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ARTICLE

Enhancing bioavailability of probiotics using microencapsulation

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Microencapsulation is a process of coating tiny solid particles or droplets of liquid or gaseous material with a continuous film of polymeric material. By microencapsulation, the core material is prevented from coming in to direct contact with the surrounding atmosphere. This process offers advantages like sustained release, taste masking, increased stability and smaller particle size. Its applications are commonly found in nutraceutics, cosmetics, perfumery, textiles, paint industry and especially in pharmaceutical and food industries. Biologically active species need to be protected from enzymes present in the body as degradation prior to reaching their targeted site can lead to decreased bioavailability. One of the most trending research areas in this regard is microencapsulation of probiotics. Probiotics are microorganisms found in the digestive system and are known to provide immunity and health benefits. However, when consumed orally, they are reported to have poor viability against the gastric pH, with almost 65% of strains of probiotics having low or moderate tolerance. This emphasizes on the need to develop effective delivery systems of probiotics into the gastrointestinal tract by by-passing the highly acidic gastric conditions, which is the major degradation site of these bacteria. Different microencapsulation techniques, like spray drying, spray congealing, extrusion method, complex coacervation and materials like chitosan, carrageenan, alginate, starch have been explored for the effective delivery of probiotics. Synthetic polymers like ethyl cellulose, hydroxypropyl cellulose, acrylates and polyvinyl acetate phthalate are also promising coating agents in microencapsulation. More techniques and material are under study to develop effective systems for delivery of probiotics. This review presents the recent advances in microencapsulation process and the coating materials being studied for increased survival and targeted delivery of probiotics.

Keywords: Microencapsulation; encapsulating polymers; probiotics; biopolymers; bioavailability;

1. Introduction

According to the World Health Organization, probiotics are defined as living microorganisms which when administered in adequate amounts confer a health benefit to the host. [1] These are called beneficial bacteria. The annual global market for probiotics was estimated to be $3.3 billion in 2015, while in 2017 it increased drastically to $42.25 billion and it is expected to reach $74.69 billion by the end of the year 2025. [2] Lactobacillus, Bifidobacterium, Saccharomyces are few of the most commonly used strains of bacteria as probiotics.

These organisms reside in the gastrointestinal tract and provide benefits like producing pathogen inhibiting substances by blocking the interaction sites of bacteria, promoting nutrient absorption and inactivation of toxin receptors. Apart from these, a major role of probiotics is the modulation of immune responses. [3] On addition of probiotics to conventional food, there is nutrient enrichment in the form of fibers, vitamins, minerals and antioxidants. [4] Due to these health benefits and their safety, probiotics are in great demand in functional foods and nutrition sector. These are marketed in a wide range of products including dairy, bakery, beverages, and confectionaries as well as in fruits and vegetables. [5] Ideally, the probiotic strain should be able to proliferate at the targeted location. It itself should not induce any side-effect. It should have the characteristics to survive and grow in the gut, surviving the bile acids. They perform functions like lactose digestion, resistance to enteric pathogens, anticolon cancer effect, antihypertensive effect, hepatic encephalopathy, neutralization of dietary carcinogens, etc. [6] Probiotics are now also been used for diabetes and obesity. [7]

However, a major challenge in delivering these bioactive compounds is the viability and bioavailability throughout

the gastric conditions and also surviving the biological enzymes. A concentration of 108-109 cfu/g per day is considered to be necessary to produce the health benefits of the strain. [8] This may not be possible unless some technique is employed to increase bacterial viability. Several approaches to increase the viability of probiotics have been developed, one such promising technique is microencapsulation. Covering the probiotic with a shielding layer so that it directly does not come in contact with the acids and enzymes that tend to degrade it, and the outer covering resists all the degradation conditions is a feasible approach towards increasing the probiotic efficiency. Entrapment or encapsulation of bacteria can be achieved by the process of microencapsulation, which is reviewed in this article. Some recent patents are listed in table no. 1.

Table 1. Patented microencapsulated probiotics

Microorganism	Patent no.
Bacillus	US8697055B2, US5455028A, US9144588,
Bifidobacterium	US20100183559, US20040202749
Enterococcus	US 20070098744 A1
Lactobacillus	US8460917 B2, US6797266 B2, US20160151434A1
Lactococcus	US20080305089,
Pediococcus	US20150246082,
Saccharomyces	US 6010695 A, US20140205581A1
Streptococcus	US20140023620,

Microencapsulation is a process in which small particles or liquid droplets are covered with a polymeric layer. This resultant structure is called as microcapsule or microsphere. The material inside is called the core or the active ingredient while the outside polymeric layer is called coating or the membrane. The primary objective achieved by this process is the protection of active ingredient from adverse surrounding conditions like pH, temperature, enzymes and other environmental conditions by the active core can be degraded or inactivated. [9]

The microcapsule exhibits properties which are dependent on the active core material, the protective outside covering as well as the microencapsulation technique used in the process. This process is widely used in perfumery, flavours, functional foods, pharmaceuticals and bioactive compounds. In this article, we have discussed the materials and techniques used in traditional processes and the advances in the process for the coating of probiotics. Most commonly used coating materials are gums, gelatin, maltodextrin, proteins like whey and soy, chitosan and starch. These are used to encapsulate carotenoids, anthocyanins, vitamins, phenolic compounds. [10-16]

Coacervation is a commonly used technique which works on the principle of separation of a solution of two polymers into different phases. Simple coacervation involves only a single polymeric material. The polymer is dissolved in a suitable solvent and the core material is dispersed in the solution. By using a desolvation technique, where in a solute is added to the mixture to selectively dissolve in the solvent and displace the polymer, the polymer is made to encapsulate the active core material. This can be initiated by addition of a solvent like water, hexane, acetone, propanol, or salts like sodium sulphate and by modulation of temperature. [17-19] The choice and concentration of the solvent depend on the stability and viability of probiotics in that particular solvent.

In complex coacervation, there are two oppositely charged hydrophilic polymers. When these two polymers neutralize each other, there is a separation of polymer-rich phase. A combination of negatively charged gelatin and positively charged gum Arabic is used in the process. [20] Here, pH and temperature are important parameters. In this technique, the polymer is dissolved in a volatile solvent and the active core is added to the volatile medium. This mixture is homogenized and then the solvent is evaporated. The result is the formation of microcapsules of the active core. Coacervation techniques offer advantages like high encapsulating efficiency, lower operating temperatures and controlled release of the active core. However, this technique is preferred only for hydrophobic compounds.

Spray drying is another widely used technique for microencapsulation. It is industrially economical, flexible, efficient and scalable. In this process, the active compound is suspended in the polymeric solution of the covering. The dispersion is homogenized and using an atomizer, the solvent carrying the polymeric solution evaporates and hence the microcapsules are formed. In spray drying, one has to take care of the temperature, drying matrix, atomization, retention time and moisture content, as these parameters are crucial for bacterial viability. It is a single step, closed process and can be operated continuously. Important parameters to be considered are the inlet temperature, feed rate and air flow, which affect the properties like particle size, residual content and yield. [21] Spray drying offers a unique benefit of employing drying along with encapsulation. However, rigorous process parameters can deteriorate the overall viability of the microorganisms.

In the extrusion process, both the active core and the polymer coating solution are sprayed at high pressure through a nozzle. Such extrusion of the coating material passing through the nozzle form the microcapsules and more the number of nozzles, higher is the speed of the process. In case of bacteria, the extrusion method is employed to produce the dispersion and the resultant droplets are fall into hardening solution. This process may be repeated until the desired thickness of the covering is obtained. However, for bacteria, literature review shows that emulsion techniques are more widely used instead of extrusion methods. [22]

2. Advances

There are three basic approaches to effectively microencapsulate probiotics for improved viability. Firstly, by coating the bacteria containing microcapsule with an additional layer of some polymeric materials discussed here. Secondly, we can optimize process parameters to increase cell bioavailability. Thirdly, incorporation of prebiotics in the microbial sphere can also add to the viability.

2.1. Encapsulation materials

Polysaccharides like starch, chitosan, carrageenan, alginate and different gums, proteins like gelatin, milk proteins like casein and whey protein, and soy protein as well as fats are few of the researched materials for microencapsulation in the food industry. [23-26]

2.1.1. Chitosan

Food-grade bio-polymers are readily available, low-cost and effective as physical barriers imparting the desired protection to the bacterial core. Hence coatings like alginates, starch, pectin, carrageenan and milk proteins are considered more suitable for bacterial encapsulation. [27-29] Chitosan is a polysaccharide of D-glucosamine and N-acetyl D-glucosamine linked with beta linkages. It is abundant in nature and obtained from shells of crabs and shrimps. Chitosan has ideal properties like high biocompatibility, high mechanical strength, non-toxicity, cationic character and biodegradability. [30] Several studies have been performed using chitosan as the coating material for probiotic bacterial strains, which have shown improvement in the survival of these chitosan-coated probiotic bacteria.

Chitosan is applied on the surface of microcapsules as a coating material which adds an extra layer of protection for the coated probiotic material. It decreases the capsule permeability, makes it less prone to temperature and pH related changes. [31] It has been observed that among all the coating materials like chitosan, alginate, whey protein and poly-L-lysine have better performance against gastric condition. Further, among all these coating materials, chitosan has given the best results. Some reports are summarized in table no 2.

2.1.2. Alginate

Alginate is a naturally occurring polysaccharide found in the cell walls of brown algae. It is a polymer of L-glucuronic acid and D-mannuronic acid joined together by 1, 4-glucosidic linkages. It is a widely used polymer due to to its ability to cross-link between the two constituent acids and because of its non-toxicity. Table no. 3 shows few studies evaluating the effectiveness of alginate as a coating material.

Table 2. Reports with chitosan as microencapsulating materials

Material	Bacterial strain	Results	Reference
Chitosan coated alginate Coating	*L. bulgaricus*	Increased storage stability	Koo et al, 2001 [32]
Chitosan coated alginate Coating	*L. casein, L. planetarium*	Best protection of bacteria under simulated GI conditions	Vodnar et al, 2016 [33]
Pectin- chitosan capsules	*L. casein*	Protection from acidic conditions and higher viability in intestines	Bepeyeva et al, 2017 [34]
Pea-protein alginate capsules with chitosan coating	*L, rhamnoses, L. helveticus (R0052)*	Increased cell viability and stable microcapsule size	Varankovich et al, 2017 [35]
Chitosan coated microspheres with selenium-enriched green tea (2g/100mL)	*L. casein, L. planatarum*	Higher number of bacteria survived at refrigeration storage	Vodnar et al, 2016, [33] Molan et al, 2009 [36]
Alginate microcapsules with chitosan	*B. bifidum*	Higher protection in gastric conditions.	Zou et al, 2011 [37]
Alginate-chitosanocarboxymethyl Chitosan	*L.casei*	Increased viability up to 10^8 cfu/g, protection again harsh conditions	Li et al, 2011 [38]
Chitosan-alginate-xanthan gum- β-cyclodextrin	*L. plantarum*	95% survival rate at pH 1.8 and release at pH 6.8 and heat resistance up to 0 C	Fareez et al, 2017 [39]

Table 3. Reports of alginate as microencapsulating material

Material	Bacterial strain	Results	Reference
Alginate + starch	*B. adolescentis, L. casein*	Better resistance against acidic Conditions	Hansen et al, 2002, [40] Sultana et al, 2000, [41] Sun et al, 2000. [42]
Alginate	*B. adolescentis*	Increased survival to gastric and intestinal simulation	Annan et al, 2008 [43]
Alginate	*L. acidophilus, B. bifidum, L. casein*	Increased survival on 5, 3, 3 Log CFU	Krasaekoopt et al, 2004 [44]

2.1.3. Whey protein (WP)

Whey protein is a milk derivative obtained as a by-product in cheese production. It is a commonly used protein along with its counterpart casein. Both these proteins are used in the application of high-density coating in microencapsulation. It has been shown that milk proteins are biocompatible with probiotics and possess desirable gelling properties. [45] Another study also showed that proteins have the buffer-like ability to create a pH condition suitable for the survival of probiotic within the microcapsule, irrespective of the pH condition outside. [46] When paired with carbohydrates like alginate, maltodextrin, pectin, it exhibits amphoteric behaviour. [47] Microencapsulation of L. plantarum 299v, 800 and CIP A159 with alginate matrix in calcium chloride solution and coated with whey protein is also studied. Simulated gastric and intestinal fluid assays confirmed that coating whey protein on the alginate coated microcapsules has significant effects in increasing the cell viability from 2.19, 1.89, 1.65 log CFU to 10.04, 10.12, 10.03 log CFU for the three strains respectively. [48]

Another study on L. plantarum showed that whey protein along with sodium alginate showed 96% survival rate for freeze drying and 87% for spray drying techniques. [49] They also performed a similar experiment using denatured whey protein and the results showed that it produced 92% viability for freeze drying technique and 80% for spray drying technique. The undenatured coating showed better survival of probiotics in simulated gastric bile conditions. Hence, whey protein in its undenatured form has better encapsulating properties than its denatured counterpart. The study also stresses that for a coating material made of sodium alginate and whey protein, in a mass ratio of 1:1.5, freeze drying is a more efficient method than spray drying.

2.1.4. Poly-L-lysine (PLL)

Poly-L-lysine is a homo-poly amino acid; consist of only a single type of 25-35 amino acid molecules, lysine. It is used as a food preservative, because of its large spectrum anti-bacterial and anti-fungal activities, [50] and also as a food additive in Japan, USA and South Korea. Although PLL is widely used in encapsulating processes, its use as an effective coating agent for probiotics is not yet experimentally verified. Cui and co-workers coated B. bifidus with both alginate and PLL. There was no significant difference between the two in terms of the viability in simulated gastric fluids. [51]

Some studies also show that the use of PLL as a coating produce positive results. PLL on alginate coatings and palm oil and alginate mixture coatings were used which resulted in a small positive change for the bacteria B. lactis, Bi-04 and Bi-07. [52, 53] These experiments were performed using different parameters, other than the coating and hence we see a wide variety of results. The use of enzyme pepsin inhibits the activity of PLL as it is a protein polymer. Hence, there was a difference in the two studies, since one of them used pepsin while the other didn't. The prior group did not see any betterment in the viability properties and the latter group found out small, yet significant (1 Log-fold) improvement. [54]

2.2. Techniques of encapsulation of probiotics

2.2.1. Extrusion

Application of this technique has produced some notable positive results in different studies for different strains. In studies performed on strains L. acidophlis and B. lactic, the survival was significantly increased when coated in calcium-induced-starch complex. [55] Another study reported that using alginates in extrusion technique resulted in increased heat resistance of L. casei. [44] E King et al. showed similar results for L. acidophilus in the production of fermented tomato juice by calcium alginate. [56]

2.2.2. Spray drying

When spray drying is used as the microencapsulation technique, optimizing the spray drying conditions according to the bacterial strain and the encapsulation material will give the best possible result. Parameters like osmotic stress and temperature need to be optimized. High temperatures can cause damage to the cytoplasm membranes and organelles of the bacterial strains, thus reducing their activity. [57] Addition of protectants like glycerol, lactose and other polymers into the feed has shown to solve this problem by preventing bacterial degradation. [58] Table no. 4 summarizes more studies.

Table 4. Reports of spray drying in microencapsulation of probiotics

Wall material	Probiotic	Temperature parameters	Result (As % survival)	Reference
Whey protein isolate	*L. planatarum* A17	Input:110°C Output:68°C–70°	25–69	Khem et al, 2016 [61]
Whey protein isolate, gum acacia, tuna oil	*L. casei* 431	Input:180°C Output:80°C	37.6-56.2	Eratte et al, 2015 [62]
Maltodextrin, orange Juice	*L. planatarum*	Input:150°C Output:70°C	100	Barbosa et al, 2015 [63]
Gelatin, whey protein concentrate, maltodextrin, modified starch, pea protein Concentrate	*S. cerevisiae*	Input:80,125°C Output:70°C	84.9– 92.8	Arslan et al, 2015 [64]
Sodium caseinate, pectin, anhydrous milk fat	*L. salivarius*	Input:165°C Output:90°C	90	Zhang et al, 2015 [65]
Skim milk powder	*L. casei*	Input:170°C Output:80°C–85°C	97	Dimitrellou et al, 2016 [66]
Sugar beet pectin, soybean oil	*L. salivarius*	Input:165°C Output:9°C	78-86	Zhang et al, 2016 [67]

2.2.3. Freeze drying

This technique is commercially used on a large scale. In this technique, the probiotics are subjected to very low temperature conditions, hence not all strains can be developed using freeze drying. The formulation is frozen to a temperature below the critical temperature. In the first stage, the pressure is lowered and the temperature is raised slightly to remove water. Then in the second step, the formulation is dried to eliminate the bound water. The product, now, is brought to ambient temperature. Some studies show that the freeze-drying technique can produce microcapsules with higher viability than those produced, of the same composition, by spray drying. [49]

2.2.4. Immobilization

Immobilization technique involves entrapment of the core material within the matrix of the polymeric material. This technique involves four main steps namely entrapment, adsorption, self-aggregation followed by mechanical containment depending upon the mechanism used. This technique provides desirable properties like pH and temperature stability, higher cell density, cell loads and faster fermentation rate. [59] This technique has been verified by using whey protein isolate gel micro-entrapment to improve the viability of L. rhamnoses. [60]

2.3. Incorporation of prebiotics

Prebiotics are those food materials which aid the bacterial growth and increase the activity in the colon. Hence these confer health benefits offered by the bacteria. [68] Some natural foods are also good sources of prebiotics, like banana, onions, leeks, raw oats, beans, soya beans, etc. Prebiotics can be classified on the basis of the types of bifidogenic, non-digestible oligosaccharides (OS), like inulin and its hydrolysates like oligofructose (OF) and oligogalactose (OG), present in them. [69]

Probiotics are combined with prebiotics and the resultant combination is called synbiotic. The prebiotic component selectively stimulates the activity of the probiotic component. [70] A major advantage is an increase in the viability of the probiotic. It also offers added advantages like improving the microbial balance of gut and immunity modulation. [71] A typical composition of symbiotic is a mixture of OS, OF and inulin as prebiotics and *Lactobacillus*, *Bifidobacterium* and *Saccharomyces* species as the probiotics. Table no. 5 shows an analysis of few symbiotic combinations and their characteristics.

Table 5. Symbiotic combination of probiotics with prebiotics

Material as Coating	**Prebiotic**	**Bacterial strain**	**Results**	**Reference**
Chitosan-alginate	Quercetin	*L. gasseri* and *B. bifidum*	Improved survival	Chávarri et al, 2010 [72]
Chitosan	Resistant Starch	*L. gasseri* and *B. bifidum*	Improved viability	Iyer et al, 2005 [52]
Chitosan-sodium Alginate	Inulin	*L. acidophilus*	Increased cell viability	Jantarathin et al, 2017 [73]
Chitosan-sodium Alginate	Jerusalem Artichoke	*L. acidophilus*	Increased cell viability	Jantarathin et al, 2017 [73]

3. Conclusion

From various research works it has been observed that microencapsulation of probiotics helps to increase the viability of various types of bacteria. Different types of techniques of encapsulation and various types of polymers help probiotics to remain stable throughout the transitions in the gastric environment. Chitosan has ideal properties among various polymers, which increases storage stability of probiotics. Alginate, whey proteins and Poly-L-lysine increase survival of probiotics in stimulated gastric conditions. Spray drying is one of the most commonly used methods for encapsulation of probiotics. Depending upon stability of probiotics in different ranges of pressure and temperature, a suitable technique is used, as each technique works on different parameters. Commercial encapsulated probiotics are prepared by spray drying and alginate gel bead encapsulation. Further research is required in order to improve the quality and to make large-scale manufacturing procedures more convenient.

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