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Encapsulation of Probiotics: Proper Selection of the Probiotic Strain and the Influence of Encapsulation Technology and Materials on the Viability of Encapsulated Microorganisms

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Abstract Probiotic encapsulation is an entire system that not only involves but also depends on many factors. Elements such as the encapsulation method itself, materials, environmental conditions, and last, but not least, the strain; all play an important role in the encapsulation process. The current paper focuses on the right selection of probiotics, the various stress factors that impact the survival capacity of probiotics during and after encapsulation, and the rational selection of appropriate protection strategies to overcome these factors and achieve the highest possible encapsulation efficiency under optimal conditions. This review discusses the effects of temperature, moisture content, and water activity as well as pH, oxygen, and pressure on the viabilities of microorganisms. The effect of the surface and structure of the capsules on the encapsulated microorganisms and the impact of the materials used for the encapsulation are discussed as well. Last, but not least, the importance of choosing the right bacteria is reviewed.

Keywords Probiotics encapsulation · Encapsulation efficiency · Viability · Protection strategy

Introduction

Probiotics are described by the Food and Agriculture Organization (FAO) of the United Nations and the World Health Organization (WHO) as "Live microorganisms (bacteria or yeasts), which when ingested or locally applied in sufficient number confer one or more specified demonstrated health benefits for the host" [1].

The health benefits of probiotics encourage its wide usage in sectors such as the food industry, pharmaceuticals, and cosmetics, as well as all agricultural sectors. However, the therapeutic effects of good bacteria appear when the number of viable organisms is higher than or equal to 10^7 CFU per milliliter or gram of product at the time of consumption [2, 3]. That is why it is of utmost importance to create new encapsulation and immobilization technologies and improve the existing ones.

Considering that living cells are going to be encapsulated, the decisions about what technologies and materials will be used play an essential role. It is crucial to ensure that the conditions are not harmful for the microorganisms, taking into consideration the fact that bacteria can be affected not only by the materials from which the capsules are made but also by different solvents, e.g., alcohols or acetone. Other important considerations include which microorganisms will be encapsulated, the environment in which the capsules will be used, which method is preferred as the cell release mechanism, and other factors that may affect the efficiency of the encapsulation and cell viability during the process as well as in storage afterwards.

Selection of the Probiotic Strain

The differences in the characteristics of different probiotic species make it crucial that the right probiotic organisms be selected for a particular encapsulation method. The criteria for the selection of probiotic microorganisms include acid, heat, and oxygen tolerance (Table 1); capacity for adherence and

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Table 1 Physical and environmental requirements for some probiotics bacteria

Probiotic strain	Physical and environmental requirements for microbial growth			References
	Atmosphere	pH	Temperature, °C	
Lactobacillus acidophilus	Microaerophilic	4.0–6.4	37–45	[4, 5]
Lactobacillus gasseri	Anaerobic	4.5–6.4	37–45	[4]
Lactobacillus helveticus	Microaerophilic	4.5–6.4	39–52	[6]
Lactobacillus paracasei	Anaerobic	5–6.4	10–40	[6]
Lactobacillus plantarum	Facultative anaerobic	5–7	15–30	
Lactobacillus reuteri	Anaerobic	4.5–6.4	37–45	[4]
Lactobacillus rhamnosus	Facultative anaerobic	4.5–6.4	15–40	[7]
Lactobacillus salivarius	Facultative anaerobic	4.5–6.4	37–45	[4]
Bifidobacterium bifidum	Anaerobic	6.5–7.0	25–45	[6]
Bifidobacterium lactis	Anaerobic	6.5–7.0	25–45	[6]
Bifidobacterium longum	Anaerobic	6.5–7.0	25–45	[6]
Bacillus coagulans	Facultative anaerobic	4–7	30–57	[6]
Streptococcus salivarius	Facultative anaerobic	6.5–7.0	37–45	[6]
Streptococcus thermophilus	Facultative anaerobic	6.5–7.0	45	[6]

colonization to the epithelium/tissue; ability to stimulate an immune response; antimicrobial activity/antagonisms to pathogens; ability to improve host digestion, etc. [8, 9].

Individual species of probiotic organisms may vary in their sensitivities to external stresses such as those encountered during homogenization. Cell wall elasticity is thought to improve their resistance to mechanical stress due to variations in cell morphology [10]. Additionally, gram-positive bacteria with thick cell walls can tolerate the shear forces generated during high-speed blending or homogenization [11]. Moreover, gram-positive bacteria are considered to be more resistant to thermal and mechanical stresses than gram-negative bacteria [12].

It is also known that bacterial resistance to stress depends on the growth phase. For example, microorganisms are considered to be most immune to dehydration during the stationary phase of growth [8].

Other criteria for choosing a probiotic for an encapsulation method are the conditions during the encapsulation process, e.g., temperature and pH. Lactobacilli and bifidobacteria produce organic acids as end products of carbohydrate metabolism, which makes these bacteria less susceptible to low environmental pH compared to other bacteria. Lactobacilli can survive and grow in acidic media with an initial pH of 6.4–4.5. Growth ceases when pH 4.0–3.6 is reached, depending on the species and strain [4]. Bifidobacteria tend to be less acid-tolerant, with most strains dying at pH values lower than 4.6 [9].

Different species also have different heat tolerances. For example, the encapsulation of two probiotic cultures under the same conditions using the spray drying method showed that *Lactobacillus paracasei* NFCB 338 survived significantly better than *Lactobacillus salivarius* UTCC 118, due to its higher resistance to heat-induced stress [13].

It is important to take bacterial respiration into consideration. Lactobacilli are more tolerant of oxygen than bifidobacteria, while for facultative anaerobes such as *Bacillus coagulans*, oxygen has no negative impact at all. That is why, when using processes that are highly aerating, it is suggested that the latter culture be used for encapsulation [9].

Viability and Survivability of Encapsulated Probiotics

There are various terms to describe different stages in the life of a microorganism: viable cells, active cells, non-viable cells, dead cells, vegetative cells, stressed cells, injured cells, dormant bacterial cells, etc. [14]. Usually, the term "viability" is equated with "culturability," which means that only active and readily culturable bacterial cells can be classified as viable. In that case, if culturability is synonymous with viability, it is difficult to achieve high viability of probiotic cells in final products. Moreover, the accurate enumeration of the population of the microbes in the preparation of the products, and the communication of this information to the consumer via the product label, then becomes complicated [15–17]. This also leads to an underestimation of total cell survivability, and this may be a major reason for the selection of the wrong encapsulation strategy. That is why the term "viable but nonculturable (VBNC)" should be taken into consideration. More than 30 years ago, Staley and Konopka noticed the differences between the numbers of bacteria that are countable by microscopic examination versus those that can form colonies on agar media. This novel phenomenon was introduced

as "the great plate anomaly" [18]. It is known that during the encapsulation process, probiotic cells are affected by various stress factors, such as pressure or temperature, and in response to those stresses, a fraction of the live probiotic microbes may enter a VBNC state in which they are dormant but metabolically active. These microorganisms are capable of replicating once acclimated to a more hospitable host environment [17]. Therefore, in pursuit of high survivability during encapsulation and accurately defined numbers of viable cells, it is important not only to choose the optimum encapsulation conditions but also to keep in mind the requirements of specialized methodologies, such as nucleic-acid-based enumeration methods, fluorescent in situ hybridization or fluorescence-activated cell sorting for final cell counting, and efficiency evaluation.

Factors Affecting Probiotics Viability during the Encapsulation Process and Possible Solutions

Environmental Conditions and Process Parameters

Temperature

There are several probiotic encapsulation methods in which probiotics are subjected to extreme temperatures: spray drying, spray freeze drying (spray freezing), freeze drying, and air-suspension coating [3, 19]. The cell damage caused by heat can vary significantly, while relatively lower temperatures are more likely to affect the cell membrane. The membrane becomes porous, which causes the leakage of intracellular substances. At relatively higher temperatures, the most thermally labile proteins, such as the α- and β-subunits of RNA polymerase, unfold, which causes the death of the microorganism [20–22].

Although capsules can be dried at relatively low temperatures, even low heat decreases the number of viable microorganisms significantly. For instance, the drying process of capsules with *Lactobacillus reuteri*, at 55 °C, resulted in a decrease in viability from 1.6×10^9 CFU g^{-1} to 2.5×10^7 CFU g^{-1} [23]. Nevertheless, Arslan et al. ascertained that the viability of *Saccharomyces cerevisiae* var. *boulardii* in acidic conditions (at pH 1.0, 1.5, and 2.0) was higher in capsules produced at 125 °C than in capsules dried at 80 °C [24].

It has been suggested that the viability of bacteria during spray drying is inversely proportional to the outlet temperature and not directly related to the inlet temperature of the dryer [25]. This can be explained by the theory that any increase of outlet temperature directly increases the temperature that the droplets are subjected to; moreover, the time needed to decrease the outlet air temperature prolongs the drying duration [24].

Despite thermal inactivation of microorganisms, spray drying technologies have advantages. The amount of energy used during the process is 6–10 times lower than is used during freeze drying. Spray drying is also 30–50 times cheaper [26, 27]. That is why it is important to find ways to save microorganisms from the damaging effects of heat. One method is the application of a mild heat treatment before spray drying. Paéz et al. state that a mild heat treatment (52 °C for 15 min) may enhance *Lactobacillus casei* and *Lactobacillus plantarum* survival during spray drying [28]. Similar studies have been conducted with other probiotic cultures, such as *Lactobacillus acidophilus* and *Lactobacillus rhamnosus*. These studies showed that when subjecting these cultures to temperatures lower than the heat shock temperature (50 °C for *L. acidophilus* and 52.5 °C for *L. rhamnosus*, for 12 min), the viability of the microorganisms during the spray drying process increased, compared with cultures that were not affected by the temperatures. This leads to the conclusion that exposing the probiotics to mild heat increases their subsequent tolerance to near-lethal thermal stresses due to the production of heat shock proteins [20].

There are various substances that can be used to reduce the thermal inactivation of microorganisms, including low-melting-point fats, sugars, skim milk, trehalose, starch, fibers, and prebiotics [29, 30]. The protective effect of sugars is explained by the water-replacement hypothesis. The sugars act as water substitutes when water molecules connected to the phospholipids of the membrane are eliminated [31]. Lactose has been reported as a very effective protectant of cell viability during spray drying due to its forming hydrogen bonds with proteins when water is removed, which helps to retain the structural integrity of cell membranes [32]. Trehalose also has a similar effect [33, 34]. Lapsiri et al. reported that trehalose and proteins have increased the viability of *L. plantarum* TISTR 2075 during and after spray drying and also during the storage period [29]. Other research has shown that gum acacia helps to maintain higher *L. paracasei* viability during drying [35]. Fats with low melting temperatures, such as margarine or shortening, are thought to absorb part of the heat energy as they melt during the spray drying process, thereby reducing the temperature of the capsules and decreasing the heat shock to the probiotics [30].

Some of the substances used for encapsulation are known for their ability to reduce the heat transfer rate. Arslan et al. have stated that gelatin is protective because it contains amino acids; the remains of amino acids, such as proline, hydroxyproline, and alanine, can reduce heat transfer. It is also known that the heat transfer rate in organic solutions decreases with increasing viscosity [24].

Spray freeze drying and lyophilization methods are used not only for the production of particles containing probiotics but also the preparation for further storage [36, 37]. During this process, a decrease in cell viability is caused by the

formation of ice crystals, high osmotic pressure, and the loss of water, which impacts the characteristics of many hydrophilic molecules in the cell [34, 38]. Damage caused by freezing can be reduced by encapsulating cryoprotectants, such as lactose, trehalose, sorbitol, saccharose, or milk proteins, together with the probiotics [37]. Cryoprotectants can reduce intracellular ice formation during freezing through the formation of hydrogen bonds with water molecules and cellular structures, thereby stabilizing the membrane and cell proteins [39–41]. Skim milk powder adsorbs on the surface of the cells and forms a viscous layer, which protects from the formation of ice crystals and maintains ice in an amorphous form close to the cell [42]. Although some scientists suggest the use of glycerol, other publications prove that glycerol is not suitable as cryoprotectant [40, 43].

Moisture Content and Water Activity

Cell injury and protein denaturation can occur only when two parameters are combined: temperature and high water activity; so the viability of probiotics is not dependent only on temperature [44]. It is known that water activity (a_w) and moisture content not only have an impact on the viabilities of microorganisms during the process but also affect the viability during subsequent storage [34]. Teixeira et al. and Ananta et al. state that to maintain stability after drying, the water activity should not exceed 0.25, and moisture content should be 4–7% [45, 46].

When water activity is higher than 0.25, the death rate of probiotic bacteria increases, supposedly due to high-molecular mobility in the matrix, which is related to the stimulation of metabolism [38, 47]. Studies showed that when the water activity is 0.7 in calcium alginate capsules coated with chitosan, the viability of encapsulated *L. plantarum* decreased after 3 days of subsequent storage, and after 10–14 days, no viable cells were detected. However, in capsules where the water activity was 0.2, bacterial viability after 45 days was $> 10^5$ CFU/g [47]. On the other hand, when the water activity is too low (< 0.1), the oxidation of membrane lipids can decrease bacterial viability [48].

Given that water content strongly correlates with drying temperature, it is important to choose the right content of moisture after the drying process [35, 44, 49]. Zayed and Ross reported that a certain amount of water must remain in dried product. Their research reveals that after 7 weeks of storage, the viability of freeze-dried *L. salivarius* was 72% lower than the viability of cells where the moisture content was 2.8% [34].

When freeze drying, a high water content can result in the decrease of the viability of microorganisms due to mechanical stress. Mechanical stress occurs during the formation of ice crystals in the external medium or inside the cells [27, 50].

To maintain as high a probiotic viability as possible, it is important to take into account the hygroscopicity of the materials used for the encapsulation [51]. It is known that the hygroscopicity of materials depends on the number of hydrophilic groups in the structure, which can bind to water molecules from the surrounding air [52]. Ersus and Yurdagel have stated that the hygroscopicity of sugars used for encapsulation depends on the molecular weight; as the molecular weight increases, the hygroscopicity decreases, and vice versa [53]. Additionally, amorphous matrices are known to be more soluble as well as more hygroscopic [54]. Even though, as stated earlier, the desirable water content is 4–7%, this value is too high when encapsulating with milk components. During encapsulation with skim milk or whey powder, the moisture content should not exceed 4% in order to avoid caking due to the absorption of water by the hygroscopic amorphous lactose, which is converted into crystals of α-lactose monohydrate, promoting the aggregation of the powder particles, which is undesirable when trying to maintain as high a viability of microorganisms as possible [55].

Studies have revealed that prebiotics, when encapsulated together with bacteria, decrease the water content and water activity in the microcapsules [51, 56]. The microcapsules produced with inulin showed the lowest dissolution in water, while the microcapsules produced with oligofructose were the most hygroscopic [51].

Pressure, Oxygen, and pH

The impact of pressure on biological systems is based on the fundamental principle that pressure affects the conversion rate of biochemical reactions. Depending on how the system volume changes when subjected to elevated pressure, a specific reaction can be either accelerated or slowed down.

It has been determined that an increase in pressure up to 50 MPa disturbed the division of *Escherichia coli* cells and the total rate of protein synthesis [57]. A negative effect of pressure may occur during spray drying; when the liquid is atomized, the probiotic cells can also be damaged due to shear forces [12]. On the other hand, studies have shown that pretreatment with pressure increases the heat tolerance of microorganisms. Ananta and Knorr reported that incubation of *L. rhamnosus* GG at an elevated pressure of 100 MPa for 5–10 min prior to exposure to lethal heat at 60 °C led to an increase in heat resistance compared to no treatment [57].

pH has also been shown to affect the survival of probiotics. To be able to survive and multiply, the microorganisms have to maintain a stable pH in the cytoplasm, which ensures optimal functionality and the integrity of the structure of cytoplasm. Transition to an alkaline environment, as well as an acidic one, is stressful for bacteria [58]. For instance, an acidic pH can result in the denaturation of cellular proteins or can decrease the pH of the cytoplasm due to proton efflux, because

of a high pH gradient [59]. Some capsule-formation methods rely on the pH change. For example, microencapsulation of probiotic bacteria using pH-induced gelation of sodium caseinate and gellan gum. That is why it is crucial to choose the right microorganisms for the encapsulation, so that the conditions of the process do not decrease their viability [60].

For anaerobic microorganisms, oxygen toxicity is an important and critical problem. Lourens-Hattingh and Viljoen have suggested that a high oxygen-consuming strain, e.g., *Streptococcus thermophilus*, be included when encapsulating anaerobic cultures. In this way, the oxygen, which is dangerous for anaerobic cultures, is effectively decreased [61]. An anaerobic environment should be set up for the entire encapsulation process, including deoxygenation of the encapsulating solution, to establish and maintain an anaerobic environment of encapsulating instruments [39]. Additionally, certain substances, such as L-cysteine or ascorbate, are added to reduce the redox potential in order to allow the growth of anaerobic organisms [62].

Materials Used for the Encapsulation

Effectiveness of the encapsulation and the viability of microorganisms depends not only on the physiology of the culture and process parameters but also on the materials used for the encapsulation. It is vital to understand how each component interacts with the others, and a good understanding of bacterial interactions with the encapsulation matrix is crucial [63] (Table 2). However, most often the encapsulation matrix itself is highly compatible with living microorganisms, and dangers come instead from other materials that are used with it, such as polymer solvents (e.g., alcohols), salts, surfactants, and pH regulators.

When a dynamic encapsulation system is used, for example, in emulsification, the capsules being prepared tend to coalesce while moving and form larger particles that flocculate to form aggregates in the absence of any emulsifier. That is why it is crucial to use surfactants, which decrease the tension of the surface [60]. Cationic or less-anionic surfactants are the most damaging, while non-ionic are the least damaging for the microorganisms. Surfactants can affect the cell wall, the cytoplasmic membrane, or the cytoplasm itself. In addition, surfactants can operate as bacteriostatic agents, i.e., to disturb fundamentally important functions, such as protein synthesis [75]. For the latter reason, non-ionic emulsifiers, such as the polysorbate-class emulsifier Tween 80, are used for the encapsulation.

While producing calcium alginate capsules, the decrease in calcium chloride concentration did not affect the ability of the capsules to protect viable cells; however, higher yields of immobilized cells could be produced [76, 77].

Studies have led to the hypothesis that encapsulating hydrophobic bacteria with components that have hydrophobic features, prevents thermal inactivation of the cells during the drying process, due to hydrophobic interactions followed by adhesion to the proteins, resulting in cells being embedded within the walls of the capsules [63].

Size, Structure, and Surface Properties of Capsules

Microbial cells are quite large (usually 1–4 μm), and thus it is impossible to encapsulate them in small particles, and the production of larger particles is risky because of the possibility of negative effects on the structural and sensorial properties of the product to which they are added [78]; an optimal range of 100–200 μm has been suggested [60, 79].

The sizes and structures of capsules depend mostly on the chosen encapsulation method. For instance, the sizes of the capsules produced by spray drying can vary from 3 to 100 μm, while those of the capsules produced by extrusion are 1–5 mm, and by emulsification, 25–2000 μm [80, 81]. Capsules produced by spray drying usually show uneven, rugged spherical surfaces [82]. Studies have revealed that those ruptures ease heat dissipation from the capsules, which decreases heat caused damage to the probiotics inside the capsules [25]. It is also assumed that wrinkles or shrinking occur because of the slow formation of films during the drying of the atomized droplets [82].

Capsule size depends not only on the technology but also on the materials that are used. The higher the viscosity of the encapsulation suspension, the larger the capsules. Additionally, capsules produced from higher viscosity materials are more spherical [83]. This is the reason why higher polymer concentrations result in the production of larger particles. Although there was no statistically significant relationship between the concentration of the polymer and encapsulation yield [79], the size of the microcapsules was affected by the concentration of inulin [84]. Encapsulating probiotics together with oligofructose resulted in a smaller capsule size compared to the ones encapsulated with the higher-molecular-weight inulin [51]. The surfaces of freeze-dried calcium alginate capsules were rugged and had a collapsed center, while the surface of capsules produced using same method and coated with low-molecular-weight chitosan had a more even surface. On the other hand, capsules coated with high-molecular-weight chitosan were rugged and partially collapsed at the center. Due to the higher viscosity of chitosan, it binds to the surface with limited binding to the alginate gel network [85].

The hygroscopicity of capsules also depends on their size; the larger surface area of the capsule, the higher the amount of moisture that can be absorbed from the environmental air [51].

Current technology allows for the production of capsules as small as a few tens of micrometers, but this often results in decreased viability of probiotics. For example, in order to obtain capsules that were as small as possible using

Table 2 List of several strains subjected to encapsulation in various methods and materials

Probiotic strain	Technology	Materials	Particle size (μm)	Functionality	Reference
L. rhamnosus	Double emulsion (O/W/O)	Canola oil Sweet whey	8–12	Protective against the stressful conditions of the gastric tract Sweet whey can be used as an adequate growth medium	[64]
	External gelation	Alginic acid Calcium chloride Chitosan	400–450	Higher thermotolerance upon prolonged wet heat exposures at 60 and 70 °C	[65]
	External gelation	Sodium alginate Sugarcane baggase Calcium chloride	293–557	Higher viability upon heat exposures at 90 °C for 40 s	[66]
	Dual aerosols method	Sodium alginate Calcium chloride Maltodextrin	35	Improve the survivability following freeze drying	[67]
	Ionotropic gelation	Pectin Whey protein	185	Protective against the stressful conditions of the gastric tract	[68]
	Spray drying	Native rice starch Inulin	6	Protecting bacteria during spray drying	[69]
		Whey protein Dextrinized starch Hydrolyzed palm stearin Tocopherol	10–20	Protective effect on survival during storage	[70]
L. acidophilus	Spray drying	Skim milk Sweet whey	13	Increase the viability of the probiotic during exposure to simulated gastrointestinal conditions	[55]
	Spray drying	Flaxseed mucilage Flaxseed protein	3.4	Enhanced viability during spray drying and incubation in simulated gastric acid and bile solutions	[71]
	Solid lipid microparticles using spray chilling	Fully hydrogenated palm-kernel oil Inulin Polydextrose	60	Protects cells from the effects of gastric and intestinal fluids and release them in the intestines during fat digestion	[56]
	Extrusion	Sodium alginate Calcium chloride Hi-maze starch	70	Increase survivability during storage of the moist and freeze-dried microparticles	[72]
	Co-extrusion	Sodium alginate Calcium chloride Apple skin polyphenol	423–486	Improve the survivability in milk (stored 4 °C for 50 days) or in acidic water (pH 2)	[73]
	Emulsification/internal gelation	Sodium alginate $CaCO_3$/Ca-EDTA Soybean oil Span 80	323–343	Higher cell survivals in both simulated gastric juice and bile salts solution	[74]

emulsification, a calcium alginate-oil emulsion was homogenized for 2 min at 8000 rpm. It was seen that after the homogenization, the viability of the bacteria was 64.6%, but increase of the rotation speed up to 13,500 rpm lowered the viability to 25.5%. This decrease in the number of viable microorganisms is related to the occurrence of shear forces [86].

It should be noted that the viability of encapsulated microorganisms in simulated gastric fluid increases with the increasing the size of the capsules [78]. Hansen et al. have reported that capsules smaller than 100 μm do not significantly protect *Bifidobacterium* spp. in simulated gastric fluid [87]. The surface characteristics of capsules define their functional performance; biocompatibility and diffusion characteristics depend on them as well [88].

Another important structural parameter is porosity. It is believed that an increase in surface porosity causes an increased rate of bioactive-compound release from the microcapsules [89]. Researchers showed that milk-based microspheres have lower porosity, and that is why leakage of the encapsulated probiotics was also smaller [90]. It is known that higher porosity is a result of low cross-linking density, which is why it is important to use alginate, which is rich in guluronic acid [78, 89].

The morphologies of capsules can differ depending on storage conditions. For example, spherical calcium alginate capsules lose their spherical shape after 60 days at − 20 °C. The physical processes that lead to shape/form changes may have led to ruptures in the microparticles, exposing the probiotic

cells to the adverse external conditions. Additionally, the porous net that composes the particle had been compressed [91].

Conclusions

In spite of the numerous methods and materials being used for encapsulation, there are many difficulties related to microorganism viability during encapsulation process that have not been solved yet. There is a growing need to find suitable technologies that provide high encapsulation efficiency and probiotic viability and at the same time provide an adequate quality of the final product. Encapsulation has some limitations on both the maintenance of probiotic viability during the process as well as long-term storage. Thus, it is essential to find properly selected systems where bacteria can be encapsulated without losing their viability. Guidelines for choosing each element included in the process shall take into consideration all aspects in order to result in the highest possible bacterial viability. Future studies should be focused on substances of encapsulation, which could protect probiotics from environmental conditions that are unfavorable for bacteria. Furthermore, the studies should be oriented towards novel encapsulation methods that are more suitable for the viabilities of probiotics.

Compliance with Ethical Standards

Disclosure Statement The authors declare that they have no competing interests.

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