THE VIABILITY OF ENCAPSULATED *Lactobacillus plantarum* DURING CUPCAKE BAKING PROCESS, STORAGE, AND SIMULATED GASTRIC DIGESTION

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ABSTRACT

In this study, the *Lactobacillus plantarum* viability in the cupcake according to baking time was investigated for the first time. *L. plantarum* was microencapsulated by the emulsion method in κ-carrageenan mixed with skim milk (CS sample) or carrageenan coated by skim milk (CcS sample). The average size, and the *L. plantarum* viability during cupcake baking, cold storage and in the simulated intestinal fluid (SGF) were investigated. The result showed that the particle size was 165μm and 320μm for the CS and CcS samples respectively. In the cupcake baking process indicated that the *L. plantarum* viability in control samples was decreased 0.21; 0.66; 2.13; and 5.33 log CFU/cake after 2; 4; 8; and 12 min baking respectively as compared to the initial, whereas the decreasing of the *L. plantarum* viability was 0.22 to 0.34; 0.80 to 0.90; 1.48 to 1.50; and 3.58 to 3.80 log CFU/cake in CS samples and CcS samples respectively. The pH value of the control samples was 6.8 to 6.5 which tended to be lower than the microencapsulated samples during 14 days of storage, though there was not a significant difference. Additionally, the *L. plantarum* viability in the control, CS and CcS samples was tended to increase slightly during storage. However, *L. plantarum* cells showed high sensitivity with the SGF medium. The longer the storage time, the more sensitive *L. plantarum* in SGF. The CcS sample showed significant higher protective efficacy in SGF than the CS samples. The same result was not observed in simulated intestinal fluid. In the sensory test, the difference between the cupcake with and without the addition of microcapsules was not significant.

Keywords: carrageenan, cupcake, baking process, microencapsulation, probiotic, skim milk

INTRODUCTION

The term probiotic is a relatively new word meaning "for life" and it is currently used to name bacteria associated with beneficial effects for humans and animals (**FAO/WHO., 2002**). Probiotic bacteria have been used both in pharmaceutical preparations and in food products (**Muzzafar *et al.,*** 2018). However, the application probiotic in food products are limited and currently provided by dairy products such as yogurt (**Adhikari *et al.,* 2000**), mayonnaise (**Lieu *et al.,* 2017**), skim milk **Maciel *et al.,* (2014)**, cheese and so on, and have few studies on bakery products, a non – dairy products. Besides, dairy products also cause lactose – intolerant (**Majzoobi *et al.,* 2018**). Moreover, the price of dairy products makes the consumers who live in third–world countries are limited in consumption. Thus, the bakery products which supplemented probiotic can be an alternative way to solve that problem. In previous studies, to increase the viability of probiotic, many authors mingling the probiotic with cream that complemented the bread after baking (**Zanjani *et al.,* 2012**), others chose to apply the mixing cream in between biscuits (**Muzzafar *et al.,* 2018**). That would keep probiotic out of the effect of the hash baking condition and maintain a numerous probiotic after supplementing. In the opposite view, the loading directly probiotic in the dough before baking will give more choices that can be chosen by the target consumer. However, the heat sensitivity of probiotic bacteria leading to the inactivation that occurs during baking decreased the viability of probiotic (**Majzoobi *et al.,* 2018; Arslan *et al.,* 2018**). Microencapsulation techniques are the method to help probiotic organisms against the disadvantaging effect of the outside environment (**González – Forte *et al.,* 2014; Lieu *et al.,* 2017; Zhang *et al.,* 2018; Muzzafa *et al.,* 2018; Arslan *et al.,* 2018**). Encapsulation involves the incorporation of food ingredients, enzymes, cells or other material in a small capsule and encapsulated material can be protected from moisture, heat or extreme conditions, thus enhancing their stability and maintaining viability (**Gibbs *et al.,* 1999**). The microencapsulation includes extrusion, emulsion or spray–drying techniques, and so on (**Lieu *et al.,* 2017; Zanjani *et al.,* 2012**). Spray – drying technique is widely utilized to encapsulated probiotics in food. However, the spray – drying process is associated with high cell mortality

resulting from simultaneous dehydration and thermal inactivation microorganisms (**Malmo *et al.,* 2013**). Besides, the dimension of microencapsulation produced by the spray – drying process usually has micron size (**Lieu *et al.,* 2017**) that decreasing the survival rate of probiotic-supplemented in the bakery during baking. On the other hand, the extrusion method deliveries the bigger beads (above 1000 μm) that support the protection of probiotic cells effectively, but it will give more negative sensory. Whereas the emulsion technique would make smaller particles which ranges between 70 to 500 μm (**Zanjani *et al.,* 2012**), and these particles are bigger than spray – drying method (**Lieu *et al.,* 2017**) but ensuring to reduce the effect of these particles on sensory properties and structures of food (**Arslan *et al.,* 2018; Zanjani *et al.,* 2012**). The wall materials such as κ–carrageenan, alginate, and chitosan are demonstrated improving probiotic microorganism against the harsh condition. κ–carrageenan was utilized for encapsulating probiotic cells by emulsion technique and was demonstrated that can be employed in protection Bifidobacteria shelf – life in yogurt by **Adikari *et al.,* (2000)**. And skim milk, the purification products made from milk, also use as a wall material to protect probiotic in the previous study of **Maciel *et al.,* (2014)** and the first time to show their potential protection. To increase the effectiveness of protecting probiotic bacteria, the combination of wall material is getting more interest. Carrageenan-locust bean gum coated milk microspheres making by extrusion method showed a high protective effect on *Lactobacillus bulgaricus* viability in SGF (Simulated gastric fluid) medium (**Shi et al., 2013**). Similarly, carboxymethyl cellulose/κ–Carrageenan blends encapsulated *Lactobacillus plantarum* by extrusion method showed higher protective effect than free carboxymethyl cellulose in SGF medium. Although many studies have been carried out, the incorporation of carrageenan and skim milk, making by emulsion method and supplement to cupcake was poorly reported. Therefore, in the present study, carrageenan was used as the main wall material, and skim milk was used as incorporating or coating agents. *Lactobacillus plantarum* ATCC 8014 was microencapsulated by the emulsion method and supplemented in the cupcake product. The thermal influence during baking, the change in the survival rate of probiotic, pH changing during storage,

and the resistance in simulated intestinal fluid after storage was also carried out in this study.

MATERIAL AND METHODS

Microorganisms

Lactobacillus plantarum ATCC 8014 was harvested from 100 ml of a 24-h culture (37°C) by centrifugation at 5000 rpm. The cells were then washed twice and resuspended in 10 ml of sterile saline water and used in the microencapsulation process.

Microencapsulation

The microencapsulation method was made according to the method described by **Adhikari *et al.*,** (2000) with slight modifies. Briefly, a 10 ml cell suspension was added into 40 ml of 2.5% (w/v) carrageenan (CcS samples) or the mix of carrageenan (2.5%) and skim milk 0.5% (w/v) (CS samples). Then, stirring and heating in a magnetic hot plate stirrer at 45÷48°C. Vegetable oil (100ml, Crisco) and Tween 80 0.1% (v/v) was added into the Becher as an emulsifier. Stirring and dispersing for 15 minutes at 45°C to allow for emulsification and microencapsulation to occur. Then, added slowly 100 mL of KCl 0.1 M solution to break the emulsion. The particles were collected (in case of CS samples) or incubated (in shaking incubator at 200 rpm) in skim milk 0.5% (v/v) (in case of carrageenan coated by skim milk; CcS samples). The particles were used immediately in the next steps. Examined microencapsulation yield in the microcapsule preparation and calculated according to the following formula:

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

Particle size examination

The particles added in 50 ml of distilled water and vortexed. Then 20 ml samples were taken and checked by HORIBA LA-size 920 machine.

Cupcake samples preparation and the survival of bacteria during baking and storage

Standard cupcake formulation was prepared with the addition of flour, sugar, softened butter and egg (1:1:1:0.5, (w/w/w/w)). Vanilla and baking powder were added at 0.5% (w/v) of the cake mix, and the ingredients were mixed using a blender. The microcapsules (4% w/w) were mingled with the dough, and then the mix was spread 50 grams into the tin. Conduct baking at 200°C for 12 minutes. The temperature of the surface and core of the cupcake was examined by FLUKE-62 MAX and TESTO 106 machines. Cupcake samples were cooled down and stored at 4°C. Cupcake samples contain free cells used as control samples. The pH of cupcake samples and the survival rate of *L. plantarum* were determined after the baking process immediately and determined every 2 days until 14 days of storage at 4°C.

pH examination

The pH value of the cupcake was determined every 2 days until 14 days of storage at 4°C by using a 10% dispersion of samples in distilled water using a pH meter. The pH meter was calibrated by standard buffer solutions 4 and 7.

Enumeration of the free and microencapsulated bacteria

The viability of microencapsulated *L. plantarum* in the cupcake was made according to the method described by **Adhikari *et al.*,** (2000) with slight modifies. Briefly, 5 grams of the cupcake was resuspended in 45 ml sterile buffer phosphate (pH: 7, 0.1 M). The capsules were disintegrated by homogenizing in a stomacher and incubating for 20 min at 42°C before making serial dilutions in 0.1% sterile peptone buffered water. The counts were determined by plating on MRS agar plates and incubating for 48 h at 37°C. The cupcake containing free bacteria were treated similarly.

Effects of simulated gastric fluid (SGF) and intestinal fluid (SIF) on *L. plantarum* after 04 and 10 days of storage

5 grams of the cupcake after 04 days and 10 days of storage were incubated in 45 ml of Simulated gastric fluid (SGF) medium (9 g/l NaCl + 3 g/l pepsin (Himedia) adjusted to pH 2.5 with 5N HCl), at 37°C and shaking speed 100 rpm for 120 minutes. Similarly, 5 grams of cupcake were incubated in 45 ml of (simulated intestinal fluid) SIF medium (0.85% NaCl, 0.3% bile salts, adjusted to pH 6.5 with 5N NaOH) at 37°C for 240 minutes. The viability of *L. plantarum* in the cupcake was immediately assayed by plating on MRS media.

Sensory evaluation

The "A, not A" test was conducted with the objective of determining if a significant difference existed between A (cupcake without containing microcapsules) and 'not A' (cupcake containing microcapsules). A sensory evaluation was conducted by 30 assessors. Each assessor was familiarized with the sensory characteristics of the target sample ('A') from the cupcake without containing microcapsules and the nontarget sample ('not A') from the cupcake containing microcapsules, and then received one test sample and asked to identify it as the 'target' or 'not the target'.

Statistical analysis

The data were subjected to analysis of variance (ANOVA) using Statgraphics 15 followed by Tukey test to compare means, with a significance level of 5% when the significant difference between treatments was noted. The Chi-square test was used for sensory evaluation. All tests were performed in triplicate and the data expressed as means ± standard deviation.

RESULTS AND DISCUSSION

Influence of microencapsulation ways on the particle size and microencapsulation yield

The effect of microencapsulation ways on the particle size and microencapsulation yield is shown in **Table 1.** The result showed that the average size of the CcS sample was 165 μm that smaller ($p < 0.05$) than the CS sample (320 μm). Similarly, the microencapsulation yield of *L. plantarum* in the CS sample was higher than the CcS sample, which was 93.50% and 89.25% respectively. In previous studies, the emulsion method produced with unequal particle size because of the influence of homogenized processing. The particle size of alginate – resistant starch particles made by the emulsion method ranges 70 to 500 μm and average size was 280 μm (**Zanjani *et al.*, 2012**) that smaller than the extrusion method that has an average size of 530 μm and particle size ranges 1.62 mm – 1.89 mm (**Krasaekoopt *et al.*, 2004**). Similarly, the result of **Valero-Cases *et al.*, (2015)** showed that microencapsulated *L. plantarum* made from the emulsion method has a smaller size than the extrusion method significantly.

Table 1 The average size and microencapsulation yield (%) made by different microencapsulation ways.

Microencapsulation ways	Average size (μm)	microencapsulation yield (%)
The mix of carrageenan and skim milk (CS samples)	320[a]	93.50 ± 1.04[a]
The beads coated with skim milk (CcS samples)	165[b]	89.25 ± 0.78[b]

[ab] Parameters with different superscript letters within the same column have significant differences (*p*<0.05)

The particle size is one of the important factors affecting food sensory properties. In the emulsion method, the particles are formed in micron size and deliver smooth texture when adding to food products (**Zanjani *et al.*, 2012**). However, the small size may not give enough protection to probiotic bacteria (**Shi *et al.*, 2013**). The small particle size (3 μm) did not show the protective effect of wall material on *L. reuteri* treating at 80°C in 5 min (**Malmo *et al.*, 2013**). The present study showed that microencapsulation ways affected significantly the particle size and the microencapsulation yield (**Table 1**). This phenomenon could explain that the incubation phase with skim milk in the shaking machine (200 rpm) that would make the particles separated that leading to decrease particle size as well as the microencapsulation yield.

Impact of baking temperature on the viability of *L. plantarum* in the cupcake

The temperature changes of bakery samples and the survival rate of *L. plantarum* were presented in **Figure 1**. Temperature values of surface and core of cupcake samples were different significantly ($p < 0.05$), they began from ambient temperature up to 108°C and 82°C, respectively. The difference was 26°C after 12 min of baking at 200°C. The heating treatment of the baking process affected significantly the survival rate of *L. plantarum*. The viability of *L. plantarum* was decreased 0.21; 0.66; 2.13; and 5.33 log CFU/cake after 2, 4, 8 and 12 min baking respectively as compared to the initial. After 12 min baking, the viable *L. plantarum* of free cells, CS, and CcS samples decreased dramatically from 10.18±0.18 to 4.85±0.15 log CFU/cake; 10.05±0.15 to 6.25±0.16 log CFU/cake; and 9.80±0.20 to 6.22±0.18 log CFU/cake respectively.
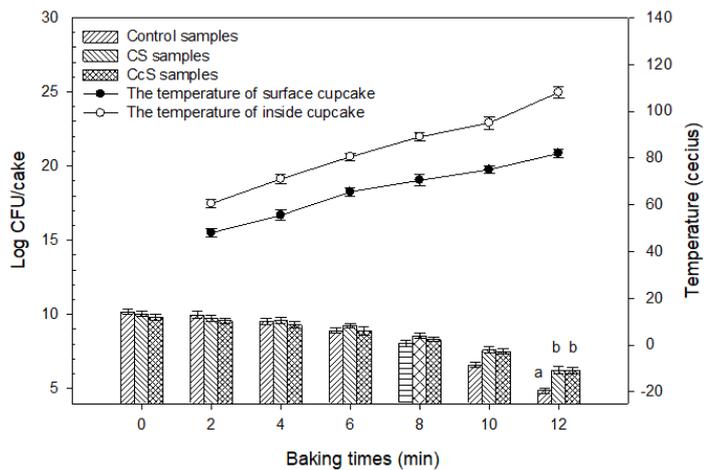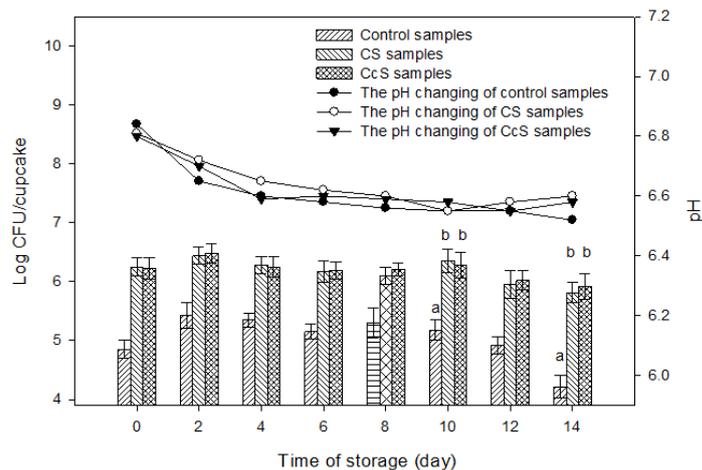
Figure 1 Impact of baking temperature on the viability of *L. plantarum* in the cupcake, [ab] Parameters with different superscript letters have significant differences (*p*<0.05)

Figure 2 Effect of storage conditions on the *L. plantarum* viability in the cupcake [ab] Parameters with different superscript letters have significant differences (*p*<0.05)

In the previous studies were shown that the probiotic bacteria inactivation appeared in thermal treatment upon two factors: temperature and humidity. **Zhang *et al.,* (2018)** demonstrated that the thermal inactivation of *L. plantarum* presented during the baking process and was lost 4 to 5 log CFU/g in their survival rate. **Reid *et al.,* (2007)** shown that WPI - based microsphere protective solution with *L. rhamonus* in biscuit dropped of 3 log after baking at 280°C in 5 min and storage for 24 h. **Malmo *et al.,* (2013)** showed that probiotic bacteria encapsulated by spray – drying method (average size about 3 μm) mere maintained 10% survival cells after 10 min baking time at 180°C. However, in the previous studies, the influence of baking time on the probiotic viability in the cupcake was not reported. In the present study, the surface and core temperature of the cupcake were significantly different (**Figure 1**). The *L. plantarum* viability was decreased rapidly when the temperature inside the cupcake was up to 70.5°C after 8 min of baking (**Figure 1**). The results also indicated that microcapsules presented the protective role to *L. plantarum* cells was higher significantly than free cells and reached over 6 log CFU/cake and achieved the requirement of probiotic-supplemented products. When the microcapsule was exposed to heat, the outer hydrophobic layer melted, and it absorbed heat during melting leading to the temperature of the microcapsule center, which contains the probiotics, could remain cooler than its surroundings (**Arslan *et al.,* 2018**). These results suggested that the component of the wall material and the particle size play an important role in the probiotic's thermal resistance. In addition, the food matrix is a factor that improves the probiotic's thermal resistance. **Malmo *et al.,* (2013)** shown that the heat resistance of *L. reuteri* DSM 17938 found in food was better than in vitro test, this phenomenon related to different food ingredients (carbohydrates and lipids) on the bacterial viability (during baking). Besides, the temperature of the center and surface of bread differ significantly in which the center temperature is cooler than the surface during bread baking (**Zhang *et al.,* 2018**). These help probiotic bacteria overcome the baking processing. However, the protective effect of carrageenan and skim milk on probiotic's thermal resistance during baking processing was poorly reported. In the present study found that have a significant difference in microencapsulated probiotic and free cells (**Figure 1**). The results also indicated that the microencapsulation made from carrageenan and skim milk and the carrageenan beads coated with skim milk were not a significant difference (*p* > 0.05). These suggested that the heat tolerance of microencapsulated *L. plantarum* was not affected by microencapsulation ways.

Effect of storage conditions on the survival of *L. plantarum* in the cupcake.

The influence of storage condition on the pH change and the *L. plantarum* viability were shown in **Figure 2**. The results showed that the pH value of the experimental samples was not changed significantly during storage. The pH value of the control samples tended to be lower than the microencapsulated samples, however, there was not a significant difference. In the other hand, *L. plantarum* viability of the control, CS and CcS samples were not changed dramatically and tended to increase slightly from 4.85 ± 0.15 to 5.18 ± 0.18 log CFU/cake; 6.25 ± 0.16 to 6.35 ± 0.20 log CFU/cake; and 6.22 ± 0.18 to 6.28 ± 0.21 log CFU/cake during 10 days of storage at 4°C, respectively. The *L. plantarum* viability was decreased after 14 days of storage and more rapidly in the next two days, and remain 4.21 ± 0.19 log CFU/cake, 5.81 ± 0.17 log CFU/cake and 5.92 ± 0.22 log CFU/cake for the control, CS and CcS samples respectively.

The pH value change during the storage of products was reported in previous studies. Yogurt, mayonnaise, and many products which supplemented with probiotic were investigated that pH value decreased during storage at 4°C (**Adhikari *et al.,* 2000; Lieu *et al.,* 2017**). Many authors reported that the metabolic phenomenon existed towards generating more organic acid during storage and the decrease in pH value was a consequence. However, the probiotic bakery products are changed slightly in the pH value unlike dairy products (**Figure 2**). **Zanjani *et al.,* (2012)** reported that the pH value of cream filling supplemented with *L. casei* ranged 6.5 to 5.88 and 6.5 to 6.38 in the free cell and encapsulated cell samples after four weeks of storage at 4°C. Similarly, the pH value of cream-filled, marmalade –filled and chocolate-coated cake contained microcapsules probiotic was decreased slightly after 90 days of storage (**Arslan *et al.,* 2018**). These results indicated that the metabolism of microencapsulated probiotic in the cupcake was very slow, which help cake sensory properties were unchanged during storage time.

The influence of storage condition on probiotic viability is received a lot of concern due to the lower probiotic viability during storage leading to decreasing of the benefit of health to the customer. In previous studies showed that the storage temperature affects significantly probiotic viability. In the storage process, probiotic bakery products were generally stored in room more temperate such as at 20°C in biscuit coated with methylcellulose (**González – Fontez *et al.,* 2014**), 23°C in biscuit (**Reid *et al.,* 2007**), 25°C in bread and in cream biscuit (**Zhang *et al.,* 2018; Muzzafar *et al.,* 2018**) all monitored that the probiotic viability decreased during storage time. On the contrary, **Zanjani *et al.,* (2012)** demonstrated that probiotic viability in the cream cake was better at 4°C than at 25°C. **Arslan *et al.,* (2018)** reported that encapsulated probiotic which supplemented in cream-filled and chocolate-coated decreased dramatically after 30 days of storage at 4°C. **Zanjani *et al.,* (2012)** reported that the *L. acidophilus* viability in cream – filled in a baked cake was lost 8.09 log CFU/g after four weeks of storage. However, in the present study, it is interesting to note that the viability of *L. plantarum* did not decrease during storage (**Figure 2**). This result agrees with **Majzoobi *et al.,* (2018)** who indicated that the frozen storage process did not affect significantly the probiotic viability in re-baked bread after four weeks of storage at -20°C. This phenomenon can explain that the probiotic survival was affected by temperature degrees and the kind of compound of the bakery. Moreover, previous studies suggested that a suitable wall matrix in food could deliver probiotic protection better than others (**Gibbs *et al.,* 1999; Adikari *et al.,* 2000; Krasaekoopt *et al.,* 2004; Malmo *et al.,* 2013**). **Zanjani *et al.,* (2012)** reported that the viability of *L. casei* that encapsulated in alginate – starch wall matrix was significantly improved which lost just 1 log after one week of storage at 4°C. In the present study indicated that the *L. plantarum* viability in the samples containing unencapsulated and encapsulated cells was no significant difference during storage (**Figure 2**). It could be explained that the food matrix and storage condition (4°C) is not becoming a proper media culture for probiotic growth. In this condition, probiotic cells returned into the anabiosis phase that leading to the metabolic activity of *L. plantarum* was slow down, and it is suitable for maintaining the probiotic viability during 10 days of storage. However, in the next days of storage, the viability of *L. plantarum* trended to decrease faster and below the minimum value requirement in probiotic products (< 6 log CFU/cake) after 14 days of storage. These indicated that cold storage condition starts inhibiting the generation of *L. plantarum* and decreasing their survival rate. Therefore, 10 days storage was necessary for ensuring that the counts of microencapsulated *L. plantarum* were maintained in the cupcake, suitably (**Figure 2**).

Effect of SGF and SIF medium on the viability of *L plantarum* in the cupcake after 04 and 10 days of storage

The effect of SGF and SIF conditions on the survival rate of *L. plantarum* in the cupcake after 04 and 10 days of storage were shown in **Table 2**. The result showed a difference significantly ($p < 0.05$) in the free and microencapsulated cells. In SGF condition, after 4 and 10 days of storage, the survival rate of *L. plantarum* in control samples was not recorded while the *L. plantarum* viability in the CS samples was 3.42±0.15 log CFU/cake and 2.65±0.15 log CFU/cake, in CcS samples was 4.39±0.17 log CFU/cake and 3.59±0.17 log CFU/cake respectively. In SIF condition, the survival rate of *L. plantarum* in control samples after 4 and 10 days of storage was reached 4.05±0.16 log CFU/cake and 3.85±0.11 log CFU/cake which lower ($p < 0.05$) significantly than CS and CcS samples, and there was no significant difference ($p > 0.05$) between CS and CcS samples. The results showed that the storage time affected significantly on the *L. plantarum* sensibility with the SGF medium (**Table 2**).

Table 2 The survival rate of *L. plantarum* in SGF and SIF after the 04th and 10th days of storage.

Cupcake samples	04th day of storage (log CFU/cake)			10th day of storage (log CFU/cake)		
	Initial	After 2h in SGF	After 4h in SIF	Initial	After 2h in SGF	After 4h in SIF
Control	5.35±0.12[Aa]	0[Aa]	4.05±0.16[Aa]	5.18±018[Aa]	0[Aa]	3.85±0.11[Aa]
CS	6.28±0.15[Ab]	3.42±0.15[Ab]	5.43±0.22[Ab]	6.35±0.20[Ab]	2.65±0.15[Bb]	5.05±0.20[Ab]
CcS	6.25±0.17[Ab]	4.39±0.17[Ac]	5.55±0.20[Ab]	6.28±0.21[Ab]	3.59±0.17[Bc]	5.28±0.19[Ab]

[AB] Parameters with different superscript letters within the same row (the same condition) have significant differences ($p<0.05$)

[abc] Parameters with different superscript letters within the same column have significant differences ($p<0.05$)

The survival rate of probiotic bacteria in SGF and SIF media plays an important role in providing healthy benefice for their host. Evaluating the survival rate of probiotic bacteria in SGF and SIF media was usually carried out in previous studies. The SGF medium has a low pH value is an acidic medium that excites large of H^+ ion which is an inhibited factor on microorganisms. **Zhu et al., (2006)** reported that the digestion enzyme (pepsin) in low pH medium that increasing the antibacterial effect against *E. coli* because of their protein hydrolyzed ability, and *E. coli* resistance related with pH value and enzymatic concentration. On the other hand, bile salt condition also affects probiotic survival. Bile salt impact the bacterial cell membrane, decreases intracellular pH value, causes plasmolysis, and positive gram microbial cells are more sensitive than a negative gram (**Begley et al., 2005**). In other studies, showed that *L. plantarum* cells were sensitive in low pH with the lost cells were 6.61 log CFU/g (**Ding et al., 2007**) or could be up to 10 log CFU/g (**Dafe et al., 2017**). Those studies also have shown that the survival rate of encapsulated probiotic was higher than free cells that incubated in SGF and SIF media because of the wall material of microcapsules displayed as a barrier against the diffusion (**Shi et al., 2007; Lieu et al., 2017**). Moreover, the incorporation of every single wall material increases the protective effect more significantly than the individual wall material. The incorporation of κ – carrageenan and whey protein that increased significantly in the survival rate of *L. plantarum* in SGF, was reported by **Hernández-Rodríguez et al., (2014)**. Similarly, **Maciel et al., (2014)** indicated that the viability of probiotic encapsulated by skim milk was not affected when incubating in the SGF medium (pH 2). This phenomenon can be explained that skim milk maintained pH value inner capsules higher than pH value outside leading to improved significantly in the survival rate of encapsulated probiotic (**Heideback et al., 2009**). The present study showed that the microencapsulation process affected significantly on the *L. plantarum* viability in which the particles coated by skim milk (CcS samples) delivered a higher survival rate than the particles (CS samples) made by the mix of carrageenan and skim milk (**Table 2**). These results could be explained that, in CS samples, skim milk interfered in the cross-linking formatting processing leading to the H^+ ion diffuse through the particle structure and destroyed probiotic cells. In CcS samples, carrageenan particles were incubated in skim milk which not only would not affect the cross-linking matrix of particles but also reduce their porous. Finally, it helped to increase the survival rate of *L. plantarum* cells in the low pH environment (**Table 2**). The influence of the cold storage condition on the probiotic viability in SGF and SIF conditions were reported in previous studies. **Cristina et al., (2002)** indicated that the sensitive degrees of the fresh and cold storing *L. johnsonii* La1 cells with SGF medium were not significantly different, but in the SIF medium, the fresh *L. johnsonii* La1 cells reached a higher survival rate than the cold storage cells. However, in the present study showed that storage condition affected the *L. plantarum* sensitivity in SGF and SIF conditions (**Table 2**). This result was similar to **Priscilla et al.,** (2015) who reported that *Bifidobacterium animalis* in ice cream after 120 days storage was more sensitive ($p < 0,05$) with SGF medium than after 30 days storage. Similarly, **Dong et al., (2017)** indicated that the longer the storage time, the more sensitive probiotic in the SGF and SIF conditions. In the present study showed that the sensible degrees of *L. plantarum* with SGF medium increased during cold storage. However, the same result was not observed in the SIF medium (**Table 2**).

Sensory evaluation

The sensory evaluation of comparison of the cupcake with and without the addition of microcapsules (4% w/w), with the Chi-square value, was 0.144 (Statistical differences are found when the chi-square values are ≥ 3.8) indicated that the difference between these samples was not significant ($p>0.05$). The sensory evaluation is an important role that ensures the addition of the microcapsules to the cupcake would not affect the original cupcake sensory property. The sensory difference due to the supplementation of microcapsules could lead to consumer rejection.

Table 3 Comparison of cupcake with and without the addition of microcapsules showed that the Chi-square was 0.144. Statistical differences are found when the Chi-square values are ≥ 3.8.

Subjects responded	Subjects received		Total
	A	Not A	
A	5	6	11
Not A	10	9	9
Total	15	15	30

In previous studies, the microencapsulated *Bifidobacterium longum* B6 supplement into yogurt showed the difference with the non-microencapsulated sample (**Adhikari et al., 2003**). However, the research of **Zanjani et al., 2012** showed that the body and texture of the cream-filled cake samples did not show a significant difference ($p> 0.05$) between the cream-filled cake containing free bacteria and encapsulated bacteria (average size was 280 μm). The same result was recorded in the present study (**Table 3**). There was no significant difference in cupcakes with and without the addition of microcapsules (**Table 3**). The significant difference was found in the cupcake adding more than 5% w/w of microcapsules (data not shown). The result suggested that the amount of the microcapsules, as well as the particle size, affect the sensory properties of the cupcake.

CONCLUSION

The present study showed that that the average particle size of the CcS sample was 165 μm that smaller ($p < 0.05$) than the CS sample (320 μm). Similarly, the microencapsulation yield of *L. plantarum* in the CS sample was higher than the CcS sample. The baking temperature impacted significantly on the survival rate of *L. plantarum*, whereas refrigeration storage did not affect the *L. plantarum* viability. Microencapsulation technique improved the survival rate of *L. plantarum* during baking processing, storage, and exposed in simulated gastric digestion, specific the skim milk coated beads provided more protective than the mixed form. The survival rate of *L. plantarum* after baking was decreased dramatically 4.85 log CFU/cake from 10.18 log CFU/cake initially, whereas the *L. plantarum* viability in microencapsulated samples reached over 6 log CFU/cake. The results also indicated that the *L. plantarum* counts in all experimental samples were not impacted by storage conditions at 4°C. However, *L. plantarum* cells showed high sensitivity with the SGF and SIF media. The longer the storage time, the more sensitive probiotic in the SGF medium. In the 4th and 10th days of storage, the *L. plantarum* viability in the control samples was not recorded after 2h in the SGF medium and only remained 4.05 log CFU/cake and 3.85 log CFU/cake after 4 h incubated in SIF that was lower than microencapsulated cell samples. The samples containing carrageenan particles coated by skim milk (CcS samples) showed significant higher protective efficacy in SGF medium than the mixed carrageenan and skim milk (CS samples). However, there was no significant difference between coating samples and mixed samples in the SIF medium after 4h incubation. κ–carrageenan particles coating by skim milk not only did not affect the gel structure matrix but also limited the diffusion of H^+ into the microcapsule structure leading to enhance the survival rate of *L. plantarum* in SGF condition. Additionally, the difference between these samples was not significant in the sensory test. The result showed that the potential application of the microencapsulated probiotic bacteria supplementing directly in the dough before baking that diversify probiotic bakery products, but it also maintained necessary probiotic survival.

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