

Nutritional Communication

Material and Techniques for Microencapsulation of Probiotics: Literature Based Review

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ABSTRACT

Probiotics are live microorganisms that are introduced to induce positive health benefits in the host. Different species relevant to various genus are used in food in order to enhance the health benefits of the food product. But, the most widely used probiotics are related to lactobacillus and Bifidobacteria genus. The health benefits attributed to the consumption of probiotics include immune system modulation, reduction of symptoms related to irritable bowel syndrome (IBS), diarrhea treatment, reduction of lactose intolerance, serum cholesterol reduction, anti-inflammatory properties, prevention of cancer and mutagenesis, and production of bacteriocins which make environment unsuitable for pathogenic microorganisms specially by lowering the pH. The claimed health benefits are related to the species and even strain of probiotics and achieved when the microorganisms are higher than the minimum satisfactory level. Moreover, the viability of probiotics is of vital importance from the time of production to the time of reaching to the target organ. In order to enhance the survival of probiotics, several techniques have been used among which the results of microencapsulation are outstanding. Microencapsulation is the process of physical protection of probiotics from harsh environmental and hostile conditions. The process is carried out by using different materials like alginate, chitosan, starch and others through different methods such as extrusion, emulsion, spray drying and freeze drying. Alginate in combination with chitosan coating is widely used through extrusion and emulsion techniques. But, in the terms of industrial use the spray drying method is outstanding. In this review, the efforts have been made to gather more relevant information and undertook studies on microencapsulation of probiotics.

KEY WORDS: MATERIALS, MICROENCAPSULATION, PROBIOTICS, REASONS, TECHNIQUES.

INTRODUCTION

Probiotics are beneficial microorganisms and have been extensively used for their beneficial health effects. The term probiotic has been derived from a Greek word which means for life or is a combination of Latin (pro=in favor of) and Greek (bios=life). So far, many definitions have been postulated for the term probiotics, but a more comprehensive and thorough definition has been given by Hill et al. (2014). They define the probiotics as “live microorganisms that, when administered in adequate amounts, confer a health benefit on the host”. In order to get maximum health benefits from the consumption of probiotics, it is recommended to get various species of probiotics at time of consumption. The most commonly used probiotics are from the genus of

lactobacillus and Bifidobacteria. In order to get the claimed health benefits from probiotics, the number of probiotics should be higher than the range of minimum satisfactory level. This level is reported in the range of 10^6 - 10^7 CFU/mL (Lee and Salminen 2009; Mortazavian et al. 2012; Arihara 2014; Sarao and Arora 2017; Kumari et al. 2020).

The claimed health benefits associated with probiotic consumption are elevation of immune system, improvement in colonic health, cancer prevention, reduction in serum cholesterol level and others (Kumari et al. 2020). Moreover, improvement in sensitivity with foods, neurological activities, diabetes mellitus, *H. pylori* infection, and prevention and treatment of oral infection are also reported to be associated with probiotic consumption (Shafi et al. 2014; Roobab et al. 2020; Chugh et al. 2020). The claimed health benefits for the consumption of probiotics are obtained when the live cells of probiotics reach to the target part of the body. Thus, the survival of probiotics is of importance during storage,

Article Information:*Corresponding Author: attaulhaqbanuree@gmail.com

Received 05/07/2021 Accepted after revision 21/09/2021

Published: 30th September 2021 Pp- 922-928

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Published by Society for Science & Nature, Bhopal India.

Available at: <https://bbrc.in/>

Article DOI: <http://dx.doi.org/10.21786/bbrc/14.3.3>

manufacturing process of food and transit through the stomach and small intestine. Various techniques have been developed for the enhancement of probiotics' survival in food and subsequent process, of which microencapsulation of probiotics are outstanding (Figueroa-Gonz' et al. 2011; Terpou et al. 2019; Han et al. 2021).

Microencapsulation is a recent method of physical protection of probiotics. The particle size of 0.2-5000 μ m is considered as microcapsule (Maleki et al. 2015). Alginate, k-Carrageenan, Gellan gum and xanthan gum, Chitosan, Starch, Gelatin, Cellulose acetate phthalate, and Milk proteins are used mainly through different chemical, physical, and physiochemical methods for the microencapsulation of probiotics (Burgain et al. 2011; Hamyouni et al. 2012; Cota and Stanila, 2013; Iravani et al. 2015; Peanparkdee et al. 2016; Yao et al. 2020). Extrusion, emulsion, spray drying, and freeze-drying are broadly used techniques (Burgain et al. 2011; Solanki et al. 2013; Rathore et al. 2013; Serna-Cock and Vallejo-Castillo, 2013; Martín et al. 2015; Pupa et al. 2021).

2. Survival of Probiotics: In order to obtain the maximum health benefits from probiotic products it is necessary that the product should have the minimum certain number of viable cells of probiotics. This number of probiotics is called the therapeutic dose of probiotics (Pupa et al. 2021). Despite of no world-wide anonymous consensus on the minimum viable probiotic cells per gram or milliliter of probiotic product, generally, the concentrations of 10^6 and 10^7 - 10^8 cfu mL⁻¹(cfu g⁻¹), respectively, have been accepted as the minimum satisfactory levels (Mortazavian et al. 2012; Marinova et al. 2019). It has also been stated that probiotic products should be consumed regularly with an approximate amount of 100 gr /day in order to deliver about 10^9 viable cells into the intestine (Terpou et al. 2019; Pupa et al. 2021). Probiotics' viability, which is crucial for reaching and colonizing the human large intestine, determines their quality in probiotic food products. Probiotics must be viable during three important stages: (1) storage; (2) the functional food's manufacturing process; and (3) stomach and small intestine transit. Thus, probiotic viability is a critical problem from both an economic and technological standpoint. A study found that freezing a probiotic at -40°C reduced its vitality from 1.8×10^{15} to 1.6×10^{10} CFU mL⁻¹, while freeze-drying followed by storage at 4°C reduced its viability from 8.9×10^{14} to 2.4×10^9 CFU mL⁻¹ (Figueroa-Gonz' et al. 2011; Pupa et al. 2021).

Many factors have been reported that affect the survival of probiotic bacteria in food throughout the three important stages indicated above. Food parameters (pH, titratable acidity, molecular oxygen, water activity, presence of salts, sugar, and chemical compounds such as hydrogen peroxide, bacteriocins, artificial flavoring, and coloring agents); microbiological parameters (heat treatment, incubation temperature, product cooling rate, packing materials and storage procedures, and manufacturing scale); processing parameters (strain of probiotic, rate and proportion of inoculation) are among the factors important factors (Tripathi and Giri 2014; Terpou et al. 2019). On the other hand, food matrix, very low pH in the stomach, bile

salts and gastro-enzymes in the small intestine, Lysozyme in saliva, and colonic conditions (competition with other bacteria including pathogens) are the key factors that determine probiotic survival in the GIT (Mortazavian et al. 2012; Stasiak-Róžańska et al. 2021).

Microencapsulation of Probiotics: Encapsulation of probiotics is one of the most effective methods for increasing the viability of probiotics (Dong et al. 2013; Yao et al. 2020). "Microencapsulation is a process by which live cells are packaged within a shell material, which confer them protection by preventing their direct exposure to unfavorable environment, but permits diffusion of nutrients in and out of the matrix, thereby supporting the viability of the cells" (Vivek 2013). Microcapsules are particles with a diameter of 0.2 to 5000 micrometers, while macrocapsules are larger than 5000 micrometers and nanocapsules are smaller than 0.2 micrometers. The procedure is known as coating when the core material is quite large. The enclosed particle is ideally spherical; nevertheless, the structure of the core material influences this (Maleki et al. 2015). The core material is the entrapped components inside the microcapsule, while polymers are referred to as wall materials, shells, coatings, carriers, or encapsulants (Peanparkdee et al. 2016; Pech-Canul et al. 2020).

The reservoir type and the matrix type are two separate types of encapsulations. the reservoir type has a shell around the core material, and therefore it is also known as a capsule. In the later type, the active agent is spread over the carrier material and can also be present on the surface. A third type of capsule is created by combining these two types in which the active substance is retrieved by a coating. Encapsulated probiotics have been employed in a variety of probiotic products so far. Microencapsulated probiotics are most commonly found in dairy products (49%), followed by fruit and vegetable-based goods (28%), meat-based products (13%), and bakery items (11%) (Burgain et al. 2011; De Prisco and Mauriello 2016; Stasiak-Róžańska et al. 2021).

Reasons for Microencapsulation: Microencapsulation is primarily used to protect encapsulated materials from extreme environmental conditions so that they can safely reach the point of ingestion and eventually pass-through GIT. The following are some of the most important reasons for encapsulation:

- It enhances probiotic viability by allowing them to pass through the GIT's acidic-enzymatic-bile conditions
- Production of high-viability bacterial starter cultures
- Increase the viability of probiotic microorganisms by protecting them from harsh environmental conditions
- Application in Fermenter: increases microorganisms' endurance to severe the conditions
- Production of food products with a high probiotic viability till its consumption
- Probiotic immobilization
- Fixation and improvement in the sensory properties of probiotic products
- Superior active agent handling
- Improvement in the stability of final product and

- throughout processing
- Improved safety (e.g., reduced flammability of volatiles like aroma, no concentrated volatile oil handling)
- Controlled release (Vivek 2013; Razavi et al. 2021)

Materials Used for Microencapsulation of Probiotics:

Depending on the substance to be coated and the characteristics needed in the final microcapsules, coating materials, which are essentially film-forming materials, can be chosen from a wide range of natural or synthetic polymers. The coating material's composition is the most important factor in determining the microcapsule's functional characteristics and how it may be utilized to increase the performance of a certain component. The following properties should be present in an ideal coating material (Poshadri and Kuna 2010; Razavi et al. 2021):

1. Good rheological characteristics at high concentrations and simple encapsulation workability
2. The capacity to disperse or emulsify the active ingredient while also stabilizing the resulting emulsion.
3. Non-reactivity with the encapsulated materials throughout processing and long-term storage.
4. The capacity to seal and hold the active ingredients within its structure throughout processing and storage.
5. Under drying or other desolventization conditions, the capacity to entirely release the solvent or other ingredients used during the encapsulation process.
6. The capacity to protect the active substance from adverse environmental conditions (e.g., oxygen, heat, light, humidity).
7. Solvents' solubility that are acceptable in the food industry (e.g., water, ethanol).
8. Chemical nonreactivity with the active core materials.
9. Inexpensive, food-grade status.

A single coating material cannot fulfill all of the aforementioned requirements. In reality, either a mixture of coating materials is used, or modifiers such oxygen scavengers, antioxidants, chelating agents, and surfactants are added (Poshadri and Kuna 2010; Razavi et al. 2021). The following are some examples of encapsulating materials:

Alginate: Alginate is a naturally produced polysaccharide that is made up of two monosaccharide units: α -L-guluronic acid (G) and β -D-mannuronic acid (M), which are linked together by a β (1–4) glycosidic bond. The technological functionality of alginate is determined by M/G ratios. On the other hand, the gel's strength is determined by the large amount of block G (Solanki et al. 2013). Alginate, particularly calcium alginate for its non-toxicity, cheapness, simplicity, and biocompatibility is used extensively in the encapsulation of probiotics (Sarao and Arora 2017). Calcium alginate is used as encapsulation material in different concentrations mainly in the range of 0.5–5% (Martín et al. 2015; Liu et al. 2020).

The use of alginate as the encapsulating material has certain disadvantages as well. The primary drawbacks associated with alginate are: the sensitivity to the acidic environment,

problems in the scaling up process, quite porousness of their microcapsules. These pitfalls can be solved by combining alginate with another polymer component or altering the alginate's structural properties. These methods can be blending starch with alginate, mixing alginate with other polymers such as corn starch, resistant starch, mixing of alginate with cryoprotectants as like glycerol in order to improve viability of at $-20\text{ }^{\circ}\text{C}$ frozen storage, or to form a semipermeable layer of chitosan around the alginate capsules (Martín et al. 2015; Sarao and Arora 2017). Ji et al. (2019) reported that alginate microcapsule coated with chitosan protected *Bifidobacterium longum* from GIT fluid and elevated temperature conditions (Ji et al. 2019; Liu et al. 2020).

Chitosan: Chitosan is a linear polysaccharide that is randomly made up of $-(1-4)$ -linked D-glucosamine and N-acetyl-D-glucosamine. Chitosan is made commercially by deacetylating chitin, a structural component of crustaceans' exoskeletons (such as crabs and shrimp) and fungi's cell walls. On average, the molecular weight of commercially manufactured chitosan ranges from 3800 to 20,000 Da. It is soluble at pH less than 6 and like alginates forms gel structures by ionotropic gelation. In the presence of anions and polyanions, chitosan can crosslink even more (Călinoiu et al. 2019).

It is most commonly employed as a coat over the produced capsule because to its failure to increase probiotic cell viability. Low-concentration chitosan solutions are usually applied to capsules. Chitosan and hexamethylene diisocyanate, as well as chitosan and glutaraldehyde, have been shown to produce stronger coatings than chitosan alone. Coating of alginate capsules with chitosan is achieved by allowing alginate microcapsules to drip into a chitosan–calcium chloride solution Calcium ions must be present for appropriate coating to occur (Vandamme et al. 2016; Călinoiu et al. 2019).

Gellan Gum and Xanthan Gum: Gellan gum is a microbial polysaccharide, which is produced from *Pseudomonas elodea*. It made up of a repeating unit of four monomers namely glucose, glucuronic acid, glucose, and rhamnose. The use of a mixture of xanthan–gellan gum for encapsulation of probiotic cells is more resistant to acidic environments than the use of alginate for encapsulation (Sarao and Arora 2017). Hoh et al. (2021) documented that the xanthan gum coating improved the survivability of *Lactobacillus rhamnosus* GG in simulated gastric juice and simulated intestinal juice (Hoh et al. 2021).

κ -Carrageenan: κ -Carrageenan is a natural occurring polysaccharide derived from marine macro algae. κ -Carrageenan is made up of repeating D-galactose-4-sulphate units and 3,6-anhydro-D-galactose linked by alternating $\alpha 1\rightarrow 3$ and $\beta 1\rightarrow 4$ glycosidic linkages (Iravani et al. 2015). The use of κ -Carrageenan necessitates a temperature range of 40 to 50°C . At this temperature, the cells are introduced to the polymer solution. When the mixture is brought to room temperature, it begins to gel formation. The addition of potassium ions stabilizes the microparticles that have formed. When probiotic bacterial

cells are encased in κ -carrageenan, they remain alive (Sarao and Arora 2017; Hoh et al. 2021).

Starch: Maize, potato, barley, oats, and other starchy foods are the most common sources of starch. This polymer is made up of amylose and amylopectin that are linked by a α -1-6 glycosidic bond. Heating is the primary cause of starch gelation. In order to protect bacterial cells and allow optimum diffusion of micronutrients and metabolites, starch is generally combined with alginate microspheres. However, under acidic circumstances and in the presence of pancreatic enzymes in the GIT, it can be destroyed. As a result, resistant starch could be utilized as an encapsulating polymer for probiotics that can be fermented in the colon and are not digested by pancreatic enzymes (amylases) in the small intestine, ensuring the transport of viable and metabolically active probiotics to the colon (Rathore et al. 2013; Hoh et al. 2021).

Gelatin: Gelatin is a kind of gum that may be used to produce a thermo-reversible gel. This has been used to encapsulate probiotic cells, either alone or in combination with other substances. Due to its amphoteric nature, it can create an ideal combination with anionic polysaccharides, such as gellan gum. At a pH greater than 6, the hydrocolloids resist each other due to the fact that they both have a net negative charge. When the pH is reduced below the isoelectric point, gelatin takes on a net positive charge, resulting in a strong interaction with the negatively charged gellan gum (Sarao and Arora 2017).

Cellulose Acetate Phthalate (CAP): CAP is a cellulose polymer in which 50% of the hydroxyl groups are esterified with acetyls and 25% are esterified with one or two phthalic acid carboxyls. At pH 6 or above, CAPs are soluble, while at pH 5 or lower, they are insoluble (Vandamme et al. 2016). When probiotic bacteria are encapsulated in cellulose acetate phthalate, they are well protected in a simulated GI state. Because of its harmless nature, cellulose acetate phthalate is used for controlling drug release in the gut (Sarao and Arora 2017).

Milk Protein: Casein and whey proteins, which are found in milk, are widely regarded as suitable materials for encapsulating probiotic microorganisms. Because of their biocompatibility, whey proteins and their gel matrices are crucial. Because of their structural and physicochemical characteristics, they can be used as a delivery system (Vandamme et al. 2016; Sarao and Arora 2017; Liu et al. 2020).

Microencapsulation Techniques: Several techniques can be used in order to encapsulate food components in coating materials. The contributing factors in the process of technique selection mainly depend on required particle average size, the physical and chemical characteristics of the carrier material, the uses of the encapsulated substance, the required release mechanism, and costs. These factors must be investigated for each probiotic and technique. There are three main stages of encapsulation of probiotics namely: incorporation of the bioactive component in a matrix, microcapsule production, and microcapsule

stabilization by a chemical, physicochemical, or physical procedure (Burgain et al. 2011; Serna-Cock and Vallejo-Castillo 2013; Liu et al. 2020). The followings are some microencapsulation techniques:

Extrusion: Extrusion is the oldest and most widely used method for microencapsulating probiotics because of its ease of use, low cost, and mild conditions that enable high entrapment of the microencapsulated probiotics. Extrusion has been effectively used to encapsulate probiotic bacteria by using biopolymers such as alginates and carrageenan in the presence or absence of minerals (calcium, potassium, etc.). In the case of alginate capsules, the extrusion process entails the following steps: production of a cell suspension from probiotic cells and a hydrocolloid solution, extrusion of the produced cell suspension into a hardening solution containing divalent cations such as calcium, and cross-linking of alginate polymers and calcium ion to form a three-dimensional lattice structure (Solanki et al. 2013; Vandamme et al. 2016; Liu et al. 2020).

Prilling is a method that involves the production of droplets in a controlled manner (as opposed to spray-drying). The pulsation or oscillation of the jet nozzle is a good way to achieve this. Another popular method for forming droplets is to employ coaxial flow or an electrostatic field (Burgain et al. 2011). Many parameters impact the size and shape of the beads in this process, including the viscosity of the alginate solution, the distance between the needle and the hardening solution, the diameter of the needle orifice, and the hardening solution's surface tension. This technique produces microcapsules with 2- to 5-mm dimensions, which are bigger than those produced by the emulsion method and, as a result, could affect the sensory properties of the product (Solanki et al. 2013; Vandamme et al. 2016).

Emulsion: Because of vegetable oil is required for emulsion formation, the emulsion process is more costly than extrusion (Iravani et al. 2015). The cell polymer suspension is mixed with a considerable amount of oil in this method. After that, the mixture is homogenized to create a water-in-oil emulsion. The water-soluble polymer is insolubilized (crosslinked) to produce the particles within the oil phase after the water-in-oil emulsion is generated. Finally, filtering is used to extract the beads. The size of the beads is determined by the agitation speed, which can range from 25 μ m to 2mm. In this technique, vegetable oils are used in food applications. White light paraffin oil and mineral oil have been used in certain experiments. Emulsifiers are also used to produce a better emulsion since these chemicals reduce the surface tension, resulting in smaller particles. In emulsion technique of microencapsulation, carrageenan and its mixes, sodium carboxymethyl cellulose, cellulose acetate phthalate (CAP), alginate and its mixtures, chitosan, gelatin, and chickpea protein can be used (Martín et al. 2015; Razavi et al. 2021).

Spray Drying: Probiotic suspension and dissolved polymer are combined together in the spray drying method. Gum arabic and starches are commonly used as polymer matrices because they tend to produce spherical microparticles during the drying process. The prepared mixture is compressed

and then atomized to produce a “mist” into the drying chamber in this process. In the drying chamber, heated gas (air or nitrogen) is also blown. This heated gas causes the solvent to evaporate. The capsules are then transferred to a cyclone separator where they will be recovered. The benefits of spray drying include the procedure's speed and cost-effectiveness. The process is extremely repeatable, and, more importantly, it is appropriate for industrial use. One drawback of spray drying is that it has a limited range of applications. However, the main issue is that it uses high temperatures, which are incompatible with the survival of probiotics. Protectants can be applied to the medium before drying to increase probiotic survival. For instance, granular starch improves the viability of culture throughout drying and storage, soluble fiber boosts probiotic viability during storage, and trehalose protects against heat. Furthermore, spray-dried capsules can be coated with an extra layer to provide protection against acidic stomach conditions or to decrease the adverse effects of bile salts (Burgain et al. 2011; Razavi et al. 2021).

Freeze Drying: Freeze drying has been used to make probiotic powders for decades but it has been recently becoming apparent to combine freeze drying and encapsulation. The method is based on sublimation, which occurs in three stages: freezing, first, and then drying. Cells are usually frozen first, then dried by sublimation in a high vacuum. As the processing requirements of freeze drying are milder than those of spray drying, greater probiotic survival rates are attained in this technique.

The solvent is frozen and removed by sublimation in this process. Freezing, on the other hand, damages the cell membrane due to crystal formation and also causes stress due to excessive osmolarity. Various types of protectants, such as skim milk powder, whey protein glucose, maltodextrin, trehalose, and others, have been added to the drying media before freeze drying to protect the viability of probiotics during dehydration (Razavi et al. 2021). Cryoprotectants can also be added to media before fermentation to help probiotics adapt to their surroundings. Cryoprotectants work by accumulating within cells and decreasing the osmotic difference between the internal and exterior environments (Martn et al. 2015).

CONCLUSION

The findings of the present study suggests that probiotics are considered as live beneficial microorganisms that induce positive health benefits in the host. These attributed health benefits are achieved when viable cells of probiotics reach to the target organ. Microencapsulation is one of the promising means for the improvement of probiotics survival in both food matrices and transit throughout GIT. It ensures the delivery of viable probiotics to the intestine. Alginate with chitosan coating is commonly used materials for the microencapsulation of probiotics through extrusion and emulsion. But, for industrial purposes, spray drying especially freeze drying is the promising technique.

ACKNOWLEDGEMENTS

This study was financially supported by the Spinghar Higher Educational Institute.

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