40]. This technique has also been studied to preserve the viability of encapsulated cells under gastric and intestinal conditions [41].

Fluidized bed processes are used for coating, granulating, or drying, in which the coating is sprayed onto solid particles in suspension. This method is time-saving, low-cost, and high-performance. It is effective for the production of alginate-chitosan microcapsules containing *L. plantarum* NCIMB 8826 and *L. acidophilus* TISTR 1338 [3].

New methods for encapsulating probiotic microorganisms are as follows.

Liposomes have been reported to encapsulate probiotics successfully. There were developed *L. rhamnosus*-loaded nanoliposomes coated with chitosan and gelatin, the size of which was between 373.2 and 495.8 nm. Viable probiotics were proven to be encapsulated in these nanoliposomes by fluorescence imaging. In addition, the encapsulation resulted in improved survival during digestion [8, 42].

Cryomilling is a mechanical grinding method of ball milling, the temperature of which was lower than −150 °C controlled by liquid nitrogen or liquid argon. For the first time**,** it was developed as the microencapsulation of *L. acidophilus* into solid lipid microparticles by the cryomilling method, improving the survival of probiotics in harsh conditions. The advantages of cryomilling are low temperature, short processing time, and lack of solvents [8, 43].

Nanofibers are a relatively novel type of encapsulation of probiotics. electrospun nanofiber mats are prepared using corn starch and sodium alginate to encapsulate lactobacilli and bifidobacteria. Multi-layer poly-lactic-co-gly-

colic acid/pullulan/poly-lactic-co-glycolic acid electrospun nanofibers were prepared and used to improve the delivery of probiotics, and the authors found a higher survival of *L. rhamnosus* GG from multilayer construct compared to a monolayer construct [8, 44, 45].

Edible film packaging is being paid attention to instead of using traditional plastic films due to the strengthening awareness of environmental protection. An edible film can act as a carrier for probiotics. For example, whey protein isolate, carrageenan, and whey protein isolate/carrageenan blend films containing *L. acidophilus*, *L. plantarum* and mixed culture (*Lactobacillus* spp., *Lactococcus* spp., and *Bifidobacterium* spp.) have been developed to study the survival of probiotics during storage at 4 and 25 °C for 30 days, and the results indicated that multi-strain mixed culture had higher survival within polymer matrices [46].

3D printing has been widely recognized in the food field, and recently, it has been applied to the encapsulation of probiotics. There has been developed a shelf-stable and gel-based carrier for probiotics, including *B. lactis* and *L. acidophilus*, by encapsulation, 3D printing, and freeze-drying, which is a feasible technology to protect probiotics in a gel matrix, and the authors found that the survival of *B. lactis* was maintained above 6 log CFU/g over two months of storage [8, 49]. As 4D/5D/6D printing progresses in food, they will also be applied to the encapsulation of probiotics, providing better survival and enriching foods [48].

Single-cell encapsulation is a precise encapsulation method that uses one cell as an encapsulation object. Generally, this encapsulation achieves nanoscale coatings on the cell surface [8, 49]. A self-assembling single-cell encapsulation improves the stability of anaerobic microbes. Its specificity is that polyphenols and iron ions form a metal network structure on the surface of probiotics. Interestingly, the anaerobic microbes could have high survival even in the absence of canonical cryoprotectants during freeze-drying and oxygen exposure [8, 50].

Spray-drying with a three-fluid coaxial nozzle is the transformation based on traditional encapsulation techniques added with the latest breakthrough: a novel spray-drying technique with a three-fluid coaxial nozzle has been developed to achieve a single step of particle formation, alginate crosslinking, and drying, streamlining the production of powdered encapsulation of probiotics. The authors found that the formulation of crosslinked alginate with sucrose exhibited the highest survival after spray-drying and simulated gastric fluid that the mechanism is which is generalized across multiple probiotics, including *L. rhamnosus* GG, *Lactiplantibacillus plantarum* [51].

The microfluidic process is an encapsulation technology that has emerged in recent years, and there has been considerable progress in the encapsulation of nutrients. Current advances in probiotic encapsulation also touch on it. The authors [52, 53] presented dual-core microcapsules to encapsulate *Lactobacillus* and *Bacillus subtilis* into separate compartments by microfluidic, which is an ideal candidate for treating metabolic syndrome and related diseases.

Electrospinning is a versatile method for the continuous production of nanofibers ranging in diameter from nanometers to micrometers, which was first introduced in 1934 by Formhals. Electrospun fibers are widely used in almost all areas of research: tissue engineering, energy storage and conversion, food packaging, drug delivery and release, catalysts, sensors, filtration, etc. Electrospinning nanofibers are mainly used for encapsulation of antioxidants, antimicrobials, enzymes, and probiotics and packaging in the food industry [54—56]. However, further research is needed to improve this delivery system for commercial use. In particular, more research is needed to realize the large-scale commercial production of functional foods and supplements fortified with probiotic microorganisms.

Coating techniques can also be used to enhance the viability of probiotic microorganisms,

both in foods and as probiotic pharmaceuticals. In this case, individual probiotic cells are covered with a layer of material. Coating technologies are particularly suitable for increasing the mucoadhesion of probiotics. Unfortunately, there is still not enough research on mucoadhesion and colonization of encapsulated bacteria in the colon. Strong mucoadhesive characteristics allow probiotics to adhere to the intestinal mucosa after their release in the colon. The microgels used to encapsulate probiotics are significant in their external dimensions with a diameter range from tens to thousands of microns [8], which is also related to their retention time in the intestinal lumen (the larger the size, the shorter the retention time).

One of the approaches involves sequential layer-by-layer deposition of oppositely charged biopolymers on the surface of probiotics by electrostatic deposition. This approach leads to the formation of nanolaminated biopolymer coatings from 2 to 10 layers around the probiotics. The thickness of each layer is from 4 to 5 nm. The biopolymers used generally have enteric and mucoadhesive properties. Commonly used food biopolymers are polysaccharides or proteins such as alginate, pectin, starch, gelatin, and whey protein. So, the probiotic microorganism *B. coagulans* was coated with double layers of chitosan/alginate using this approach. In addition to increased viability in the upper GIT, this coating also exhibits greater mucoadhesion to the mucosa, which is associated with the strong adhesive properties of chitosan and alginate. In addition, the number of bilayers (chitosan/alginate) affects viability in GI conditions. A single bilayer is less effective for protecting probiotics than a double bilayer, while a triple bilayer coating delays the exponential growth phase of microorganisms by 10 hours [8, 48—53].

An alternative approach is to encapsulate live probiotic cells in an alginate-based nanofiber shell that can be easily swallowed. Cross-linked alginate materials are stable in the low-pH environment of the stomach, and the crosslinks are reversed in high- pH environments such as the intestines. The controllable and reversible nature of these cross-links makes alginate a promising encapsulating polymer for targeted delivery of probiotics to the gut. Alginate hydrogel microbeads have been investigated for the encapsulation of drugs, proteins, and bacteria. Unfortunately, the size of these microbeads, which range from a few microns to several hundreds of microns, limits their use in foods, as the chewing process will immediately break the beads and release the encapsulated cargo. Electrospinning can be used to form nanofibers that are much smaller than such microgranules [57].

Nanofiber-based technologies are of growing interest in the drug delivery industry due to their high surface area- to-volume ratio, which allows control of the diffusion of active ingredients from the material. During electrospinning, solid nanofibers are formed due to the action of an electric field on a polymer solution. Fiber formation, diameter, and morphology depend on the properties of the solution such as concentration, density, conductivity, and surface tension, as well as on such experimental parameters as the solution flow rate, collector tip, distance, and applied voltage. The high surface area- to-volume ratio and high porosity of these sheath nanofibers make them suitable for many biomedical applications including protective clothing, wound dressings, vascular grafts, and drug delivery. Electrospinning can be used to encapsulate a wide variety of viable bacteria, both gram-negative and gram-positive, into the cells of dry mats of nanofibers [57].

Unfortunately, many of the delivery systems described in the literature are unsuitable for commercial use because they are produced using ingredients or processes that are not economically viable, do not maintain viability under the real processing and storage conditions, and adversely affect the organoleptic characteristics of foodstuffs (appearance, texture) and stability.

Encapsulated probiotic microorganisms in the food industry. In recent years, the global market for nutritional supplements and functional foods fortified with probiotic microorganisms has grown exponentially due to their potential health benefits. In 2019, the global market for probiotics was valued at US$ 48.4 billion and is expected to reach US$ 77.09 billion by 2025 [3]. For a positive effect on the host organism, it is generally accepted that the number of viable probiotic cells present in the food matrix should reach a minimum concentration of 10^6 — 10^7 CFU per gram or mL (Food and Agriculture Organization (FAO)/ World Health Organization (WHO), 2002). Various food matrices have been used to deliver probiotic cells. Thus, NextFoods under the GoodBelly Probiotics brand produces juices, supplements, yoghurts, and cereals containing strains of *L. plantarum* 299v or *B. animalis* subsp. *lactis*. KeVita manufactures fermented kefir drinks containing *B. coagulans* GBI-30, 6086, a natural probiotic ingredient. The Korean company Binggrae makes a fermented milk product known as Dr.Capsule 1000, which, according to the company, contains strains of *Lactobacillus* which have a 1,000-fold higher survival rate [58]. However, these companies do not report methods for encapsulating probiotic cells.

Micropharma Inc. (Canada) and Danone Research have jointly developed fermented milk containing Cardioviva™, which is a culture of encapsulated *L. reuteri*. The Mexican company Yoplait Inc. produced yogurt containing capsules of bifidobacteria [58]. The Institut Rosell & Lal'food produced chocolate containing encapsulated probiotic cells using the Probiocap® technology. Balchem Encapsulates and Institut Rosell have produced nutrients and chocolate bars in which microorganism delivery reaches about 100%. The Kerry group in Ireland partnered with Chr Hansen to develop an orange juice containing encapsulated probiotic strains (Probio-Tec®), which kept the probiotic viable throughout the product's shelf life [59]. Thus, cell encapsulation, as an alternative to improving the survival of probiotics added to foods, is a promising method (Table 2).

Encapsulated probiotic microorganisms in dairy products. Historically, probiotic microorganisms were found in fermented foods or non-fermented dairy products. These foods, especially fermented dairy products, are considered ideal for delivering bacteria with probiotic properties, as the amount of carbohydrates, proteins, and lipids present in this food matrix can improve the survival of bacteria added to milk.

Thus, the food industry is interested in developing dairy products containing probiotic cells [69]. Generally, dairy products are acidic and, depending on pH, may not be the ideal environment for the stability of probiotic microorganisms. Thus, encapsulation techniques can be used to improve the survival of encapsulated bacteria in stressful environments. The production and storage of fermented milk products containing *L. casei* ATCC 393 encapsulated in alginate by extrusion were studied by the authors [66]. Encapsulated cells showed greater viability than free cells after 28 days of refrigeration. In addition, fermented milk products have shown an improvement in sensory performance through the production of *L. casei* aromatic compounds. The production of ice cream enriched with microencapsulated *L. acidophilus* ATCC 4356 in alginate and carrageenan has been reported as well [58]. Encapsulation improved the survival of the encapsulated probiotic in ice cream compared to unencapsulated cells during refrigerated storage and passage through a simulated GIT. The authors considered both materials for encapsulation, however, alginate particles showed better release compared to carrageenan.

In the production of cell-encapsulated probiotic fermented milk by spray drying, adding the encapsulated cell to the product did not affect its organoleptic aspects [66].

Encapsulated probiotic microorganisms in meat products. Meat and meat products are important

foods in the human diet and are included in the diets of consumers around the world. However, the current excessive consumption of them leads to health disorders such as cardiovascular disease, type 2 diabetes, and cancer, especially colorectal. The use of probiotic microorganisms is one of the available approaches to increase the value of meat products and increase their health benefits, and in this sense, encapsulated probiotics can be used. Fermented meat products consumed without heat treatment are considered effective vehicles for probiotic bacteria since the absence of heat helps to maintain the cellular viability of microorganisms. The use of an encapsulated probiotic in salami has been studied. The use of *L. plantarum* ATCC 7469 and *L. rhamnosus* ATCC 10012 encapsulated forms during the refrigerated storage (10 days) of beef patties [4]. The use of alginate to encapsulate *L. curvatus*, which produces bacteriocins, by extrusion did not affect the production of bacteriocins and the viability of bacteria during the product storage. The incorporation of encapsulated *L. plantarum* by extrusion and emulsion methods into chorizo (a typical dry-fermented Spanish sausage) was reported in [70]. Alginate particles obtained by extrusion showed higher survival rates of the encapsulated probiotic during product maturation and storage. In addition, chorizo containing such particles showed higher organoleptic characteristics than that with emulsified particles.

Thus, the inclusion of encapsulated probiotic cells in meat products is an alternative to conventional products.

*Table 2.***Encapsulated probiotics in food matrices**

Probiotic	Method	Encapsulating material	Food matrix
L. acidophilus LA-5, *Bifidobacterium* BB-12	fluidized bed	whey protein hydrolysate—xantham gum	ready-to-reconstitute functional beverage [3]
L. curvatus MBSa2	extrusion	alginate	salami [3]
L. rhamnosus GG	extrusion	chitosan-alginate-inulin	apple juice [3]
L. casei ATCC 39392, *B. adolescentis* ATCC 15703	emulsion	alginate, wheat, rice, and high amylose corn starches with chitosan and poly L-lysine coatings	ice-cream[59]
L. plantarum ATCC 2331	emulsion	alginate-starch	dry fermented sausage [60]
L. reuteri	spray drying	alginate	tuna burger [61]
L. plantarum HM47	spray drying	maltodextrin, moringa oleifera gum, tender coconut water	milk chocolate [62]
B. animalis ssp. *lactis* BB12	spray drying	maltodextrin and/or inulin	powdered passion fruit juice [63]
S. boulardii, *L. acidophilus* LA-5, *B. bifidum* BB-12	spray drying/ spray chilling	arabic gum—β-cyclodextrin/ hydrogenated palm oil—Tween 80	cake [40]
L. acidophilus LA-5	spray drying	inulin	mousse [34]
L. rhamnosus ATCC 53103	extrusion	alginate—quince seed mucilage	dairy dessert [64]
L. reuteri PTCC 1655, *L. reuteri* ATCC 32272-DSM 200016	extrusion	alginate—inulin-lecithin	chewing gum [65]
L. casei ATCC 393	extrusion	alginate	fermented milk [66]
L. rhamnosus	extrusion	alginate	reduced-fat cream cheese [67]
L. rhamnosus GG	spray drying	whey protein isolate and modified huauzontle's starch	ready-to-drink green tea beverage [68]

Table 3. **Application of probiotic microorganisms, as the basis of functional food products, in microbiota-targeted therapies**

Pathological condition	Microbiota modulation tool	Probiotic microorganisms
	Infectious disease	
Clostridium difficile	Antibiotics Fecal microbiota transplantation Microbiota-based products	Multi-strain probiotics consortium (VSL#3 contains *B. breve*, *B. longum*, *B. infantis*, *L. acidophilus*, *L. plantarum*, *L. paracasei*, *L. delbrueckii* subsp. *bulgaricus*, and *Streptococcus thermophilus* in a concentration of 450 billion live bacteria per sachet) [77, 78, 79]
Mycobacterium tuberculosis	Combinatory therapies with probiotics Postbiotics (antimicrobial peptides of probiotic microorganisms)	*Bifidobacterium* spp., *Lactobacillus* spp. [80, 81] *B. adolescentis DSM 20083* resistant to anti-tuberculosis drugs [82]
SARS-Cov2	Fecal microbiota transplantation (Pre-COVID19 as preventive setting) Probiotics (Post-COVID19 as therapeutic for the cytokine storm)	[78, 83, 84] *Bifidobacterium* and *Lactobacillus* genera [78]
	Immune compromised condition	
Cancer therapy-associated	Probiotics	*L. acidophilus, L. rhamnosus, L casei* DN-114001 [77]
Mucositis and Colitis	Fecal microbiota transplantation	*Bifidobacterium* spp., *Enterococcus gallinarum* [80, 85]
Hematopoietic stem cell transplantation / Graft versus host disease	Probiotics Prebiotics Fecal microbiota transplantation	*L. rhamnosus* GG [86—88]
HIV and AIDS	Probiotics Fecal microbiota transplantation	*L. rhamnosus* GR-1 and *L. reuteri* RC-14 (2×10^9 CFU) [89, 90]
	Dysbiosis-induced inflammation in central nervous system disorders	
Multiple Sclerosis	Probiotics Fecal microbiota transplantation	*B. fragilis* or *Bifidobacterium*, multi-strain probiotics consortium (VSL#3 contains *B. breve*, *B. longum*, *B. infantis*, *L. acidophilus*, *L. plantarum*, *L. paracasei*, *L. delbrueckii* subsp. *bulgaricus*, *Streptococcus thermophilus* in a concentration of 450 billion live bacteria per sachet) [77, 78, 79]
Parkinson's disease	Probiotics	*Bifidobacterium* spp., *Lactobacillus* spp. or *Lactococcus* spp. (*B. bifidum, B. longum, L. rhamnosis, L. rhamnosus GG, L. plantarum* LP28, and *L. lactis* subsp. *lactis*)) [91]
Alzheimer's disease	Antibiotics Probiotics Fecal microbiota transplantation	*Lactobacillus* and *Bifidobacterium* multistrain probiotic (200 mL/day probiotic milk containing *L. acidophilus, L. casei, B. bifidum*, and *L. fermentum* (2×10^9 CFU/g for 12 weeks) [92]
	Cardiovascular diseases	
Atherosclerosis and coronary artery disease	Probiotics Fecal microbiota transplantation	*L. fermentum* MTCC:5898 [93], *S. thermophilis, L. acidophilus* LA-5 and *B. bifidum* BG-12 [94] *L. plantarum* DR7 or *L. plantarum* PH40 [95]
Chronic and acute heart failure	Probiotics Fecal microbiota transplantation	*L. rhamnosus* GG [96]

Continuation of Table 3

Pathological condition	Microbiota modulation tool	Probiotic microorganisms
Chronic inflammatory disorders		
Rheumatoid Arthritis	Probiotics Fecal microbiota transplantation	*L. casei* (2×10^9) CFU/g) *L. rhamnosus* GR-1 and *L. reuteri* RC-14 [97]
Type 1 Diabetes	Probiotics Synbiotics Fecal microbiota transplantation	*L. rhamnosus* and *B. lactis* Bb12, *L. sporogenes* with *FOS* [98, 99]
Inflammatory Bowel Disease	Antibiotics Probiotics Fecal microbiota transplantation	Multi-strain probiotics consortium (VSL#3 *Lactobacillus casei, L. plantarum, L. acidophilus,* and *L. delbrueckii* subsp. *bulgaricus*), three strains of bifidobacteria (*B. longum, B. breve,* and *B. infantis*), and *S. salivarius* subsp. *thermophilus*) *S. boulardii* [100, 101]

Encapsulated probiotic microorganisms in food films and coatings. While edible films are defined as thin layers used as a coating or wrap, edible coatings are formed directly on the surface of products. Both contain polysaccharides, proteins, and/or lipids and are classified as any packaging or coating material applied to foodstuffs to extend shelf life and safety while maintaining and/or improving their nutritional and organoleptic qualities. Thus, the use of food films and coatings allows the aggregation of various functional products with foods [71]. The use of dispersion of probiotic bacteria in a polysaccharide-based solution to coat freshly cut fruit, the bacteria that entered the edible package were actually encapsulated was considered in [72]. The authors [73] described the production of an alginate pectin-based food film containing *L. plantarum* KMC 45. Alginate coatings enriched in inulin and oligofructose containing *L. rhamnosus* CECT 8361 were used to coat fresh blueberries. The use of prebiotic compounds in the edible casing improved the survival of *L. rhamnosus*, maintaining its viability above 10^6 CFU/g when stored in the casing. The addition of a bioactive coating to blueberries does not affect the organoleptic characteristics and has antimicrobial activity in the final product, reducing the amount of *Listeria innocua* [74]. Edible coatings have also been applied to preserve and extend the shelf life of freshly cut vegetables. Freshly cut carrots were coated with a food-grade alginate coating containing *L. acidophilus* La-14 to retain moisture and reduce discoloration when stored in the refrigerator, which is a determining factor for the commercialization of this type of product. The use of food films and coatings containing probiotic bacteria has also been reported for meat and fish products. It is associated with extending the shelf life of processed meat and fish, mainly by maintaining physicochemical and organoleptic characteristics such as water activity, moisture, and color, or by the antagonistic activity of probiotic microorganisms captured by the film/coating. against bacteria that cause food spoilage. Whey protein isolate food casing containing *B. animalis* Bb-12 and *L. casei* 01 in sliced ham has been reported to retain a high and constant number of probiotic bacteria over the shelf life, suggesting that consumption might contribute to beneficial effects for consumers [75].

Application of functional food products enriched with encapsulated probiotic microorganisms in microbiota-targeted therapies. A crosstalk between the microbiota and the immune system begins before birth and develops throughout life as a consequence of geographic, cultural, and dietary habits, as well as an individ-

ual's genetic background. For this reason, each person has their own gut microbiota, making it difficult to identify a fixed health-related microbial ecology. A change in the composition of the intestinal microbiota leads to such a condition as dysbiosis. Dysbiotic events occur throughout life (for example, with the use of antibiotics, due to infections, or when taking medications). However, the gut microbiota, resistant to aggressive conditions, is able to restore its physiological composition, possibly with the help of the immune system. Due to the unique composition of the microbiota of each person, an individual approach based on stratification (division of patients into groups with different characteristics of the qualitative and quantitative composition of the intestinal microbiota) of the intestinal microbiome is necessary.

The use of functional food products enriched with microencapsulated highly effective probiotic microorganisms is based on the conception of 3p - pathophysiology-based individualized use of probiotics and prebiotics for metabolic syndrome: implementing a predictive, preventive, and personalized medical approach [76].

Table 3 shows the use of such products to restore the microbiota and eliminate inflammation in several pathological conditions.

Conclusions. Changes in the composition of the gut microbiota are associated with a wide range of pathologies, including inflammatory, neurological, metabolic, and autoimmune disorders. Both intra-intestinal (for example, inflammatory bowel disease, *C. difficile* infection) and extra-intestinal (for example, cardiovascular disease, central nervous system disease, COVID-19) diseases are associated with dysbiosis, changes in intestinal permeability, and exacerbation of local or systemic immune responses.

The modulation of dysbiosis is emerging as a new strategy for the treatment of the above diseases, with a particular focus on inflammatory diseases. Extraintestinal diseases are often associated with bacterial metabolites or derivatives produced in the intestine but transported to distant sites due to disruption of the intestinal barrier in dysbiosis. Violation of the physiological functioning of the intestinal microbiome is also observed in many pathological conditions, including those unrelated to intestinal pathologies. Thus, the use of dietary supplements and functional food products enriched with highly effective encapsulated probiotic microorganisms, as well as prebiotic compounds, to restore both the normal intestinal microflora and its integrity, also indirectly affects the positive outcome of many pathological conditions mediated by an imbalance in the intestinal microflora.

Encapsulation of microorganisms with probiotic properties is an effective method for maintaining their viability and stability. Positive results are experimentally shown when using various encapsulation methods and encapsulating materials. Microencapsulation is one of the most effective methods for preserving the biological activity of live probiotic strains under conditions of industrial processing, storage, and protecting them from the gastrointestinal environment.

Alginate is the most commonly used to encapsulate probiotics. Its properties and application conditions are relatively mild and favor the encapsulation of heat-sensitive agents such as microbial cells. At the same time, the growth of the global market for nutritional supplements, functional foods, and probiotic products requires new products to meet the needs of consumers. Therefore, in addition to traditional dairy products, plant-based meat products are also actively studied as carriers of encapsulated probiotics. Despite technological challenges, several studies have shown that a suitable encapsulation approach turns non-dairy products into alternative matrices for probiotic cell delivery.

The use of functional food products enriched with microencapsulated highly effective probiotic microorganisms is based on the conception of 3p — a prognostic, preventive, and personalized therapeutic approach.

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ІНКАПСУЛЬОВАНІ ПРОБІОТИЧНІ МІКРООРГАНІЗМИ У ФУНКЦІОНАЛЬНИХ ПРОДУКТАХ ХАРЧУВАННЯ

Зміни у складі мікробіоти кишечника пов'язані з широким спектром патологій, включаючи не тільки запальні захворювання шлунково-кишкового тракту, а також діабет, ожиріння, рак, захворювання серцево-судинної та центральної нервової систем. При дисбалансі мікробіоти (дисбіозі) спостерігаються зміни проникності кишечника та порушення місцевих або системних імунних реакцій. Одним із можливих способів корекції мікробіоти кишечника є застосування дієтичних добавок та функціональних продуктів харчування, збагачених високоефективними інкапсульованими пробіотичними мікроорганізмами, а також пребіотичними сполуками. Такі продукти сприяють відновленню нормальної мікрофлори кишечника та його цілісності, а також впливають на позитивний результат багатьох патологічних станів, опосередкованих порушенням балансу кишкової мікробіоти. Збереження активності пробіотиків у харчових продуктах-носіях або продуктах функціонального харчування, розроблених для профілактики та комплексної терапії різних патологічних станів має важливе значення як для нормалізації кишкової мікрофлори, так і для здоров'я організму в цілому. У цьому контексті інкапсуляція є ефективним підходом до підтримки життєздатності та стабільності пробіотиків у несприятливих умовах шлунково-кишкового тракту. Розробка продуктів функціонального харчування, збагачених високоефективними інкапсульованими пробіотичними мікроорганізмами є пріоритетним напрямком нових досліджень у галузі профілактики та лікування мікробіото-таргетної терапії. Використання таких продуктів засновано на концепції «3р» — патофізіологічно обґрунтованого персоналізованого застосування пробіотиків та пребіотиків не тільки при метаболічному синдромі, а також при різних патологічних станах, опосередкованих порушенням якісного та/або кількісного складу кишкової мікробіоти як реалізація прогностичного, превентивного та персоналізованого терапевтичного підходу.

***Ключові слова**: продукти функціонального харчування, пробіотики, інкапсуляція, мікробіото-спрямована терапія.*