Microencapsulation of probiotics in hydrocolloid gel matrices: a review

Wunwisa Krasaekoopt
Assumption University, Faculty of Biotechnology, Hua Mak Campus, Hua Mak, Bangkapi, Bangkok 10240, Thailand

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ABSTRACT: The use of hydrocolloid gel matrices to encapsulate probiotics is of interest due to their gentle and simplicity of gel formation as well as mild condition used. This technique enhances the viability of entrapped cells during the product shelf life at least above therapeutic minimum level as well as in the gastrointestinal tract to ensure the health benefits of consumers. This review describes the advantages of microencapsulation, hydrocolloid gel matrices such as alginate, carrageenan and whey protein, microencapsulation processes, special treatments for further improvement in encapsulation efficiency of gel matrices as well as food applications of microencapsulated probiotics.

INTRODUCTION

Probiotics are living microorganisms that have been used in a manufacturing of functional food products such as yogurt and cheese because they improve the intestinal microbial balance of the host (1). The health benefits of probiotics include intestinal infection control, cholesterol level control, immune system stimulation, lactose utilization improvement in lactose maldigestors (persons who cannot digest lactose), providing antitumorigenic activity, and reduction of inflammatory bowel disease as well as Helicobacter pylori infection (2-4). In order to achieve these benefits, probiotic bacteria should be metabolically stable and active both in the product and host, which were influenced by food composition (acid, oxygen and hydrogen peroxide (5)) and digestive system of host (acid, enzyme and bile salt). To make a health claim, the therapeutic minimum level should be at least 10^7 cfu/g or ml of the product (6), which can be achieved by using microencapsulation technology. Simultaneously, low level or poor survival of free probiotic bacteria was demonstrated by many studies (7-13). It was influenced by many factors such as pH, post acidification, hydrogen peroxide and storage temperature, including food compositions.

Microencapsulation of probiotics is a process that probiotic bacteria (core material or internal phase) is coated with or entrapped within another material (supporting material or wall material). This technique provides a physical barrier to the bacterial cells and has been proved to improve the survival of probiotic bacteria in adverse conditions such as in food products and simulated digestive system (14-17). Selection of microencapsulation technique is of consideration. Since probiotics are living microorganisms, therefore, the microencapsulation technique should provide the non-toxic condition in order to maintain cell viability and assist the releasing of cell in the GI tract where probiotics can colonize and provide the health benefits. Physical methods of microencapsulation techniques, such as spray drying, freeze-drying and spray-freeze drying methods, involve with the conversion of cell suspension in to solid dried powder (18). The encapsulated cells are released by rehydration as soon as they are wet when the powders are applied in foods. Conversely, microencapsulation of probiotics by using hydrocolloid gel matrices is achieved through chemical reaction. The microencapsulated cells remain in the gel when they are applied in wet condition of foods and will be released when the gel matrices are broken due to the changes in ionic condition. Moreover, the nutrient and metabolites can transport through the semipermeable membrane of gel matrices easily, minimizing cell release and contaminations. Therefore, this paper reviews the microencapsulation techniques of probiotics in hydrocolloid gel matrices including microencapsulation process, drying methods of microcapsules as well as the application of microencapsulated probiotics in foods.

BENEFITS OF MICROENCAPSULATION IN HYDROCOLLOID GEL MATRICES

Since the microencapsulation in hydrocolloid gel matrices uses and provides friendly environment to probiotic cells, the entrapped cells are saved from the harmful organisms and environment such as bacteriophages, freezing, freeze-drying, storage condition, harmful compounds in foods and gastric condition. Microencapsulated probiotics are protected from bacteriophages due to the smaller pore size of gel matrices than the smallest size of bacteriophages (19). Calcium alginate gel, for example, provides the pore size of 7-20 nm in diameter, whereas the smallest lactic acid bacteriophages are approximately 150 nm in length and exhibit a head diameter of approximately 50 nm (20, 21). Processing techniques also considerably influence to the survival of probiotic cells. Increasing in number of entrapped cells after freezing and freeze-drying are recognized. The viability of microencapsulated probiotics was remarkably improved by 30-40% during freezing and freeze-drying (22-24). In addition, the greater stability of entrapped cells was of interest. Many researchers have reported that probiotic bacteria encapsulated in hydrocolloid gel matrices, such as alginate and xanthan gum, provide better stability during storage than free cells (25-27). Kebary et al. (28) also reported that the survival of bifidobacteria increased by 10-20% during the storage at 20°C. Moreover, injured probiotic cells caused by high osmotic pressure and freezing injury in frozen dessert were
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The survival of probiotics in food products and after exposure to gastric condition are also extremely important; since probiotic bacteria are required to be at least at therapeutic level in the products, assuring their colonization in the large intestinal tract in order to provide the health benefits to the host. An increase in survival of probiotics in acidic condition of yogurt by using microencapsulation in hydrocolloid gel matrices was reported by many researchers (30-33). Additionally, the presence of microencapsulated probiotic bacteria may have little or no effects on the post-acidification during yogurt storage (14, 17). The high level of microencapsulated probiotics, such as Lactobacillus casei and L. acidophilus TISTR 450, in the condition that contain high acid and natural antimicrobial substances (such as phenolic compounds) like fruit juices was also reported by Krasaekoopt et al. (15). Furthermore, the environment inside the hydrocolloid gel matrices was also favor for the growth of probiotic bacteria (16, 32).

The viability of probiotics after exposure to gastric condition was also enhanced by microencapsulation technique in hydrocolloid gel matrices. Many studies reported a decrease in death rate of entrapped cells in simulated gastric environment with and without bile salt (25, 32, 34-36). Alginate gel with chitosan coating offers an effective delivery of viable probiotics to the colon and increases their survival during simulated gastric and intestinal conditions (37, 38) as well as in gelatin coated with alginate (39, 40) and xanthan-chitosan-xanthan beads (41). Similar result was obtained when L. rhamnosus ATCC 53103 and Bifidobacterium longum ATCC BAA-999™ were encapsulated with the mixture of xanthan gum and gellan gum (42). Moreover, a great protection of eight strains of probiotics encapsulated in alginate-hi-maize resistant starch was demonstrated (43).

HYDROCOLLOID GEL MATRICES

There are various types of hydrocolloids gel matrices used for microencapsulation of probiotics. These biopolymers are alginate, gellan-gum, xanthan, κ-carrageenan, whey protein, casein and more (44). Some chemical properties of these hydrocolloids are shown in Table 1.

**Alginate**

Alginate is the most widely used hydrocolloid for microencapsulation of probiotics due to its gentle environment, low cost, simplicity, biocompatibility with the probiotics and proper release in the intestine to release encapsulated cells (35, 45, 46). It is linear heteropolysaccharides of 1-4 linked b-D-mannuronic (M) and a-L-guluronic acid (G) extracted from various types of algae (47). This polymer is found in three forms as homopolymeric M-block (M-M-M) (Figure 1)

Alginate forms a strong heat-stable gel matrix with cation such as calcium ions, which can develop and set at room temperature. The functional properties of gel matrix strongly depend on the source of alginate, the composition and the sequence in L-guluronic acid (G) and D-mannuronic acid (M). The binding sites for ions of alginate related to the cavities formed by diaxially linked G residues, providing junction zone in gel network. This binding zone between the G-block is described as “egg-box model”. Therefore, the length of D-mannuronic acid polymer is the major structure contributing to gel formation (48). Probiotic cells were entrapped instantaneously in a three-dimensional lattice of alginate. There are many factors affecting to gel formation of alginate such as concentration of alginate and CaCl2, timing of gel hardening (35, 49). Low concentration either alginate or calcium chloride reduced mechanical strength of gel (50). Calcium chloride 0.5 M and 1-2% alginate provided the highest gel strength. Moreover, the concentration of alginate mainly influences the bead size. As the concentration of alginate or viscosity increases, the size of the microcapsule decreases. The size of microspheres was also affected by alginate composition. Small microcapsules were resulted from alginate low in guluronic acid (51). On the other hand, Poncelet et al. (52) reported that the size of the microspheres was depended on the physiochemical properties of alginate rather than the concentration (53). In acid environment, alginate microcapsules were shrunk because alginate precipitate or form gels in acidic condition (32). The pH values of mannuronic and guluronic acids are 3.38 and 3.65, respectively.

Even though alginate is the most popular wall material for microencapsulation of probiotics, there were some disadvantages as low stability and high porosity of the structure that limit the use of this compound alone. The alginate gel was not only destabilized in the presence of high affinity ions, for example, phosphate, lactate and citrate, resulting in releasing of entrapped cells; but also by high concentration of non-gel-inducing ions as sodium and magnesium ions that replace calcium ions in the structure. Moreover, the leakage of microencapsulated cells was recognized due to the macroporous structure of alginate gel. These limitations of alginate can be efficiently overcome by either blending or coating with other polymers such as resistant starch and chitosan (13, 54, 55).

**Xanthan gum and gellan gum**

Xanthan gum is exopolysaccharides produced by fermentation of Xanthomonas campestris (56). Although xanthan gum has a b-D-glucose backbone similar to cellulose, it is attached with a trisaccharides composed of mannose, glucuronic acid and mannose at every two glucose units. The mannose bound to the backbone contains an acetic acid ester on carbon 6, and the mannose at the end of the trisaccharide is linked through carbons 6 and 4 to the second carbon of pyruvic acid (Figure 2). Although xanthan gum in solution can form intermolecular associations, consequential in complex network formation of weakly bound molecules; the rigid or strong gel is not formed. Increasing in viscosity of the solution is only observed. To improve the gel strength of xanthan gel, cooperation with other hydrocolloids, such as gellan gum, is required.

Gellan gum is produced by Pseudomonas elodea (56). The structure comprises of four linked monosaccharides,
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including 17 one molecule of rhamnose, one molecule of glucuronic acid (an oxidized glucose molecule), and two molecules of glucose (Figure 2). The exact 19 molecular formula of gellan gum may vary slightly, depending on the degree to which the glucuronic acid is neutralized with various salts (29). Gellan gum form gel through chemical reaction with cations such as sodium, potassium, calcium and magnesium. These ions enhance aggregation of the gellan double helices to form a three-dimensional network, leading hard and brittle gels, which is affected by the amount of acyl groups in the structure of gellan. Low acyl gellan gum form hard gel, in contrast with high acyl gellan gum that forms soft and elastic gel. Gel formation of gellan does not require only cations, but also high temperature for gel-setting as high as 80-90°C, resulting in heat injuries of probiotics when gellan alone is used (30).

Since alginate has low acid resistance, the use of xanthan-gellan mixture is of interest for microencapsulation of probiotics by using chemical gelation with the ratio of xanthan to gellan as 1:0.75 (13, 57).

Whey protein

Microencapsulation of probiotics in whey protein gel matrices is applicable (58, 59) because of their biocompatibility (60). Whey proteins generally composed of α-lactalbumin, b-lactoglobulin, immunoglobins and serum protein. The globular structure of major whey proteins is principally heat sensitive. Protein unfolding and aggregation occurs simultaneously when whey proteins are heated. Whey protein can form gel, precipitates or colloidal dispersions depending on many factors such as temperature, pH, ionic strength and protein concentration. For microcapsule formation of whey protein, pH is the most important factor to control heat-induced whey protein aggregation. Heat treatment at pH 5.5-6.3 at ionic strength lower than 15 mM gives stable microcapsule of whey protein with the size of 220 nm. Whey proteins have been used as wall material for the microencapsulation of bacteria (59, 61, 62) and nutraceuticals (63) because whey protein gel provides protection of probiotics both during processing and storage. The presence of protein in wall materials also protects probiotics against acid condition of the gastrointestinal tract (64, 65) and fruit juice (66). Using whey protein with and without resistant starch provide better protection to L rhamnosus GG in apple juice than the use of resistant starch alone during the storage at 4°C. Microencapsulation of probiotics in protein gel matrices can be performed in both physical methods, such as spray drying and freeze drying methods, and chemical method as extrusion method. Whey protein provides the highest potential for microencapsulation with 96% yield of probiotics in the whey protein microcapsules by using extrusion method (26). Recently, Rodrigues et al. (67) reported the high survival of L. acidophilus Ki, L. paracasei L26 and B. animalis BB-12 during 6-month storage by using whey protein and spray drying method. On the other hand, whey protein did not improve the survival of L. rhamnosus GG at high water activity (68).

Renetted casein

Caseins are proline-rich, open structured rheomorphic proteins. Caseins contain both hydrophilic and hydrophobic parts and 95% of caseins are naturally self-associated as casein micelle. Gelling of caseins by rennet enzyme to form water-insoluble microcapsules for microencapsulation of probiotics is of interest. There was no significant loss of probiotics during the microencapsulation process. A high density gel network is obtained when a high concentrated aqueous casein solution is used, resulting in better probiotic protection. High pH within the casein gel matrices, caused by buffering capacity of casein, protect the cells from the severe condition of simulated gastric juice at low pH. Moreover, the microcapsule size is controlled by the unique functional properties of caseins (69).

Many researchers have reported high survival rate of microencapsulated probiotics using renneted casein as wall material with or without non-dairy wall materials (64, 70, 71). Recently, Heidebach et al. (44) used renneted casein and freeze drying method to encapsulated Lactobacillus F19 and Bifidobacterium Bb12 and the improved survival rates of these microorganisms during freeze-drying and storage at 4°C were reported.
**Carrageenans**

Carrageenans are family of linear sulfated polysaccharides, extracted from red seaweeds Rhodophycea. Their structures are composed of high molecular weight linear polysaccharide with repeating of galactose units and 3, 6-anhydrogalactose (3, 6 AG), both sulfated and non-sulfated, liked by alternating α-(1, 3) and β-(1, 4) glycosidic linkage. Carrageenans naturally exist as kappa (κ), iota (ι), and lambda (λ) (72). These carrageenans vary in their components, influencing on gel strength, texture, solubility, synergism, and melting temperature. As wall material for microencapsulation of probiotics, k-carrageenan is the most widely used. k-carrageenan requires high temperature as 60-90°C to dissolve when the concentration as high as 2-5% is used (73) (Figure 3).

k-carrageenan gel is thermoreversible. To use as wall material for microencapsulation of probiotics, the solution is needed to cool down from 40-45°C to room temperature immediately after addition of probiotic cultures, resulting in gel formation. Gelation of k-carrageenan can also be performed using chemical method by reacting with monovalent ions such as potassium (KCl), providing brittle gel, with low ability to withstand stresses (29). Nevertheless, addition of KCl inhibits the growth of some lactic acid bacteria such as Streptococcus thermophilus and L. delbrueckii ssp. bulgaricus (74), which can be eliminated by the combination with locust bean gum with the ratio of k-carrageenan to locust bean gum as 1:2 (75, 76). The mixture provides high gel strength with lower susceptibility to lactic acid produced during fermentation, promoting high stability of microencapsulated probiotics (77, 78). However, survival of some probiotics as bifidobacterium may be reduced since gelation of this mixture requires calcium ions (30).

**Cellulose acetate phthalate**

Cellulose acetate phthalate (CAP) is one of the most widely material used for controlled release formulation in pharmaceutical industries due to its resistance to acidic condition of gastric juices and its dissolvability in the intestinal juice (pH>6), caused by its negative charges (79). Its structure composes of cellulose polymer that 50% of hydroxyl groups esterified with acetyl and 25% is esterified with one or two carboxyls of a phthalic acid (Figure 4). Gel formation of this compound bases on its solubility in the hardening solution, especially in the presence of carbohydrate as starch. At the same time, CAP can be used as wall material in microencapsulation using physical methods such as spray drying. The success of microencapsulation of probiotics using CAP as wall material has been reported in different chemical and physical methods. For example, Rao et al. (1989) reported high survivability as high as 10^9 cfu mL^-1 after incubation in simulated gastric juice of B. pseudolongum encapsulated in CAP using emulsion technique. The similar result was obtained from the studies of Favaro-Trindale and Grosso (80) when B. lactis (Bb-12) and L. acidophilus (La-05) were encapsulated in CAP using spray drying method.

**Gelatin**

Gelatin is a protein gum derived from collagen, the main protein component of animal connective tissues. Commercial gelatins can be divided into two groups depending on the treatment to obtain as type A (acid pre-treatment) and type B (basic pre-treatment) (81). The structure of this compound has shown in Figure 5. Gelatin is water-soluble, providing high viscosity. A thermoreversible gel exist only a small temperature range depending on gelatin grade and concentration. Gelation of gelatin is caused by conversion of random coiled-helix when it is cooled. This compound can be used for microencapsulation of probiotics either alone or in combination with other compounds like anionic polysaccharides such as gellan gum due to its amphoteric properties.

<table>
<thead>
<tr>
<th>Hydrocolloids</th>
<th>Gel formation or microencapsulation mechanism</th>
<th>Acid resistance</th>
<th>Thermal stability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alginate</td>
<td>Require Ca^{2+}</td>
<td>No</td>
<td>Stable</td>
</tr>
<tr>
<td>Xanthan-gellan</td>
<td>Require Ca^{2+}</td>
<td>Yes</td>
<td>Stable</td>
</tr>
<tr>
<td>Whey protein</td>
<td>Thermal gelation</td>
<td>Yes</td>
<td>Sensitive</td>
</tr>
<tr>
<td>Rennet casein</td>
<td>Rennet enzyme</td>
<td>Yes</td>
<td>Stable</td>
</tr>
<tr>
<td>k-carrageenan</td>
<td>Require Ca^{2+}</td>
<td>Yes</td>
<td>Sensitive</td>
</tr>
<tr>
<td>Cellulose acetate phthalate</td>
<td>Require starch</td>
<td>Yes</td>
<td>Stable</td>
</tr>
<tr>
<td>Gelatin</td>
<td>Thermal gelation</td>
<td>Yes</td>
<td>Sensitive</td>
</tr>
</tbody>
</table>

Table 1. Some chemical properties of hydrocolloids used as wall material for microencapsulation of probiotics.
nature. Since these hydrocolloids carry net negatives charges and repel each other, they are miscible at pH>6. Nevertheless, when pH is below the isoelectric point of gelatin, the net charge of gelatin becomes positive, resulting in strong interaction formation with negatively charged gellan gum and stabilization of gel obtained (82).

MICROENCAPSULATION PROCESSES

Microencapsulation of probiotics in hydrocolloid gel matrices is the technique that the probiotic cells are entrapped during gel formation of wall materials using chemical gelation, leading to spherical droplet containing probiotics. These methods generally produce the beads of micron to millimeter size. These microcapsules can be further dried using freeze- or spray drying methods and the cells are still entrapped in the gel matrices after rehydration due to their water insoluble.

Extrusion

Extrusion is the oldest and most common methods widely used to form microcapsules of hydrocolloid gel matrices due to its ease, simplicity, low cost and gentle condition, ensuring high level of entrapped probiotics. The mixture of hydrocolloid solution (such as alginate and carrageenan) and the suspension of probiotic cultures is extruded through a syringe needle as droplets into a hardening solution such as calcium chloride (55, 83). The size and shape of the beads were influenced by many factors as the needle diameter, distance between needle and hardening solution as well as the surface tension of the hardening solution. This method produced the beads size of 2-5 mm, affecting to sensory characteristics of the applied food products. Nevertheless, the microencapsulated probiotics were well protected in the beads against the harmful condition of simulated gastric and intestinal juices as well as in food products (14-16, 32). Slow microcapsule formation caused the complexity to scale up.

Air atomization

Air atomization or concentric air-jet technique is the process that the mixture of probiotics and wall material solution is forced pass through the air atomizer by pressurized air in order to form the micron-size beads when dropped to the hardening solution (84) (Figure 6). The beads produced from this process were moderately uniform in size and shape with the diameter more than 200 µm, depending on nozzle size, rate of feeding, wall material concentration, distance between the orifice and hardening solution as well as the air pressure (85, 86). The beads can be produced under mild and sterile condition (87, 88) with the microencapsulation efficiency of approximately 30% (85, 86, 89).

Electrostatic atomization

Electrostatic atomization is the method that the electricity, either static or pulse, is used to create the electrostatic potential between the needle and the hardening bath in order to disperse the mixture of probiotic cultures and wall material solution to form the fine aerosol by acting on the charged surface of the mixture (90, 91) (Figure 6). Strong electrical field applied also had no effect on the survival of probiotic cells (92). There are many factors influencing to the size of the beads such as magnitude of the voltage (93), distance between the needle and hardening bath, concentration of wall material used, feeding rate of the mixture and diameter of needle used. This method is only suitable for the low viscosity mixture, even though it is a cost effective technique (94).

Jet break-up methods

These methods were developed to increase the productivity of bead formation with the small size of the microspheres (95). These methods are vibration nozzle and jet cutter (Figure 6). For vibration nozzle, the microspheres are formed by oscillating and forcing the mixture of probiotic culture and wall material through a nozzle into the hardening solution, resulting in narrow size distribution of beads as 0.1-3.0 mm in diameter (96). Similar to electrostatic atomization, this method cannot be used with high viscosity mixture (97). Conversely, jet cutter method was developed to use with high viscosity solution as high as to several thousand mPa•s by Genia Lab BioTechnology, Germany (98). The mixture of probiotics and wall material was forced through a nozzle to form liquid jet and then cut by a rotataing cutting tool or wire into
hardening solution to form the beads with the size less than 1 mm. The size of the beads is controlled by the number of cutting wires and the number of rotations of cutting tool as well as the feeding rate. The rate of bead formation and size distribution can be increased by applying of electrostatic charge (96).

**Spinning disc**
The beads are formed by feeding the mixture of probiotics and wall material onto the high velocity spinning disc (Figure 6). The droplets are then formed due to the centrifugal force at the edge of the disc and dropped into the hardening solution to form solid beads (99, 100). The size of the beads is controlled by the speed of rotating disc, in which the slow rotation is preferred. This method produces the beads with the size ranges from few hundred microns up to several millimeters. This method is suitable for the low viscosity fluid lower than 200 mPa•s, although it has a very high productivity.

**Vortex bowl**
In this method, the mixture of probiotics and wall material is fed through the atomizing disk located in the center of rotating bowl that fixed to the top of an agitation shaft, creating a vortex film of the mixture that is then broken down into smaller droplets prior to dropping into the hardening bath (101) (Figure 6). Formation of filaments that cannot be form droplet later is the limitation of this method, which caused by high viscosity and high flow rate as well as high speed used.

**Micro nozzle array**
In this method, the mixture of probiotics and wall material is forced through silicone micro-nozzle array with the size of 30 µ x 30 µ x 500 µm and then cut off by the flow of oil to form droplets, which are formed gel later in the hardening bath at the downstream of oil flow (102-104) (Figure 7). This method can be used with the high viscosity solution as high as 50 Pa due to the centrifugal induction and high pressure conditions (105).

**Impinging aerosol**
This method is a continuous, easy and scalable technique that has been developed to produce small beads with the size of less than 50 µm, aimed to reduce sensorial detection limits (106). The mixture of probiotics and wall material and the hardening solution are sprayed in the opposite direction (Figure 7). The mixture is sprayed at the top of the chamber, simultaneously the hardening solution is sprayed from the bottom, resulting in an immediate gel formation of the microspheres, which can be collected at the outlet of the chamber base. High productivity can be achieved by this method.

**Coacervation or emulsion method**
This method is based on the relationship between the discontinuous (the mixture of probiotics and wall material) and the continuous (vegetable oil) phases. A water-in-oil emulsion of both phases is formed and then broken by the addition of a solidifying agent, resulting in formation of microspheres within the oil phase (7, 29, 107) (Figure 8). Emulsifiers can be added to form better emulsion, leading to smaller size of beads (108). The most common emulsifier used is Tween 80 at 0.2% (28). The microbeads are then usually obtained by the membrane filtration. The size of the beads is in the range of 20 µm to 2 mm controlled by agitation speed, the type of emulsifier used and the ratio between water and oil (107). This method is easy to scale up for large production, however cost of the oil and its disposability could be a limitation (55).
SPECIAL TREATMENTS

Special treatments such as coating or addition of other polymers are applied to improve the mechanical and chemical stabilities and dense structure of the microcapsules to assure the high survival rate of microencapsulated probiotics in food products and in simulated digestive system (15, 16). This has less effect to the size of the beads. Chitosan, a positively charged polycation, is the most widely used as coating material for microencapsulation of probiotics. It forms a semipermeable membrane around a negatively charged polymer, improving survival and stability of entrapped cells (36, 49, 63, 109). Low-molecular-weight chitosan diffuses faster into the hydrocolloid gel matrix structure than high-molecular-weight chitosan, leading to a denser structure (32). Whey protein is also of interest as a potential coating material due to its biodegradable, especially if whey protein derived from bovine milk, which was stable and not digested in digestive system. Moreover, the high survival rate of probiotics after incubation in simulated gastric and intestinal juices was reported (110). The use of resistant starch, which is not digested by pancreatic enzymes in the small intestinal tract, can be used as co-polymer wall material with other compound such as alginate. It offered good enteric delivery by providing the sufficient diffusion of nutrients and metabolites, maintaining the growth of entrapped cells (13, 111). Moreover, the presence of starch reduces oxygen toxicity to B. lactis due to the oxygen diffusion restriction through the gel matrices as well as prebiotic functionality (112).

DRIYING METHODS OF MICROCAPSULES

The gel microcapsules of probiotics can be further dried to form dry particulate by using spray drying and freeze-drying methods in order to increase storage stability. The dried microcapsules of B. lactis [BI 01] and L. acidophilus [LAC 4], produced by emulsion method using the mixture of casein and pectin as wall material, showed greater stability in simulated gastric juices and during the storage at 7°C for 120 days (70). Goderska and Czarnecki (113) also reported very high survival rate of probiotics encapsulated in modified starch and alginate using extrusion method after spray drying at the inlet and outlet air temperature as 185°C and 85°C, respectively. The similar result was obtained by the studied of Li et al. (114) who encapsulated L. casei ATCC 393 in alginate-gelatin using extrusion method, followed by spray drying. Recently, Sohail et al. (115) reported only 3 log reduction occurred during the 6 month storage of the microencapsulated L. rhamnosus GG (ATCC 53130) and L. acidophilus NCFM in alginate matrix using impinging aerosols method, followed by spray drying at 120°C/60°C. Freeze-drying also can be used to convert the encapsulated probiotics in hydrocolloid gel matrices into the powder form for improvement of storage quality. The storage quality of L. bulgaricus KFRI 673 encapsulated in chitosan coated alginate beads was improved by freeze-drying (49). Addition of resistance starch and inulin as cryoprotectants during microencapsulation in hydrocolloid gel matrices was recommended to increase survivability during freeze-drying (116).

FOOD APPLICATIONS OF MICROENCAPSULATED PROBIOTICS

Microencapsulated probiotics has been used in many kinds of foods in order to increase their functionality (Table 2). There are two factors concerning for the food application of these probiotics, encapsulated in hydrocolloid gel matrices, as the therapeutic minimum level (107 cfu/g or ml of foods) and effect on sensory quality of the products (17, 117). Among dairy product, yogurt is considered as a good probiotic carrier. Due to a poor survival of these organisms in yogurt caused by its acidity and oxygen toxicity, addition of microencapsulated probiotics has been recommended (29, 109, 118). Direct addition of microcapsules after yogurt fermentation is suggested due to its easy production management (14). Although the big size of the probiotic beads from some microencapsulation method influenced the sensory properties of the product, the high consumer acceptance as high as 82.3 and 94.9% obtained for plain and fruit yogurts, respectively (119). Addition of GOS as prebiotics also enhances the growth of microencapsulated probiotics inside the beads (16). Although cheddar cheese is also a good carrier of probiotics due to its high pH (pH 5.5), good buffering capacity and high fat content, the application of microencapsulated probiotics is limited due to uneven distribution in the product (120). Moreover, the stability of encapsulated probiotics reduced during ripening process of cheese (121). Ozer et al. (122) also reported a great viability of microencapsulated probiotics during the process and storage of Kaser cheese as well as in white-brine cheese (123). Application of microencapsulated probiotics in frozen dairy dessert is also of interest due to its enhancing the survival in the product. Homayouni et al. (124) also reported the possible used of microencapsulated probiotics in frozen dairy dessert without effect on sensory quality of the product. In addition, the microencapsulated probiotics remained above the therapeutic minimum level in the product during 6 month storage at -18°C without sensorial effect (125).

Apart from dairy products, fruit juice was suggested to serve as a good carrier for probiotic delivery (126). Probiotics in fruit juice was not successful due to its unacceptable sensory characteristics (127); therefore direct addition is of interest. Similar to dairy product, the number of probiotics should not be less than therapeutic minimum level in order to validate the beneficial effects in the product. Krasaeenkoopt et al. (13) demonstrated the 4-log higher than free cells of microencapsulated probiotics in alginate beads coated with chitosan in various types of fruit juices without changes in acidity of the products during the refrigerated storage for 4 weeks, which was dissimilar to the studies of Sohail et al. (115). Moreover, high percentage of consumer acceptance as high as 85% of fruit juices containing probiotic beads was reported, indicating a potential market for these products (128), which was parallel to the studies of An-Eri King et al. (129) and Tsen et al. (130). They demonstrated that the sensory quality of fruit juice was not affected when the microencapsulated probiotics was applied. Mayonnaise, chocolate and meat products are also considered as a good probiotics delivery systems. Probiotics were well protected against the bactericidal effects of vinegar (131, 132). Recently, Malmo et al (133) also demonstrated that microencapsulation protects probiotics from the severe condition of chocolate production. For meat products, although high survival rate of microencapsulated probiotic bacteria in dry fermented sausages without the effect on sensory qualities was reported, the inhibitory potential of probiotics against Escherichia coli O157:H7 was reduced (134, 135).

CONCLUSIONS AND PERSPECTIVES

Microencapsulation of probiotics in hydrocolloid gel matrices is a successful method enhancing the survivability of these microorganisms not only in food products, but
also in the gastrointestinal tract to assure the beneficial health effects. Complex wall materials of hydrocolloid gel matrices and microencapsulation processes have been developed to assure a better protection of cells from the harsh environment and easy for large-scale production of industrial purpose. Therefore, the challenges are the selections of appropriate wall materials and microencapsulation process, including coating materials, in different products as well as optimization of processing factors to assure the highest viability and sensory satisfaction due to negatively relation between size and the protection potential of microcapsules. In addition, application of microencapsulated probiotics in hydrocolloid gel matrices into a wider range of foods are limited due to incompatible food matrix and non-favorable storage conditions as well as by the regulation of each country for addition of hydrocolloid gels in food, especially in dairy products, while an increasing of probiotic market is recognized. Therefore, more research work in food applications of microencapsulated probiotics in hydrocolloid gel matrices is required as well as its safety and environmental friendly production.

REFERENCES AND NOTES

Readers interested in a complete list of references are kindly invited to go to our website: www.agro-food-industry.com
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