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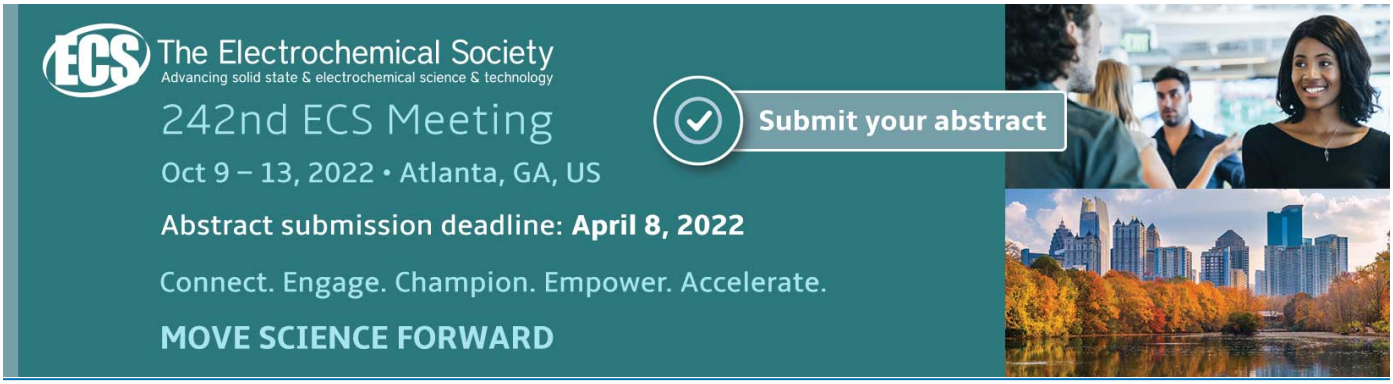

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Biopolymeric encapsulation of probiotics for improved release properties in the gastrointestinal digestion system

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Abstract. Encapsulation of probiotics in the biopolymeric system is an excellent technique to enhance the protective effect, prolong the shelf life and deliver the probiotics in the human gastrointestinal tract at a specific time. Probiotics are usually loaded in a biopolymeric system or the food itself as a natural carrier for food applications. Probiotics are well-known for a healthy intestinal tract and digestibility of nutrients. The fate and viability of these bacteria in the digestion system are explored through in vitro evaluations. Probiotics encapsulated with natural biopolymers such as alginate, chitosan, gelatine, whey protein are found to improve their release properties in either emulsion or hydrogel system. This review emphasized on the release properties of encapsulated probiotics loaded with biopolymers using different dispersion methods including emulsification, suspension, extrusion, and drying. Biodegradable polymers or the food itself could be a potential protective agent and promote the controlled-release properties of probiotics.

1. Introduction

To date, encapsulation is an engineered material using a variety of methods to preserve the probiotics along with the processing and storage. The attempt is also to improve the viability of the probiotics throughout the gastrointestinal transit. Hydrogel beads or known as an ionic gelation method is a prevalent encapsulation method formulated to increase the protective effect and the efficacy of probiotics. This method prevents the possible toxicity of unwanted conditions as the hydro gelation process depends on physical cross-linking between oppositely charged of ionic biopolymers such as alginate, carrageenan, and cellulose [1-3].

Encapsulation is an excellent technique in entrapping probiotics cell within an encapsulant matrix which can be developed using two common methods; extrusion and emulsion, or also known as droplet method (external ionic gelation) and two immiscible liquid (internal ionic gelation) [4]. Prebiotics, non-digestible food are being used as ingredients to produce functional food that could enhance survivability and protection of the microorganism, potential probiotics along with the gastrointestinal transit [5].

Recently, various biopolymers had been used for coating encapsulation agent and evaluated on their effectiveness. For example, sugarcane bagasse (SB), a source of cellulose or biopolymers promoted higher cell survivability of *Lactobacillus rhamnosus* (NRRL 442) than pineapple core (PC) [2]. This scenario is due to a great holding capacity of the biopolymers during the immobilization of NRRL 442. Furthermore, the immobilized *L. rhamnosus* in SB was encapsulated with a sodium

alginate solution (NaA) to produce a heat resistant microcapsules with enhanced encapsulation efficiency and survivability [1].

2. Encapsulation techniques for probiotics

The application of encapsulation technologies in the food and beverage industry is successful due to their ability to promote the stability of bioactive ingredients, which is the encapsulation matrix could be a physical barrier to protect against distinct temperature, UV, moisture and oxygen conditions. Various studies are required to design and improve the quality of the functional food products: 1) A possible interaction between bioactive compound and encapsulation matrix to provide safer and more effective functional food production, 2) Physical and chemical stability of the delivery systems incorporated with bioactive compounds, 3) Absorption, bioavailability, and safety analysis of encapsulated bioactive compound. Encapsulation of bioactive ingredients is beneficial in improving the bioavailability of functional properties by increasing the water solubility of bioactive ingredients including probiotics, protecting them against severe conditions at a different part of the digestive tract and releasing them in a targeted area such as intestine [6,7].

3. Method of encapsulation of probiotics

The most potential methods used for encapsulation of probiotics are including hydrogel (for example in figure 1), hydrogel combine with emulsion, spray drying, freeze-drying, microencapsulation. Alginate, a polyanionic copolymer of mannuronic and guluronic acid residues is extensively used as a coating matrix for encapsulation of probiotics (Table 1), which are cheap, non-toxic, biocompatibility, and high gelling capability [4]. Nevertheless, the physical stability of alginate is reducing when exposed to severe environmental conditions or monovalent ions or chelating agents [8]. According to recent studies (refer to Table 1), the efficacy of encapsulated probiotics was being enhanced through co-encapsulation with prebiotics, entrapping the beads using other polymers or by the combination of alginate with another polymer or compound [9]. The combination of prebiotics and biopolymers, such as alginate generate a synergetic effect [10].

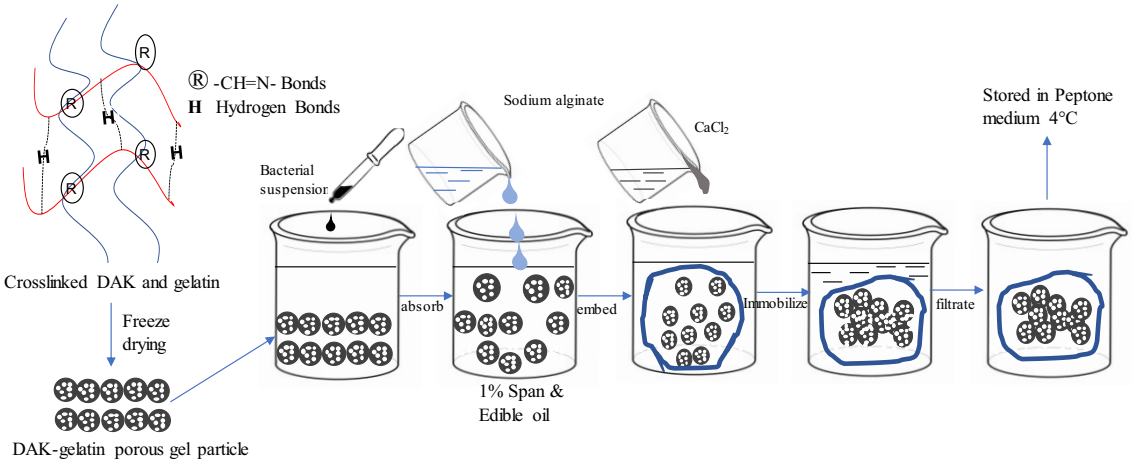

Figure 1. Encapsulation of probiotic by hydrogel method (adapted from Mu et al., 2018) [11]

4. Characterization of encapsulated probiotics

The particle size of encapsulated probiotic was evaluated using laser diffraction particle size analyzer (SALD-2001, Shimadzu, Japan) and the result varied from 18.2 to 23.01 μm according to different biopolymers concentration; 2% alginate (Al), 1% alginate: 1% gellan gum (Al-GG) and 1.5%alginate: 3% gum Arabic (Al-GA) [12].

Table 1. Release properties of probiotics encapsulated with biopolymers and various techniques.

Probiotic	Prebiotic	Biopolymers as encapsulation material	Technique	Survivability and release properties in simulated gastrointestinal fluid
Lactoferm Aby 6-*Streptococcus thermophiles* (80%), *Lactobacillus acidophilus* (13%), *Bifidobacterium bifidum* (6%), *Lactobacillus delbrueckii ssp. Bulgaricus* (1%) [13]	-	Whey protein concentrate-alginate (WPC-alginate) and whey protein hydrolysate-alginate (WPH-alginate)	Electrostatic extrusion technique	WPH-alginate- provide better protection on probiotics
Lactobacillus acidophilus [11]	Konjac oligosaccharides (KO)	Konjac glucomannan, Sodium alginate (SA), edible oil	Hydrogel and water-in-oil emulsion	Survival rates of L. acidophilus (with and without encapsulated) were significantly ($P < 0.01$) increased during freeze-drying situation
Lactobacillus plantarum CECT 220, *Lactobacillus casei* CECT 475 [14]	-	Soybean protein-maltodextrin-oligofructose-inulin	Coacervation	Enhanced tolerance at 3-4 log cycles
Lactobacillus acidophilus [15]		Alginate-shellac	Hydrogel, extrusion, co-extrusion	Co-extrusion-higher viability
Bifidobacterium BB-12 [16]	Sugarbeet and chicory	Full-fat goat's milk and/or prebiotics (inulin and/or oligofructose)	Hydrogel	The best probiotic **survival rate-** microcapsules produced with full-fat goat's milk (94.29%)
Bifidobacterium breve, *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis* [17]	-	Multilayer alginate hydrogel beads (MAHBs)	Hydrogel, emulsification	MAHBs-the promising encapsulating material for the oral delivery system
Lactobacillus acidophilus [18]	-	Resistant starch (hi-maize)-chitosan-sodium alginate (SA)	Hydrogel	Microparticles of SA with hi maize and chitosan-better protective effect at up to 6.35 Log CFU/g
Lactobacillus rhamnosus GG [9]	Inulin	Chitosan-alginate-apple juice	Hydrogel	4.5 times better survival rate than unencapsulated
LAB, *Lactobacillus brevis* WK12 and Lactococcus lactis WK11 [10]	-	Sodium alginate Food-grade cryoprotective agents (skim milk, soy powder, yeast extract, and trehalose)	Hydrogel beads	The greatest viability-10% soy powder
Bifidobacterium animalis subsp. Lactis [19]	-	Goat's milk ice cream	-	84.7% survival rate, 6-7 log CFU/g viability sustained within 120 days
Bifidobacterium-BB-12 [20]	Inulin and polydextrose	Liquid sweet whey only, liquid sweet whey and inulin, liquid sweet whey and polydextrose	Spray drying	Better protective effect- sweet whey, sweet whey and inulin
Lactobacillus plantarum [12]	-	Sodium alginate (Al), gum Arabic (GA), gellan gum (GG)	External ionic gelation	Al-GA-the greatest survival rate, 98.11% after 1 month storage

According to SEM analysis, Shaharuddin and Muhamad (2015) [1] has found that immobilized probiotic in sugarcane bagasse (SB) could tolerate heat treatment after being encapsulated using alginate solution (NaA) at a concentration of 1%. Besides, the structure of *L. rhamnosus*-loaded NaA-SB microcapsule before and after heat treatment was demonstrated by SEM images while FTIR

analysis had proved the changes in functional bonding of the microcapsule containing *L. rhamnosus*-NaA-SB.

The investigations on the survivability and the release properties of free and encapsulated probiotics were done after the treatment of simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) [21]. Simulated gastric juice was prepared using 0.2% (w/v) NaCl, at pH 2.5 and pH 2.0 before encapsulated probiotic cells were being added. Then, the samples were collected at a specific time, 0, 10, 30, 60, 90 and 120 min and further dispersed into simulated intestinal juice after being washed with saline water to rupture the samples, which the condition is in 50 mM KH_2PO_4 at pH 6.8. The survival rate or released rate of encapsulated probiotic in was directly evaluated and reported in the percentage of log CFU/g. Furthermore, for free prebiotic cell, the sample was treated in simulated gastric juice and incubated for 0, 1, 3, 5, and 10 min at 37°C. The survival rate was determined on 100 mL of the sample taken from each incubation time.

The encapsulated probiotic was mixed with the simulated bile juice that was initially prepared using 1 or 2% bile (w/v) with the incubation condition is at 37°C for 1 and 2 h. Thus, the sample was further dispersed into simulated intestinal juice (pH 6.8) after being washed with saline water and was directly analyzed for its release properties. Moreover, for free prebiotic cell, the 100 mL of the sample was treated in simulated bile juice and incubated for 1 and 2 h. The survival rate of the sample was determined for each incubation time.

The release properties were observed by adding the encapsulated probiotic into SIF medium at pH 6.8 and 37°C. After the samples were shaken at 100 rpm, 100 mL of solution was taken from each incubation time, 0, 10, 30, 45, 60, 120, 180 and 240 min and the same volume must be topped up with the fresh medium. The samples released were directly examined for activity by plotting the cumulative amount against time. The free and encapsulated probiotics were kept at 48°C and were collected at different days from 0, 3, 6, 9, 14, 21 to 30. Finally, the activity and the viability of probiotics was measured and reported in the log CFU/g unit.

5. Release properties of encapsulated probiotics in the gastrointestinal environment

Several studies to evaluate the release properties of various types of probiotics encapsulated in various techniques were presented in Table 1 and discussed in the section below.

5.1 Encapsulation of Streptococcus salivarius ssp. thermophilus as potential probiotic

Lactoferm Aby 6 including *Streptococcus salivarius ssp. thermophilus* (80%), *Lactobacillus acidophilus* (13%), *Bifidobacterium bifidum* (6%), *Lactobacillus delbrueckii ssp. Bulgaricus* (1%) were incorporated into two types of carrier material such as alginate and whey protein (A-WPC), and alginate and whey protein hydrolysate (A-WPH) to identify the fermentative activity of probiotics cultures and the properties and survivability of the carrier material after treated through simulated digestion medium [13]. The microcapsules of Lactoferm Aby 6 was prepared with the use of A-WPC and A-WPH as the carrier materials by the application of the electrostatic extrusion technique [13].

5.1.1 Release properties of encapsulated Streptococcus salivarius ssp. Thermophiles. Probiotics of Lactoferm Aby 6 incorporated with whey protein and alginate have high survivability (96%) after being exposed to simulated gastrointestinal conditions (SGIC) as compared to their free culture (37.43%). In this study, Krunić, Obradović, & Rakin (2019) [13] described that controlled enzymatic hydrolysis promotes a distinctive interaction between peptide and alginate and led to better microcapsules properties compared to non-hydrolysed protein such as A-WPH exhibited better protection towards the probiotics during gastric condition at 4h than A-WPC.

5.2 Encapsulation of Bifidobacterium BB-12, *Bifidobacterium animalis subsp. Lactis* BLC1 *and Bifidobacterium breve as potential probiotic*

Verruck et al. (2017) [16] investigated the survivability of *Bifidobacterium* BB-12 loaded in microencapsulation using full-fat goat's milk with and without the addition of inulin and oligofructose through in vitro simulated gastrointestinal and thermal treatments. *Bifidobacterium breve* and both *S. aureus* and *E. coli* were encapsulated using multilayer alginate hydrogel beads (MAHBs) and determined on their viability and pH-dependent resistance [17]. MAHBs was also applied to encapsulate *B. subtilis* to test its potential as a delivery system through the fermentation process.

Moreover, Pinto et al. (2015) [20] also performed a similar evaluation with a different coating agent containing a liquid sweet whey protein and prebiotics such as inulin and polydextrose to encapsulate *Bifidobacterium* BB-12. A caprine ice cream loaded with or without *B. animalis* had been investigated on physicochemical characteristics, meltdown behaviour and sensory characteristics, as well as the viability of the probiotic was evaluated through the treatment in the simulated gastrointestinal environment [19].

The microencapsulation of *Bifidobacterium* BB-12 was spray-dried using four types of coating agent such as full-fat goat's milk (GM), full-fat goat's milk and inulin (GMI, 1:1), full-fat goat's milk and oligofructose (GMO), and full-fat goat's milk, inulin, and oligofructose (GMIO, 1:0.5:0.5) [16]. *B. breve* was encapsulated through the hydrogel method by mixing the probiotic with sodium alginate (SA), which then was dropped into CaCl2 solution [17]. The rinsed and collected core beads were immersed in SA and then added into oil phase containing liquid paraffin, Span 80 and Tween 80. The beads were collected and wash after $CaCl_2$ solution is added into the emulsion. These processes were repeated to produce multilayer alginate hydrogel beads (MAHBs). A previous study by Pinto et al. (2015) [20] applied a spray drying technique with a similar condition. The coating agent solution such as sweet whey, sweet whey and 100 g/L of inulin, and sweet whey and 100 g/L polydextrose were homogenised and heat-treated for 30 min at 80°C. Then the Bifidobacterium BB-12 suspension (50 mL/L) was inserted into the solution through continuous stirring after it has cooled down to room temperature. The feed solution consisted of the coating agent solution was used as a carrier material to encapsulate Bifidobacterium BB-12 with 150°C of inlet temperature and 50 ± 3°C of outlet temperature of the spray dryer. Furthermore, dried goat's milk was used to produce regular ice cream (RIC) and probiotic (*B. animalis*) added-ice cream (PIC) [19]. *B. animalis* (109 CFU/g) was incubated in the goat's milk for 3 h at 37°C before being incorporated into the formulation of ice cream.

5.2.1 Release properties of encapsulated Bifidobacterium. Verruck et al. (2017) [16] reported that encapsulated *Bifidobacterium* BB-12 with GM provided the greatest efficiency concerning the viability after being exposed to the simulated gastrointestinal environment and thermal treatment when comparing to other coating agents including GMI, GMO, and GMIO. The microcapsule produced with GM exhibited the highest probiotic survival rate (94.29%) while with GMI, the survival rate was found at 86.77% after the incubation in the simulated gastrointestinal environment. Also, the survival of *Bifidobacterium* BB-12 in the thermal treatments was promoted by all the coating agents. The encapsulated *Bifidobacterium breve* by using MAHBs has improved the viability and could maintain the viability after 12 h in the broth culture that the pH similar to the gastric condition when compared to the free culture one [17]. Similarly, the encapsulated *S. aureus* and *E. coli* were significantly enhanced their survivability against the extreme pH. The promising encapsulating material of MAHBs for the oral delivery system was also proved by the increasing and sustaining of α-amylase yield after 240 h of the fermentation process with the encapsulated *B. subtilis.*However, sweet whey is a potential carrier material as it has promoted the greatest viability of *Bifidobacterium* BB-12 after being encapsulated through spray drying technique, exposed to both gastrointestinal medium and heat treatments [20]. The encapsulated *Bifidobacterium* BB-12 with sweet whey expressed the viability at 9.54 log CFU/ g and the encapsulation yield at 95.43% after spray drying process. Sweet whey and also sweet whey and inulin possessed better protection for the probiotic after treated with gastrointestinal medium, with a low reduction level at 0.49 and 0.97 logs, respectively while a carrier material of sweet whey and polydextrose has obtained high reduction level (2.45 logs). Goat's milk ice cream was proposed to be a good delivery system for *B. animalis* due to 84.7% of survival rate recorded after 120 days of storage in frozen [19]. Moreover, the viability of the probiotic ice cream was measured at 6-7 log CFU/g despite the little loss for about 4 log cycles when the ice cream exposed to bile and pancreatin solution.

5.3 Encapsulation of Staphylococcus succinus and Enterococcus fecium as potential probiotic
Staphylococcus succinus (*S. succinus*, MAbB4) and (*E. fecium*, FIdM3) were isolated from human feces [22]. The probiotics were tested for the endurance capability towards pH and bile and also the survivability throughout the simulated gastrointestinal condition. *S.succinus* and *E. fecium* were co-encapsulated by hydrogel method with prebiotics, sugarbeet, and chicory in 2 g/100 mL alginate [22].

5.3.1 Release properties of encapsulated Staphylococcus succinus and Enterococcus fecium. Sathyabama et al. (2014) [22] has found that encapsulated *S. succinus* and *E. fecium* with both sugarbeet and chicory exhibit strain-dependant survivability. The result showed a good tolerance in pH 2 to 3 (acidic condition) and 0.3, 0.6 and 0.8 g/100 mL (bile condition) while the survival rate of the probiotics in simulated gastric fluid (SGF) was reported from 88.75 % to 98.75 % which were maintained for about 30 days of storage period at 4°C. This study has first reported the use of oligosaccharides rich source of prebiotics, sugarbeet, and chicory as a coating material to encapsulate probiotics. Chicory (7.9 log CFU/mL) shown a higher survival rate of *S. succinus* through 6 h SIF treatment rather than sugarbeet (7.1 log CFU/mL) while *E. fecium* showed equal stability for both sugarbeet and chicory beads (7 log CFU/mL).

5.4 Encapsulation of Lactobacillus brevis and Lactococcus lactis as potential probiotic
The isolation of lactic acid bacteria (LAB), *Lactobacillus brevis* (WK12) and *Lactococcus lactis* (WK11) were initiated from well ripen kimchi and had been evaluated on their viability rates and the storage stability when encapsulated with food-grade cryoprotective agents, including skim milk, soy powder, yeast extract and trehalose [10]. The encapsulation of probiotics in alginate was prepared through the gelation process (Ca-alginate) and then the cryoprotective agents were added [10].

5.4.1 Release properties of encapsulated Lactobacillus brevis and Lactococcus lactis. L brevis (WK12) and *L. lactis* (WK11) in Ca-alginate beads shown the greatest viability rate after being immersed in 10% soy powder when compared to skim milk, yeast extract, trehalose [10]. LAB WK12 had depicted 1.85×10^{11} CFU/mL and WK11 showed 1.89×10^{11} CFU/mL. Also, the storage studies for 4 weeks proved the suitability of soy powder in enhancing the protective effect of encapsulated LAB WK12 and WK11 which exhibited the highest viability at 1.80×10^{11} CFU/mL and 1.78×10^{11} CFU/mL, at -18°C respectively.

5.5 Encapsulation of Lactobacillus rhamnosus as potential probiotic
Gandomi et al. (2016) [9] identified the effect of alginate and chitosan as well as the addition of inulin as prebiotic on the viability of *Lactobacillus rhamnosus* (GG) in apple juice and simulated gastrointestinal fluid. Likewise, *Lactobacillus rhamnosus* (NRRL 442) was collected from USA Agricultural Research Service (ARS) and was tested for its viability in the immobilization using biofiber or biopolymer such as sugarcane bagasse (SB) and pineapple core (PC) [2]. In another study, *L. rhamnosus* (NRRL 442) encapsulated using alginate and SB was determined for encapsulation efficiency, particle size, morphology, FTIR, and cell viability [1].

L. rhamnosus (GG) suspension was extruded drop by drop into calcium chloride solution and then was immersed in a chitosan solution [9]. However, *L. rhamnosus* (NRRL 442) was prepared in cell suspension and SB and PC powder was added with the ratio of 8:1 and 4:1, respectively [2]. *L. rhamnosus* (NRRL 442) was immobilized in SB and encapsulated using 1, 2 and 3% of alginate (NaA) and the ratio of NaA:SB was controlled at 1:0, 1:1, and 1:1.5, to invent new *L. rhamnosus*-loaded NaA-SB microcapsule by external ionic gelation method [1].

5.5.1 Release properties of encapsulated Lactobacillus rhamnosus. Gandomi et al. (2016) [9] suggested that the survival rate of *L. rhamnosus* (GG) could be improved when encapsulated with alginate and chitosan as well as with and without inulin. The encapsulated *L. rhamnosus* (GG) showed 4.5 times greater survival rate compared to the free bacteria which was suffered 13.6% of decreasing after being stored in apple juice for 90 days. The encapsulated *L. rhamnosus* (GG) with inulin addition which was stored at 4°C showed a significantly higher survival potential compared to without inulin. The probiotic survival of the encapsulated *L. rhamnosus* (GG) without inulin was recorded at 27.7%. The immobilized *L. rhamnosus* (NRRL 442) in PC exhibited a better structure than in SB through the morphology observation by screening electron microscope. Nevertheless, SB attained 93.6% of cell survivability compared to only 64.1% by PC which was related to the solution holding capacity (SHC) [2]. Therefore, Shaharuddin and Muhamad (2015) [1] improved the encapsulation efficiency and survivability of *L. rhamnosus* (NRRL 442) by the immobilization in SB and the encapsulation in NaA (*L. rhamnosus* -loaded NaA-SB). *L. rhamnosus* was heat resistance due to enhanced survivability at

90°C for 30s of heat exposure and the resistance could be obtained at low NaA concentration, only 1%.

5.6 Encapsulation of Lactobacillus plantarum and *Lactobacillus casei as potential probiotic*
Lactobacillus plantarum CECT 220 and *Lactobacillus casei* CECT 475 were isolated from corn silage and cheese, respectively [14], *Lactobacillus plantarum* DKL 109 was isolated from kimchi [12] while *Lactobacillus plantarum* was used to evaluate the encapsulant medium comprised of different blends of coating materials [23]. *L. plantarum* and *L. casei* had been encapsulated to examine their viability in a simulated gastrointestinal medium which is high acid and bile conditions as well as the stability during storage at ambient temperature.

González-Ferrero et al. (2018) [14] loaded *L. plantarum* and *L. casei* in soybean protein and calcium chloride solution. After the incubation process was done, the mixture was transferred to a spray dryer. *L. plantarum* was dried with maltodextrin (MD) and oligofructose-enrich inulin (OEI) of different ratio; 1:0, 2:1, 1:2, 0:1 while *L. casei* was only dried with MD. However, three types of formulas using different biopolymers as coating material; 2% alginate (Al), 1% alginate: 1% gellan gum (Al-GG) and 1.5% alginate: 3% gum Arabic (Al-GA) were applied by extrusion method with an atomizing spray gun to develop encapsulated *L. plantarum* [12]. After probiotic cell suspension was mixed with biopolymers solution, the mixture was dispensed into the atomizing spray device before being sprayed into the 2% calcium chloride solution. The collected microcapsule slurry was mixed with 10% reconstituted milk and then was freeze-dried.

5.6.1 Release properties of encapsulated Lactobacillus plantarum. González-Ferrero et al., (2018) [14] found that *L. plantarum* CECT 220 had 52.4% of viability enhancement but *L. casei* CECT 475 only showed 11% when compared to non-encapsulated probiotic. The encapsulated *L. plantarum* and *L. casei* also showed greater tolerance during the treatment in gastrointestinal condition. Thus, soybean protein could provide good protection for LAB strain against a harsh environment. Moreover, soybean protein-based microparticles with OEI as a drying agent (Sp 750) exhibited the best protection as it showed the most significant viability improvement throughout storage. In a different investigation, Al-GA could produce the highest encapsulation efficiency (98.11%) of encapsulated *L. plantarum* which the viability was maintained after 1 month storage at 25° but slightly decrease by 10% at 37°C [12]. This phenomenon indicates the improvement of the survivability of the encapsulated *L. plantarum* in a harsh environment.

5.7 Encapsulation of Lactobacillus acidophilus LA3 as potential probiotic
L. acidophilus was encapsulated by the mixture of alginate and shellac through the extrusion and co-extrusion method [15]. The probiotic was investigated on its characterization and viability after the treatment of the gastrointestinal condition. Konjac glucomannan hydrogel (KGM) and sodium alginate (SA) was evaluated on its suitability as a good wall material for oil-in-water emulsion and protective effect on *L. acidophilus* [11]. *L. acidophilus* survivability was also examined through the microcapsules containing hi maize (resistant starch) and chitosan as a coating material [18]. Previous studies [11, 18] have evaluated the survivability rate of *L. acidophilus* during storage and after treated with the gastrointestinal medium.

Silva et al. (2018) [15] incorporated *L. acidophilus* with alginate or alginate and shellac as encapsulant material by extrusion. The mixture of the probiotic and the encapsulant material was extruded through the encapsulator using compressed air to produce droplets of equal particle size. The droplets were collected into a calcium chloride solution. Additionally, the co-extrusion process produced with the same encapsulator system but *L. acidophilus* was mixed with sunflower oil. Then, the mixture of *L. acidophilus*-sunflower oil and the mixture of encapsulant material were pumped at the same time. Finally, the droplets for both of extrusion and co-extrusion method were dried using a fluidized bed. In a study by Mu et al. (2018) [11], concentrated *L. acidophilus* suspension was mixed with KGM hydrogel to prepare the water phase. Dialdehyde glucomannan was mixed with gelatine to form a crosslinked KGM gel. Then, the water phase was added into the edible oil to produce the water-in-oil emulsion. The microcapsules of *L. acidophilus* were produced through the extrusion technique with two types of the coating solution, only sodium alginate and the combination of sodium

alginate and 1% hi maize, which then were spray-dried using $CaCl_2$ and $CaCl_2$ with 0.4% chitosan, respectively [18].

5.7.1 Release properties of encapsulated Lactobacillus acidophilus. LA3 encapsulated or the dried particles by co-extrusion showed higher viability when comparing to the extrusion process due to the additional barrier was provided by the use of sunflower oil as the coating material [15]. Also, the viability of the dried particles of the co-extrusion method in gastrointestinal fluids was recorded at 7.2 Log CFR/g and 6.2 Log CFU/g when using a blend of alginate-shellac and alginate, respectively, after being stored for 60 days at 25°C. *L. acidophilus* has 62.5% of encapsulation rate when microencapsulated with crosslinked KGM gel [11]. KGM could well protect and improve the acid resistance and survival rate of the probiotic through the gastric fluid simulation. The moist microparticles containing hi maize and chitosan promoted better protection of *L. acidophilus* at up to 6.35 Log CFU/g against simulated gastric and intestinal environment compared to the lower counts achieved by the freeze-dried microcapsules [18]. Both moist and freeze-dried microparticles of *L. acidophilus* are viable during storage at room temperature (25°C) and expressed at more than 6 logs for about 135 days and 30 days, respectively.

5.8 Encapsulation of Lactobacillus bulgaricus as potential probiotic
Lactobacillus bulgaricus is suspended into biopolymers such as with skim milk and alginate [21], pure milk and carrageenan-locust bean gum [24], and pure milk-alginate microspheres [25], by hydrogel method which then were further evaluated for their in vitro survivability in simulated gastrointestinal fluid. Hangzhou Wahaha Group from China had provided frozen *L. bulgaricus* cultures to all groups of researchers. The finding showed that skim milk and alginate, pure milk-carrageenan-locust bean gum and pure milk-alginate are a potential encapsulant material that could be used to entrap probiotic for oral administration and suitable for food application.

Whey protein from milk is discovered to be the most prevalent material used for the encapsulation of probiotics. In Pan et al., (2013) [21] investigation, *Lactobacillus bulgaricus* is extruded into biopolymers such as alginate and skim milk to produce microspheres by the hydrogel method, however, research on pure milk-based coating medium is still lacking. Shi, Li, Zhang, et al. (2013) [24] have investigated to evaluate the efficacy of *Lactobacillus bulgaricus* as probiotic after being encapsulated with pure milk and $CaCl_2$ solution for the first layer of coating medium while the mixture of carrageenan and locust bean gum was used for the second layer. Furthermore, *L. bulgaricus* was extruded into different encapsulation medium, microspheres consist of alginate and pure milk of different ratio (1% and 1%, 1% and 2%, 1% and 3%, 1% and 4%.) and finally solidified in $CaCl_2$ solution [25]. The microspheres were prepared by an Inotech Encapsulator IER-50 (Inotech Biosystems Intl. Inc., Reppischhof Switzerland) in all investigations.

5.8.1 Release properties of encapsulated Lactobacillus bulgaricus. *L. bulgaricus* was well protected in milk microspheres with biopolymers in the second coating layer when the viability was found to be sustained after 2 h incubation in simulated gastric fluid (SGF), which is more than 8 Log CFU/g. Moreover, after 1 h and 2 h treated in 2% bile salt solution, the viability of encapsulated *L. bulgaricus* showed a decrease at only 1 Log CFU/g and 1.5 Log CFU/g, respectively. *L. bulgaricus* was completely released from microspheres after 45 min treated in simulated intestine fluid (SIF) [24]. In another study by (Shi, Li, Li, et al., 2013) [25], *L. bulgaricus* was coated with microspheres containing alginate and milk mixture had shown a similar behavior through the exposure towards gastrointestinal environment conditions. The microspheres could also be an effective encapsulation technique due to a complete rapid release of *L. bulgaricus* after 1 h being treated in SIF. However, Pan et al., (2013) [21] found that *L. bulgaricus* was completely released in SIF after 2 h. This study also found lesser viability of *L. bulgaricus* when it decreased for about 2 Log CFU/g in 1% bile solution and 2.6 Log CFU/g in 2% bile solution.

6. Conclusion

Encapsulation of probiotics in the biopolymeric system is an excellent technique to enhance the protective effect of probiotics, to improve the viability of probiotics during processing and storage and also to deliver the probiotics to human gastrointestinal tract at a specific time with sustained-release

properties. The effect of microencapsulation on the survival of probiotics in an in vitro model simulating gastrointestinal digestion is widely investigated. Knowledge on the bioavailability of encapsulated probiotics loaded with biopolymeric through in vivo studies is limited.

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