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The Effects of Extrusion and Internal Emulsion Microencapsulation Methods on the Viability of *Lactobacillus acidophilus*

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ABSTRACT

Background: The present study aimed to investigate the effects of various encapsulation methods on *Lactobacillus acidophilus* viability using wall materials variably.
Methods: *L. acidophilus* was encapsulated using the extrusion and emulsion encapsulation methods with calcium-alginate and skim milk via mixing or coating. The particle size, encapsulation yield, and viability of *L. acidophilus* in the simulated gastric and intestinal fluids were investigated.
Results: The mean particle size in the emulsion and extrusion methods was within the range of 161 µm to1.95 mm, and the coated samples were smaller than the mixed samples. The encapsulation yield of the extrusion method was higher compared to the emulsion method. In addition, skim milk was essential to improving the *L. acidophilus* viability, which significantly improved in the calcium-alginate particles coated by skim milk compared to the mixed samples.
Conclusion: According to the results, the coating process by skim milk not only improved probiotic viability, but it also reduced the preparations particle size, which in turn decreased the adverse effects of the preparations on the sensory properties of food.

1. Introduction

Probiotics are living microorganisms that are beneficial to human health when administered in adequate amounts [1]. To exert such effects, the viability of probiotic bacteria in the product should be above 10^6 CFU/g (CFU/ml) upon consumption, and the microorganisms must also be able to survive through digestive processes [1, 2].

Most probiotic microorganisms belong to lactic acid bacteria, which are more important to the human gastrointestinal tract [2]. However, their viability may be affected by the changes in temperature, pH, acidity, dissolved oxygen, and hydrogen peroxide [3]. To enhance the viability of probiotic bacteria, various microencapsulation methods are used, which separate probiotics from adverse environments and improve the probiotic viability during production and storage, as well as in the human gastrointestinal tract [4].

Microencapsulation involves extrusion emulsion techniques. Additionally, wall materials such as alginate, chitosan, and carrageen play a key role in the protection of probiotic viability. Alginate is extracted from seaweeds containing 1,4 β-D-mannuronic link and α-L-guluronic link, which produce a gel with the calcium ion as an inexpensive and nontoxic material to be used widely in emulsion and extrusion methods [5]. According to the literature, emulsion and extrusion methods could protect probiotics against adverse conditions [6, 7]. The extrusion method often protects probiotic bacteria more efficiently compared to the emulsion method although the large size of the particles prevents its application in food products. Moreover, the survival of probiotic bacteria is affected by microencapsulation and the applied wall materials.

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The comparative studies regarding various encapsulation methods have yielded poor results. The evaluation of encapsulation methods is considered essential to determining the effective protective approaches that could enhance probiotic viability.

The present study aimed to investigate the effects of extrusion and internal emulsion microencapsulation methods on the viability of *Lactobacillus acidophilus* ATCC 4356.

2. Materials and Methods

2.1. Bacterial Strain and Materials

Lactobacillus acidophilus ATCC 4356 was obtained from the strain collection of the Faculty of Food Science and Technology at Ho Chi Minh City University of Food Industry. *L. acidophilus* was harvested from 500 milliliters of MRS broth (Himedia, Mumbai, India; late log phase) via centrifugation at 5,000 rpm. Afterwards, the cells were used immediately in the microencapsulation process. Alginate, skim milk, and pepsin were purchased from Himedia Company. Alginate was used as the main encapsulated agent, and skim milk was used as the mixed or coating agent. The particle size, encapsulation yield, and *L. acidophilus* viability in the simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) were evaluated.

2.2. Microencapsulation of L. acidophilus

2.2.1. Extrusion Encapsulation Method

Extrusion microencapsulation (EM) was developed using the method described by Lieu *et al.* (2019) with slight modifications [8]. Briefly, a 10-milliliter cell suspension (11.50 log CFU/ml) was added to 40 milliliters of 2% (w/v) alginate (EMs sample) or the mixture of 2% (w/v) alginate and 0.5% (w/v) skim milk (EMm sample). Following that, the mixture was injected into 50 milliliters of a solution containing $CaCl_2$ 0.1 M using an aseptic syringe and incubated for 15 minutes. The particles were collected (EMs and EMm samples) or incubated (in a shaking incubator at 150 rpm) in 0.5 (w/v) of skim milk in the case of the calcium-alginate coated by skim milk (EMc). The mean particle size in diameters was measured using an electrical caliper (Mitutoyo, Kanagawa, Japan), and the particles were stored at the temperature of 4°C.

2.2.2. Internal Emulsion Microencapsulation Method

Internal emulsion encapsulation (IM) was developed using the method described by Martín *et al.* (2015) with slight modifications [9]. Briefly, a 10-milliliter cell suspension (11.50 log CFU/ml) was added to 40 milliliters of 2% (w/v) alginate (IMs samples) or a mixture of 2% (w/v) alginate and 0.5 (w/v) skim milk (IMm samples). The mixture was stirred using a magnetic stirrer and dispersed for 15 minutes in 100 milliliters of palm oil containing 1.5% (v/v) Tween 80 at the speed of 900 rpm. Afterwards, 100 milliliters of 0.1 M $CaCl_2$ was slowly added down the side of the beaker to break the emulsion. The particles were collected (IMs and IMm sample) or incubated (in a shaking incubator at 150 rpm) in 0.5 (w/v) of skim milk in the case of the calcium-alginate coated by skim milk (IMc sample).

The preparations were collected via centrifugation at 5,000 rpm.

2.2.3. Determination of the Encapsulation Yield

The encapsulation yield was calculated using the following formula:

$$\textbf{Encapsulation yield}\ (\%) = \frac{\sum logCFU_{after\ encapsulation}}{\sum logCFU_{before\ encapsulation}} x100\%$$

The mean particle size was measured using a HORIBA LA-920 device (HORIBA, Kyoto, Japan), and the particles were stored at the temperature of 4 °C.

2.3. Survival of the Microencapsulated L. acidophilus in SGF and SIF

The SGF consisted of sodium chloride (9 g/l), which contained pepsin (3 g/l) and was adjusted to the pH of 2.0 using 5.0 M HCl. The SIF consisted of sodium chloride (9 g/l), which contained bile salts (3 ml/l) and was adjusted to the pH of 6.5 using 5.0 M NaOH. The survival rate of the encapsulated *L. acidophilus* was investigated after two hours of incubation in the SGF and four hours of incubation in the SIF, and the free *L. acidophilus* cell samples were used as controls.

2.4. Enumeration of the Encapsulated Probiotics

The enumeration of the encapsulated probiotics was carried out in several steps [8]. Initially, one gram of the preparations was resuspended in 45 milliliters of phosphate buffer (0.1 M; pH 7.0), followed by homogenization in a stomacher (IUL, Barcelona, Spain) for 10 minutes. In addition, probiotic viability (CFU/g) was determined by spreading on the MRS agar at the temperature of 37 °C for 48 hours.

2.5. Statistical Analysis

Data analysis was performed using the analysis of variance (One way ANOVA) in SigmaPlot software version 11.0, followed by Tukey's test to compare the means. The differences between the mean variables were considered significant at $P \leq 0.05$. All the tests were performed in triplicate, and the obtained data were expressed as mean and standard deviation.

3. Results and Discussion

3.1. Effects of the Microencapsulation Methods on the Encapsulation Particle Size

Table 1 shows the particle size of the encapsulated beads. Accordingly, the mean particle size of the EM samples was 1.95 millimeters, and no significant difference was observed in the size of the three material matrixes (EMs, EMm, and EMc samples). However, the mean particle size of the IM samples was significantly different depending on the material matrix ($P < 0.05$).

The size of the calcium-alginate (IMs samples) and mixture of calcium-alginate and skim milk (IMm samples) was 311 and 325 micrometers, respectively, while the size

of the calcium-alginate coated by skim milk (IMc samples) was 161 micrometers (Table 1). According to the findings, the particle size was influenced by the encapsulation methods. In this regard, the results obtained by Valero-Cases *et al.* (2015) indicated that the particle size prepared by the EM method was 1.86-2.25 millimeters, while the particles prepared by the IM method had the mean particle diameter of 151.1 micrometers [2]. Furthermore, the particle size of the EM method was controlled and equaled more easily compared to the IM method, and the particle size in the IM method was 25-2.000 micrometers [9].

In the present study, the particle size prepared by the EM method was also influenced by the alginate concentration, diameter of the needle, pressure on the syringe, and $CaCl_2$ concentration [6,7,10]. In another study, Muthukumarasamy *et al.* (2006) used a syringe with the diameter of 0.813 millimeters, which resulted in the mean particle size of 2.37 millimeters [6]. On the other hand, Cai *et al.* (2014) reported that the particle size of the IM method was influenced by the stirring speed and concentration of the wall materials during the encapsulation process, and the mean particle size was estimated at 343 micrometers in the mentioned study [7]. Particle size significantly affects organoleptic properties with the addition of probiotics to food products [11]. In the present study, the particle size of the IM method was smaller than the EM method (Table 1). In the IM method, the mean particle size was influenced by the wall material matrix, in which the calcium-alginate coated by skim milk (IMc samples) was significantly smaller compared to the IMs and IMm samples (Table 1). This could be due to the fact that the calcium-alginate preparations were incubated in skim milk to form the coating layer and separate the particles for the reduction of the mean size of the preparations.

3.*2. Effects of the Encapsulation Methods on the Encapsulation Yield*

Figure 1 depicts the effects of the encapsulation methods on the encapsulation yield. According to the findings, the encapsulation yield in the EM method was better than the IM method, and the optimal encapsulation yield in the EM method was estimated at 98.38 ± 0.10%, while it was 93.86±0.11% in the IM method (Figure 2). The encapsulation yield was also affected by the encapsulating approaches, and the calcium-alginate coated by skim milk had the lowest encapsulation yield.

Previous findings have denoted the effects of encapsulation methods on the encapsulation yield.

Table 1: The Average particle diameter prepared by Extrusion (EM) and Internal Emulsion (IM) methods

Microencapsulation methods	Wall materials	Average particle diameter
Internal Emulsion (IM)	calcium-alginate (IMs)	311 µm
	calcium-alginate mixed with skim milk (IMm)	325 µm
	calcium-alginate coated by skim milk (IMc)	161 µm
Extrusion (EM)	calcium-alginate (EMs), calcium-alginate mixed with skim milk (EMm) and calcium-alginate coated by skim milk (EMc)	1.95 mm

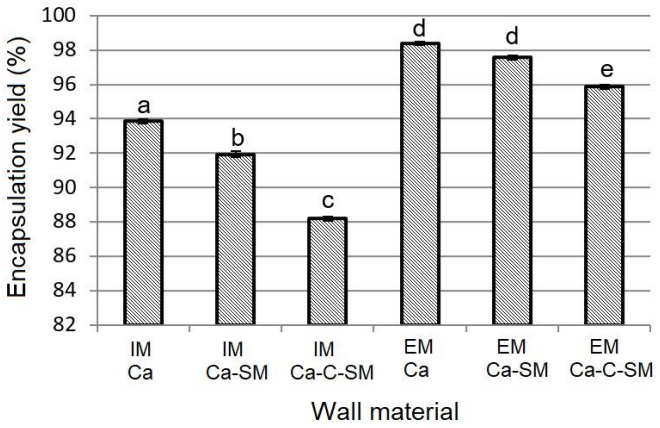

Figure 1: Encapsulation Yield in IM and EM Methods (Ca: calcium-alginate; Ca-SM: mixture of calcium-alginate and skim milk; Ca-C-SM: calcium-alginate coated by skim milk; Superscript letters (a-e) above the error bars that are significantly different [$P < 0.05$])

Ribeiro *et al.* (2014) reported that the encapsulation yield of *L. acidophilus* LA prepared by the IM method using pectin coated by whey protein as the coating agent was 91.6 ± 0.24% [12]. In addition, Zhang *et al.* (2015) observed that the encapsulation yield of *Lactobacillus salivarius* prepared by the IM method using pectin material was 90% [13]. Similarly, the study by Silva *et al.* (2016) indicated that the encapsulation yield of *Lactobacillus paracasei* BGP-1 prepared by the EM method was up to 93% [14]. Probiotic encapsulation yield is considered to be of utmost importance. The high encapsulation yield that traps higher amounts of probiotic bacteria in the particle reduces the need for the addition of particles to food products. According to the results of the present study, the encapsulation efficiency of the IM method was lower than the EM method (Figure 1), which could be due to the shaking incubation process in skim milk to form the coating layer that would leak the *L. acidophilus* cell from the calcium-alginate matrix, thereby decreasing the encapsulation yield (Figure 1).

3.3. Effects of the Encapsulation Methods on the Survival of L. acidophilus in the SGF and SIF

Figures 2 and 3 show the survival rate of the encapsulated and non-encapsulated cells after two hours of incubation in the SGF and four hours of incubation in the SIF. As is observed, the survival rate of the encapsulated cells and free cells was significantly different ($P < 0.05$). In the free cell samples, the survival rate of *L. acidophilus* decreased rapidly, and no living cells were observed after two hours of incubation in the SGF although cell loss was also observed in the encapsulation samples. Notably, the encapsulation method improved the *L. acidophilus* viability, with 2.98-3.85 log CFU/g remaining in the EM samples and 2.09-3.18 log CFU/g remaining in the IM samples (Figure 2). The cell loss in the SIF was not significant compared to the SGF, in which 6.05 ± 0.22 log CFU/g free cell samples remained, while in the EM and IM samples, 8.24-8.56 and 8.08-8.11 log CFU/g free cell samples remained, respectively (Figure 3).

Previous studies have demonstrated the viability of probiotics in the SGF. In a study, the survival rate of *Lactobacillus* paracasei BGP-1 after two hours of incubation in the SGF caused 3.3 log CFU/g of the initial concentration to remain (8.6 log CFU/g) [14].

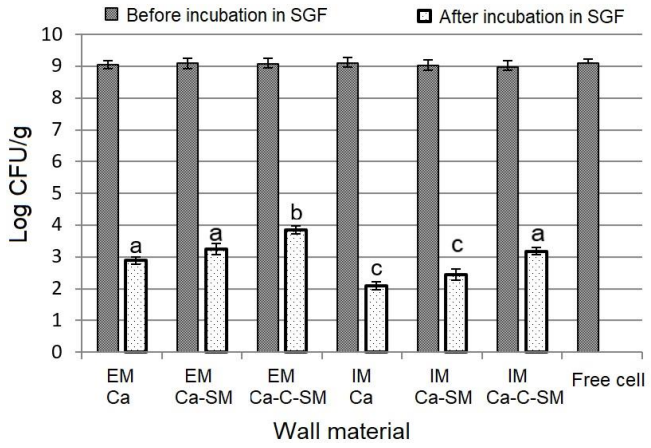

Figure 2: Effect of Microencapsulation on Viability of *L. acidophilus* in SGF (Ca: calcium-alginate; Ca-SM: mixture of calcium-alginate and skim milk; Ca-C-SM: calcium-alginate coated by skim milk; Superscript letters (a-c) above the error bars that are significantly different [$P < 0.05$])

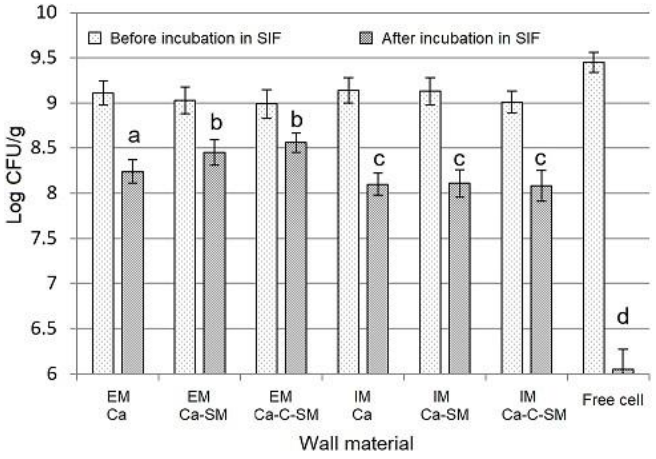

Figure 3: Effect of Microencapsulation on Viability of *L. acidophilus* in SIF (Ca: calcium-alginate; Ca-SM: mixture of calcium-alginate and skim milk; Ca-C-SM: calcium-alginate coated by skim milk; Superscript letters (a-d) above the error bars that are significantly different [$P < 0.05$])

Similarly, the viability of *Lactobacillus* plantarum after two hours of incubation in the SGF and SIF has been reported to decrease by 2.9 and 2.7 log CFU/ml, respectively compared to the initial concentration (10 and 11 log CFU/g) [15]. Therefore, it could be inferred that the viability of probiotics is significantly affected by the SGF and SIF media, and the encapsulation of probiotic cells to enhance the survival rate is essential. In the current research, the encapsulated probiotic viability in the IM method was lower compared to the EM method at the same wall material concentration [2]. The reduced probiotic viability due to the diffusion of the digestive liquid into the encapsulated particles caused cell loss since at the pH of lower than four, the calcium ions cross-linking in the calcium-alginate particle is readily exchanged with H^+ ions, thereby causing calcium-alginate to convert into alginic acid, followed by particle breaking [16].

In the present study, the particles prepared by the EM method were larger compared to those prepared by the IM method, which increased their protective efficacy against the SGF and SIF conditions (Figure 3). However, the results also indicated that the wall material matrix significantly affected the probiotic viability, and the calcium-alginate coated by skim milk showed higher *L. acidophilus* viability compared to the mixture of calcium-alginate and skim milk or calcium-alginate alone (figures 2 and 3).

According to the current research, the encapsulated bacteria survived well in the SGF and SIF compared to the non-encapsulated bacterial cells (figures 2 & 3). However, the gel matrix structure of calcium-alginate was porous, which allowed the diffusion of the hydrogen ions into the cells. To control this problem, coating or incorporated materials have been used in the previous studies in this regard. According to the proposed findings, skim milk or whey protein could improve the survival of probiotics in storage conditions and in-vitro digestion through the high buffer of milk protein [17, 18].

In the present study, the L. acidophilus viability in the mixture of calcium-alginate and skim milk was slightly higher compared to calcium-alginate alone, while no significant difference was observed in this regard. This could be due to the interference of skim milk with the cross-linking of the calcium ions with alginate, thereby degrading the particle structure and decreasing the *L. acidophilus* viability. The survival of *L. acidophilus* in the encapsulation particles coated by skim milk improved significantly compared to the uncoated particles (Figure 2). This could be attributed to the fact that skim milk largely decreased the diffusion of H^+ ion into the structure of the encapsulated particles [17], which in turn enhanced the survival rate of *L. acidophilus* (Figure 2).

4. Conclusion

According to the results, the encapsulation yield of the *L. acidophilus* prepared by the EM method was 98.38%, which was higher than the IM method (93.86%). On the other hand, the incubation during the calcium-alginate coating process by skim milk caused the encapsulation yield to decrease. The encapsulation methods had significant effects on the *L. acidophilus* viability. In addition, the EM method resulted in larger particles with higher protective efficiency compared to the IM method in the SGF and the SIF media.

The survival rate of *L. acidophilus* was significantly affected by the SGF environment, in which 6 log CFU/g decreased in the EM samples, and 7 log CFU/g decreased in the IM samples. The survival rate of *L. acidophilus* was also influenced by the wall material matrix. The skim milk that was incorporated into the calcium-alginate matrix degraded the particle structure, readily diffusing the H^+ ions into the encapsulation particle and causing cell loss.

The skim milk used as the coating agent reduced the pore size of calcium-alginate, thereby preventing the permeation of the H^+ ions into the beads. In the IM method where calcium-alginate was coated by skim milk, the incubation process for the formation of the coating layer separated the particles and decreased the particle size. The coating process by skim milk not only improved the probiotic viability, but it also reduced the particle size of the preparations, as well as the negative impact of the preparations on the sensory properties of food.

Authors' Contributions

This article was carried out by all the authors. L.M.D., L.T.H.Q., T.D.T., and D.T.K.T., contributed to carry out data collection and data analysis, and LMD wrote the manuscript.

Conflict of Interest

The Authors declare that there is no conflict of interest.

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References

1. Hotel ACP, Cordoba A. Health and Nutritional Properties of Probiotics in Food Including Powder Milk With Live Lactic Acid Bacteria. *Prevention*. 2001; 5(1): 1-10.

2. Valero CE, Frutos MJ. Effect of Different Types of Encapsulation on the Survival of Lactobacillus Plantarum During Storage with Inulin and in Vitro Digestion. *LWT-Food Sci Technol.* 2015; 64(2): 824-8.

3. Salminen S, Von WA. Lactic Acid Bacteria: Microbiological and Functional Aspects. *Marcel Dekker Inc, New York: USA*; 2004: 66.

4. Pinto SS, Verruck S, Vieira CR, Prudêncio ES, Amante ER, Amboni RD. Influence of Microencapsulation with Sweet Whey and Prebiotics on the Survival of Bifidobacterium-Bb-12 Under Simulated Gastrointestinal Conditions and Heat Treatments. *LWT-food Sci Technol.* 2015; 64(2): 1004-9.

5. Zhang JP, Wang Q, Xie XL, Li X, Wang AQ. Preparation and Swelling Properties of Ph-Sensitive Sodium Alginate/Layered Double Hydroxides Hybrid Beads for Controlled Release of Diclofenac Sodium. *J Biomed Mater Res B Appl Biomater*. 2010; 92(1): 205-14 .

6. Muthukumarasamy P, Allan WP, Holley RA. Stability of Lactobacillus Reuteri in Different Types of Microcapsules. *J Food Sci.* 2006; 71(1): 20-4.

7. Cai S, Meng Z, Yapeng F, Katsuyoshi N, Glyn OP, Fatang J. Microencapsulation of *Lactobacillus acidophilus* cgmcc1. 2686 Via Emulsification/internal Gelation of Alginate Using ca-edta and $Caco_3$ as Calcium Sources. *Food Hydrocoll*. 2014; 39: 295-300.

8. Lieu DM, Hang HTT, Quyen NTT, Luan NT, Ly NT, Tran NHN, Letal. Study on the Effect of Calcium-alginate and Whey Protein on the Survival Rate of Bifidobacterium Bifidum in Mayonnaise. *Vietnam J Sci Technol*. 2019; 57(3B): 33-41.

9. Martín MJ, Lara Villoslada F, Ruiz MA, Morales ME. Microencapsulation of Bacteria: a Review of Different Technologies and Their Impact on the Probiotic Effects. *Innov Food Sci Emerg Technol*. 2015; 25: 15-25.

10. Chandramouli V, Kailasapathy K, Peiris P, Jones M. An Improved Method of Microencapsulation and its Evaluation to Protect *Lactobacillus* spp. In Simulated Gastric Conditions. *J Microbiol Methods*. 2004; 56(1): 27-35.

11. Vivek K. Use of Encapsulated Probiotics in Dairy Based Foods. *Int J Food, Agric Vet Sci*. 2013; 3(1): 188-99.

12. Ribeiro MCE, Chaves KS, Gebara C, Infante FN, Grosso CR, Gigante ML. Effect of Microencapsulation of *Lactobacillus acidophilus* la-5 on Physicochemical, Sensory and Microbiological Characteristics of Stirred Probiotic Yoghurt. *Food Res Int.* 2014; 66: 424-31.

13. Zhang Y, Lin J, Zhong Q. The Increased Viability of Probiotic *Lactobacillus* salivarius nrrl b-30514 Encapsulated in Emulsions with Multiple Lipid-Protein-Pectin Layers. *Food Res Int.* 2015; 71: 9-15.

14. Silva MP, Tulini FL, Ribas MM, Penning M, Fávaro Trindade CS, Poncelet D. Microcapsules Loaded with the Probiotic Lactobacillus Paracasei Bgp-1 Produced by Co-Extrusion Technology Using Alginate/Shellac as Wall Material: Characterization and Evaluation of Drying Processes. *Food Res Int*. 2016; 89: 582-90.

15. Coghetto CC, Brinques GB, Siqueira NM, Pletsch J, Soares RMD, Ayub MAZ. Electrospraying Microencapsulation of *Lactobacillus* plantarum Enhances Cell Viability under Refrigeration Storage and Simulated Gastric and Intestinal Fluids. *J Funct Foods*. 2016; 26: 316-26.

16. Østberg T, Lund EM, Graffner C. Calcium Alginate Matrices for Oral Multiple unit Administration: Iv. Release Characteristics in Different Media. *Int J Pharm*. 1994; 112(3): 241-8.

17. Yu WK, Yim TB, Lee KY, Heo TR. Effect of Skim Milk-Alginate Beads on Survival Rate of Bifidobacteria. *Biotechnol Bioprocess Eng*. 2001; 6(2): 133-8.

18. Lieu MD, Dang TKT, Nguyen TH. Viability of Microencapsulated Lactobacillus Casei in Synbiotic Mayonnaise. *Food Res.* 2017; 1(6): 234-9.