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Effect of Yeast Cell Microcapsules as a Potential Carrier for Improving *Lactobacillus plantarum* Viability in Muffin Adding *Stevia rebaudiana*

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Abstract

In this study, the role of *Saccharomyces cerevisiae* treating by ultrasound on *Lactobacillus plantarum* viability during muffin baking process, during storage, as well as in the simulated digestive fluid (SDF) was reported. *Stevia rebaudiana* was added during muffin making as a substitute sugar compound. The results showed that the ultrasonic treatment on *S. cerevisiae* was necessary to improve the viability of *L. plantarum* in simulated gastric fluid (SGF) conditions. The results showed that the *L. plantarum* viability was remained to be 2.63 logCFU/mL in the SGF medium with *S. cerevisiae* present at a high concentration (10 logCFU/mL). While the yeast cell treatment by ultrasonic improved the *L. plantarum* viability up to 5.44 logCFU/mL. The *L. plantarum* viability in all samples was maintained above 4.5 logCFU/cake after the baking process. The result also indicated that stevia could help improve the encapsulated *L. plantarum* viability in the muffin. During 3 days of storage, the survival rate of *L. plantarum* was not significant difference among samples. The *L. plantarum* viability tended to increase slightly after a day of storage and then decrease gradually in the following days of storage. The result also showed that there was no significant difference in sensory properties between the samples with and without probiotic supplements. The addition of stevia into muffins containing encapsulated *L. plantarum* has both positive efficiencies. Firstly, improved the *L. plantarum* viability in the SDF after 3 days of muffin storage. Secondly, making muffins with low calories by replacing part of the sugar.

Keywords: *Saccharomyces cerevisiae*, Encapsulation, *Lactobacillus plantarum*, Probiotic, *Stevia rebaudiana*, Muffins

Introduction

Probiotics are defined as living microorganisms that have beneficial effects on host health when administered in adequate amounts [1]. Many health benefits from probiotics such as: adhering to the intestinal wall associated with the intestinal epithelium; helping to improve the immune system, forming antagonistic bacteriocin with harmful bacteria; prevent process mutation and production of carcinogens [2]. Probiotic bacteria were commonly used in the dairy industry. However, consumers are recently trending to use non-dairy probiotic foods due to concerns about high cholesterol, lactose intolerance, or following a specific diet [3]. Bakery product supplement probiotic bacteria is considered a potential approach for both non-dairy foods and innovative products. In today's market, muffins are popular with consumers for their softness and great taste, which is a potential vehicle to supply probiotic sources to consumers. In muffins, sucrose is the main sweetener in the cake because it affects the texture, color, moisture, and flavor of the product [4]. Nevertheless, the excessive use of sugar is one of the causes of obesity [5]. Obesity is becoming a worldwide concern, turning these subjects into victims of type II diabetes, high blood pressure, atherosclerosis, and even cancer [6]. Therefore, reducing the sucrose content in muffins while maintaining the sensory properties is a challenge. Many low-calorie sweeteners have been studied to replace sugar including acesulfame-potassium, aspartame, cyclamate, saccharin, etc. [7]. However, synthetic sweeteners have a lot of controversy about potential hazards to health, such as cyclamate which is converted to cyclohexylamine capable make toxic, saccharin capable of causing bladder cancer in mice [8]. Hence, finding other alternative sweeteners, especially from natural sources would be of great value in this regard.

Stevia was a low-calorie sweetener, and its sweetness can be about 300 times that of sucrose, is very heat resistant, low pH, and non-toxic to human health [9], which shows a potential source. Besides, the compound present in stevia has the ability to stimulate the growth of probiotic bacteria [10].

For probiotic supplement production, the viability of these strains is important concerning the health benefits they brought. Previous studies showed that probiotic bacteria were easily affected by heat treatment and gastric digestion [11,12]. Therefore, the protection of probiotics during food production, and storage, as well as under gastric digestion is essential in which microencapsulation techniques such as extrusion, emulsification, and drying spray are getting a lot of attention. However, the large particle size created by the extrusion technique has a great impact on the sensory property, while the emulsification technique causes the oil in preparation particles which is not suitable for diet products. Besides, the spray drying technique would affect probiotic bacteria viability, undergoing two times of the heat process by the spray-drying and baking process. Therefore, finding a suitable microencapsulation technique for the muffin line for a diet person is essential. Besides microencapsulation techniques using organic compounds as carriers, the use of microbial cells as carriers in which *Saccharomyces cerevisiae* is receiving a lot of attention. *Saccharomyces cerevisiae* is generally regarded as safe (GRAS), easy to scale at low cost, and available in the market [13]. Previous studies have shown the potential of *Saccharomyces cerevisiae* in the microencapsulation of compounds such as chlorogenic acid, curcumin, anthocyanin, etc [14-16]. But so far, the use of *Saccharomyces cerevisiae* cells for probiotic microencapsulation has been very little reported. The study aim was to evaluate the viability of the *Lactobacillus plantarum* ATCC 8014 microencapsulated into *Saccharomyces cerevisiae* cells for use in the production of muffins supplemented with stevia in baking and storage conditions, as well as under gastrointestinal fluid conditions

Materials and methods

Materials

Stevia (*Stevia rebaudiana)* after collecting samples in Hung Yen province, which is located at 20°50′38″N105°58′30″E in the northern part of Vietnam. Stevia was dried and crushed by the blender (Philips HR2118/01, Indonesia) at high speed. The samples were then sieved and kept in sealed plastic bags until used.

Saccharomyces cerevisiae DT and *Lactobacillus plantarum* ATCC 8014 strain was obtained from the strain collection of the Faculty of Food Science and Technology, Ho Chi Minh City University of Food Industry. The trains were cultured on Man Rogosa Sharpe (MRS) broth (Himedia Corp, Mumbai, India) at 37 °C in 24 h (in case of *L. plantarum*) or Hansen broth at 30 °C in 24 h (in case of *S. cerevisiae*). The biomass was then collected by centrifuge (Z206A, Hermle, Baden-Württemberg, Germany) and was used for the microencapsulation process.

Microencapsulation process

The microencapsulation process was carried out following. Brief, yeast biomass was diluted with sterile saline water (0.9 % w/v) and then was conducted ultrasound treatment (VC 750, Sonics, Newtown, USA, with a maximum power of 750 W, frequency of 20 kHz) at 225 W in 8 min (10 sec pulse on and 10 sec pulse off). The suspended liquid was centrifuged (5,500 rpm), collected the precipitate part, and resuspended with saline water.

The *L. plantarum* biomass (9 Log CFU/mL, approximately) was added to the *S. cerevisiae* suspend after ultrasonic treatment (the ratio *S. cerevisiae: L. plantarum* was 1: 1) and was shaken on a shaking machine (200 rpm in 40 min) at 30 °C for the encapsulation process. The encapsulation efficiency was evaluated by testing the *L. plantarum* viability under simulated gastric fluid in 2 h at 37 °C. After incubation, the mixture was centrifuged and resuspended in 10 mL of phosphate buffer (0.1 M, pH 7.0) and vortexed for 10 min. The *L. plantarum* viability (CFU/g) was determined by spreading on the MRS agar at the temperature of 37 °C for 72 h. The free cell of *L. plantarum* was used as control samples, and the mix of *S. cerevisiae* (without ultrasound treatment) and free cell of *L. plantarum* as the different ratio (*S. cerevisiae: L. plantarum* was 1: 100, 1: 10, 1: 1, and 10: 1) were used to evaluate the ultrasonic impact on *L. plantarum* viability.

Preparation of muffin sample and investigation of the baking and storage survival rate

The muffin cake (M sample) recipe prepared includes flour, sugar, eggs and baking powder (1: 1.3: 2: 0.03, (w/ w/ w/ w), and in muffin containing stevia (MS sample) includes flour, sugar, stevia, eggs and baking powder (1: 1: 0.02: 2: 0.03 (w/ w/ w/ w/ w). Vanilla was added at 0.5 % (w/v). Then, *L. plantarum* was added and mixed for 5 min until homogeneously. There were 4 muffin samples including: muffin

samples containing *L. plantarum* free cell (MF samples), muffin-stevia samples containing *L. plantarum* free cell (MSF), muffin samples containing encapsulated *L. plantarum* (ME samples), and muffin-stevia samples containing encapsulated *L. plantarum* (MSE). Then, the mix was spread 50 g into the tin and baked at 150 ℃ for 15 min and was cooled down.

The survival rate of *L. plantarum* was determined after the baking process immediately and after 1, 3, and 5 days of storage at 4 °C. Ten gram of muffin was resuspended in 90 mL of phosphate buffer (0.1 M, pH 7.0), followed by homogenization in a stomacher (IUL, Barcelona, Spain) for 10 min The *L. plantarum* viability (CFU/g) was determined by spreading on the MRS agar for 72 h at the temperature of 37 °C.

Evaluation for the viability of *L. plantarum* in the simulated digestive fluid

The experiment was carried out as described by Dong *et al.* (2020) [12]. Simulated gastric fluid (SGF) consisting of 9 g/L NaCl adjusted pH to 2.5 with 5M HCl and Simulated intestinal fluid (SIF) consisting of 9 g/L NaCl + 3 mL/L salt bile adjusted pH to 6.5 with 5M NaOH. The 10 g muffin sample was incubated in 90 mL of SGF or SIF at 37 °C for 2 and 4 h, respectively. The mixture was centrifuged and resuspended in 90 mL of phosphate buffer (0.1 M, pH 7.0), followed by homogenization in a stomacher (IUL, Barcelona, Spain) for 10 min. The *L. plantarum* viability (CFU/g) was determined by spreading on the MRS agar in 72 h at the temperature of 37 °C.

Sensory evaluation

The "A, not A" test was conducted to determine if a significant difference existed between A (MS samples) and 'not A' (MS containing probiotic). The sensory evaluation was conducted by 30 assessors. Each assessor was familiarized with the sensory characteristics of the target sample (‘A’) from the muffin-stevia and the nontarget sample (‘not A’) from the muffin-stevia containing probiotic, and then received 1 test sample and asked to identify it as the ‘target’ or ‘not the target’. The Chi-square test was used to determine whether consumers recognize the difference between the 2 samples.

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.

Results and discussion

Effect of ultrasonic treatment on *L. plantarum* viability under stimulated gastric digestion

The morphology of *S. cerevisiae* after ultrasonic treatment under the microscope was presented in **Figure 1**. The results showed that the ultrasonic treatment affected the yeast cell wall and caused cracks in the cell wall, though this impact did not destroy all yeast cells. The impact of SGF and SIF conditions on *L. plantarum* viability was presented in **Table 1**. In the SGF condition, the free cell *L. plantarum* was significantly affected by low pH conditions, and the *L. plantarum* viability was not recorded. The results showed that the *L. plantarum* viability was improved with the presence of *S. cerevisiae* (**Table 1**). However, this phenomenon only recorded when increasing the density of yeast up to 10 log CFU/mL with 2.63 log CFU/mL of *L. plantarum* viability. The ultrasonic treatment on yeast cells significantly impacted the *L. plantarum* viability, which reached 5.44 log CFU/mL after 2 h of incubation in SGF, the highest recording of survival (**Table 1**).

Figure 1 The morphology of *S. cerevisiae* after ultrasonic treatment under the microscope (400×).

Gastric digestion fluid has always been a major challenge for probiotic bacteria. This is considered one of the criteria for evaluating probiotics which were reported in previous studies [12,17,18]. Probiotic bacteria were very sensitive to SGF conditions, and the probiotic viability was significantly decreased in this condition [12,19]. These results suggest that the low pH of digestion condition is an adverse medium for probiotic bacteria and its survival under this condition is necessary to perform its beneficial activity to the host. Studies on improving the probiotic viability by encapsulation technique were approached including, extrusion, emulsion, and spray drying methods, etc [11,12,17,19]. Probiotic cells in alginate bean survived better than free cells after sequential incubation in simulated gastric and intestinal juices, with and without bile salt [19]. The *L. plantarum* viability was decreased by 6 logs in SGF conditions compared to that of 4 logs in encapsulation by alginate [11]. Similarly, microencapsulated *Bifidobacterium* BB-12 in reconstituted skim milk by spray drying showed 2 logs higher than that of free cells in simulated gastric conditions [17]. These suggested that microencapsulation technique significantly improves the viability of probiotic bacteria. Besides wall material from organic sources, microbial cells were considered potential materials in which yeast cell is well known for its ability to encapsulate many important compounds such as chlorogenic acid, curcumin, etc [14,15].

Table 1 The survival rate of *L. plantarum* in different forms under SGF medium.

Samples	Log CFU/mL	
	Initiation	**After 2h incubated in SGF medium**
Control	9.16 ± 0.07	0^{c}
S. cerevisiae: L. plantarum (1: 100)	9.12 ± 0.06	0^{c}
S. cerevisiae: L. plantarum (1: 10)	9.14 ± 0.08	0^{c}
S. cerevisiae: L. plantarum (1: 1)	9.16 ± 0.12	0^{c}
S. cerevisiae: L. plantarum (10: 1)	9.08 ± 0.16	2.63 ± 0.25^{b}
Encapsulation	9.07 ± 0.14	5.44 ± 0.27^{a}

*a,b are the characters representing significant differences among samples ($p < 0.05$).

However, studies on the role and effect of *S. cerevisiae* on the *L. plantarum* viability under SGF condition have not been fully reported. The *L. plantarum* viability was improved in the presence of *S. cerevisiae* (non-ultrasonic treatment) at a high concentration (10 log CFU/mL). This showed that the yeast presence created a synergistic effect on the *L. plantarum* viability. However, these survival rates were significantly lower ($p < 0.05$) compared to yeast cells under ultrasonic treatment (**Table 1**). Although ultrasonic treatment would not kill all yeast cells, the high-power impact caused the cracked appearance on many cell walls (**Figure 1**).

The ultrasonic process created the compression, expansion, and burst of air bubbles, which impacted the cell surface and led to erosion, surface peeling, and destruction of cell structure [20]. Besides, the result from the transmission electron microscopy showed that the ultrasound impacted the yeast cell wall, ruptured many cells, and created holes in yeast cells [21]. The holes on the yeast cell wall would allow probiotic cells to penetrate the yeast cell for the encapsulation process. This process improved the *L. plantarum* viability in the low pH condition (**Table 1**). The results showed that the ultrasonic treatment on yeast cells was necessary to enhance *L. plantarum* viability. The encapsulated *L. plantarum* cell by treated yeast cell was selected for muffin production in the next steps.

The viability of *L. plantarum* during the muffin-baking process

The effect of the baking process on *L. plantarum* viability in muffin cake was shown in **Figures 2** and **3**. The results showed that after 15 min of baking, the *L. plantarum* viability in MF, MSF, ME, and MSE from 9.96 ± 0.17 to 4.55 ± 0.26 log CFU/cake; 9.85 ± 0.13 log CFU/cake remained 4.89 ± 0.17 log CFU/cake; 9.82 ± 0.32 log CFU/cake remained 4.99 ± 0.34 log CFU/cake; 9.89 ± 0.17 log CFU/cake remained 5.36± 0.23 log CFU/cake, respectively. The encapsulated *L. plantarum* viability was higher than

free cell samples, but there was no significant difference ($p > 0.05$). The significant difference was only recorded in MSE samples compared to MF samples (**Figure 2**).

The high temperature is a big challenge for probiotic bacteria, which are sensitive to temperature. The viability of probiotic bacteria in cake samples was significantly reduced after the baking process (**Figure 2**). This could be due to dehydration and heat shock by high temperatures [22]. Ding *et al.* (2007) indicated that a temperature higher than 65 in 1 h caused the cell death of probiotics [11]. Besides, Zhang *et al.* (2018) suggested that the *L. plantarum* viability was reduced by 4 to 5 log CFU/g during bread baking at 235 °C at 8 min [23]. A study by Fritzen *et al.* (2013) also indicated that the survival rate of free cell Bifidobacterium BB-12 was reduced by 1 log CFU/g after 10-min treatment at 55 °C, and more than 4 log CFU/g after 1-min treatment at 75 °C [17]. These studies showed that the viability of probiotic bacteria was influenced by temperature conditions and test strain.

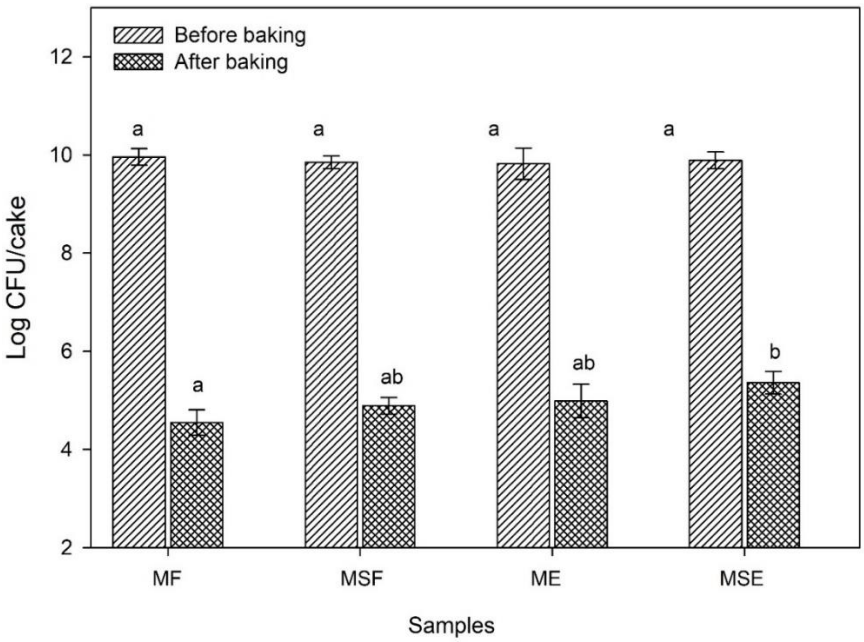

Figure 2 The *L. plantarum* viability in muffins during the baking process.
*a,b are the characters representing significant differences among samples ($p < 0.05$).

The present study showed that the *L. plantarum* was maintained above 4.5 log CFU/cake after baking (**Figure 2**). The probiotic viability after baking is related to the food types its containing. The previous studies showed that food matrixes are one of the factors which significantly improved probiotic viability [19,24]. In addition, the temperature and humidity in the crust and the core are not the same. The probiotic viability in the core was higher than in the crust due to the temperature in the crust was high, and the moisture was significantly reduced [12,23]. Therefore, with the lower temperature and high humidity of the cake core, the probiotic bacteria could overcome the baking process.

Figure 3 The muffin containing *L. plantarum* after the baking process.

In previous studies, encapsulation techniques significantly improved probiotic viability. Malmo *et al.* (2013) indicated that *Lactobacillus reuteri* was encapsulated by the spray drying method in an alginate matrix and coated with chitosan maintained a 10 % survival rate after 10 min baking at 180 °C [24]. Similarly, the encapsulation in chitosan coated alginate-starch capsules also enhanced *Lactobacillus acidophilus* and *Bifidobacterium pseudocatenulatum* viability by 3.4 log CFU/mL and 5.43 log CFU/mL, respectively, which were higher than the non-encapsulation cell after 30 min incubation in 60 °C [25]. Besides, the encapsulated anthocyanins by yeast cells were stable in colors under the influence of temperature compared to unencapsulated color solutions [16]. The ability to protect *L. plantarum* from SGF condition by *S. cerevisiae* cells was significantly effective compared to the control (**Table 1**). However, the muffin baking test showed that although the *L. plantarum* viability in ME samples was higher than that of MF samples, there was no significant difference (**Figure 2**). This indicated that yeast cells were not very effective at protecting the bacteria *L. plantarum* against high temperatures. The present study also indicated the MSE samples that contained stevia significantly improved the *L. plantarum* viability compared to that of free cell samples (MF samples) (**Figure 2**). The role of stevia on probiotic viability during the baking process was not reported fully. The compounds of stevia contain fructooligosaccharides (FOS) [10], a prebiotic which has been shown to improve the probiotic viability during spray drying [26]. Similarly, another prebiotic such as galactooligosaccharides (GOS) combine with maltodextrin as wall material showed improved viability of *L. plantarum* CIDCA 83114 up to 93 % compared to 64 % of samples without GOS [27]. These results showed that stevia was related to positive protection on the probiotic from high temperature. This could help to improve the *L. plantarum* viability encapsulating by *S. cerevisiae* in the MSE samples, which supplement stevia during muffin making (**Figure 2**).

Effect of storage time on *L. plantarum* viability in muffin

The change in *L. plantarum* density during storage was shown in **Figure 4**. In general, the *L. viability* tended to increase slightly after a day of storage and then decrease gradually in the following days of storage. After the baking process, the survival rate of *L. plantarum* in MSE samples was higher than that of MF samples (**Figure 2**). However, the *L. plantarum* in MF samples tended to increase during storage, and there were no significant differences ($p > 0.05$) among samples in this evaluation (**Figure 4**). After one day of storage, the *L. plantarum* viability in MF, MSF, ME, and MSE samples was 4.90 ± 0.22 log CFU/cake; 5.22 ± 0.0.28 log CFU/cake; 5.11 ± 0.35 log CFU/cake; and 5.45 ± 0.11 log CFU/cake, respectively. And after 5 days of storage, *L. plantarum* viability in MF, MSF, ME, and MSE samples was slightly reduced by 4.69 ± 0.30 log CFU/cake; 4.92 ± 0.28 log CFU/cake; 4.86 ± 0.27 log CFU/cake; and 4.98 ± 0.18 log CFU/cake, respectively.

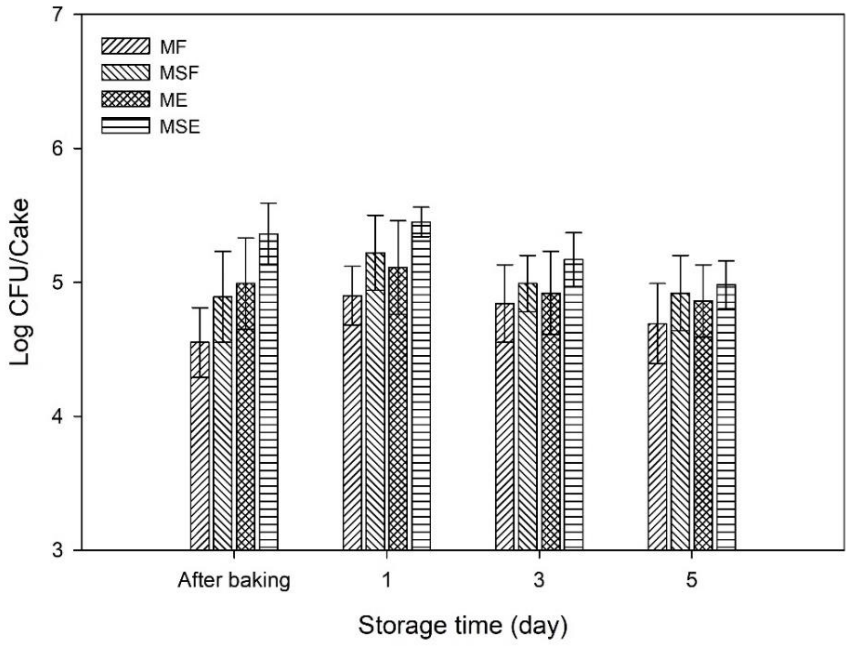

Figure 4 The *L. plantarum* viability in muffin during storage at 4 °C.

The viability of probiotics under storage conditions is important for ensuring their health benefit values to the host. For probiotic supplements food, low-temperature storage is often applied to maintain viability and limit their metabolic that affects the product's sensory properties. The previous studies showed that the storage condition significantly affected probiotic viability. Silva *et al.* (2017) indicated the viability of *Lactobacillus acidophilus* LA3 and *Bifidobacterium lactic* BLC1 in chocolate was reduced by 1.4 and 0.7 log CFU/g respectively compared to 8 log CFU/g initial density after 120 days of storage at 25 °C [18]. Similarly, cream biscuits containing the probiotic bacterium (*Lactobacillus acidophilus*, *Lactobacillus rhamnosus* and *Bifidobacterium longum* in 1:1:1 proportion), which was reduced 1.4 log CFU/g after 8 weeks of storage at 25 °C [28]. However, a study by Reid *et al.* (2007) showed that the *Lactobacillus rhamnosus* R011 viability in biscuits remarkably reduced by 1.46 log after 24 h of storage at 23 °C [29]. Zanjani *et al.* (2012) suggested that the survival rate of probiotics in cream cake at 4 °C was higher than 25 °C [30]. This could be explained that low-temperature limits probiotic's metabolism, and produce less waste, leading to improve probiotic viability during storage at 4 °C [31]. The present study showed that the *L. plantarum* was increased slightly during storage (**Figure 4**). This would explain that muffin and its components were a suitable environment for probiotic growth [32].

A study by Zhang *et al.* (2018) showed that *L. plantarum* in bread was increased by 2 - 3 log CFU/g after 4 days of storage [23]. A similar study was reported by Pereira *et al.* (2011) indicated that the survival rate of probiotic increased to 8 log CFU/mL during 42 days of storage [33]. After the baking process, surviving cells need time to recover [34]. During storage, the redistribution of moisture between crust and core of muffin with the environment [35]. Soukoulis *et al.* (2014) indicated that the moisture moves from core to crust, which creates a favorable condition for bacteria growth [36]. However, after 3 days of storage, the *L. plantarum* viability was tended to decrease gradually (**Figure 4**), and the structure of the muffin was changed (data not shown). Therefore, the storage time of 3 days was suitable for probiotic supplement muffin in this study.

The *L. plantarum* viability in simulated digestive fluid after 3 days of storage

The effect of SGF and SIF condition on *L. plantarum* viability in muffin cake after 3 days of storage was shown in **Table 2**. In the SGF test, the survival rate of *L. plantarum* was significantly different among the samples. After 2 h of incubation under SGF, the *L. plantarum* viability in MF, MSF, and ME samples was below 3 log CFU/cake, whereas in the MSE samples reached 3.5 log CFU/cake. In the SIF test, the *L. plantarum* viability showed higher than that of the SGF test, and there was a significant difference ($p < 0.05$) among samples (**Table 2**). After 4 h of incubation in SIF, the *L. plantarum* viability in MSF, ME, and MSE samples were 3.13 ± 0.08; 3.70 ± 0.17; and 4.10 ± 0.16 log CFU/cake, whereas in MF samples was below 3 log CFU/cake.

Table 2 The survival rate of *L. plantarum* in SGF and SIF conditions after 3 days of muffin storage.

Samples	LogCFU/Cake		
	After 03 days of storage	SGF medium	SIF medium
MF	4.84 ± 0.29	Na	Na
MSF	4.92 ± 0.28	Na	3.13 ± 0.08^{c}
ME	4.92 ± 0.31	Na	3.70 ± 0.17^{b}
MSE	5.17 ± 0.20	3.25 ± 0.15	4.10 ± 0.16^{a}

*a,b are the characters representing significant differences between samples ($p < 0.05$).
Na: the survival rate below 3 LogCFU/Cake

The viability of probiotics under digestion fluid is very important to ensure their health benefit to the host. However, the sensibility of probiotics to low pH conditions leading to a significant decrease in probiotic viability [11,12,19]. In SGF conditions, hydrochloric acid is a strong oxidizing agent that could kill microorganisms [37]. This caused probiotic bacteria in free cell form to be completely lost in SGF conditions [12,38]. Besides, the SIF condition also contributes to the impact on the microorganism viability by affecting the membrane, causing the protoplasmic shrinking [39]. In addition, the sensitivity of probiotic bacteria to simulated digestion was also affected by the storage time which the sensitivity increased with the storage time [12]. This results in the health benefit from probiotic bacteria being unattainable.

Encapsulation techniques have been proven to improve the probiotic viability in simulated digestive fluid. A study by Tee *et al.* (2014) indicated the viability of *Lactobacillus plantarum* encapsulating in κ-carrageenan was improved 2 Log CFU/mL under pH 2.0 in 2 h incubation compared to that of free cell [40]. Similarly, *L. plantarum* was encapsulated in the mix of alginate (2 % w/v) and maltodextrin (1 % w/v)

by emulsion method, which improved the viability to 2.08 Log CFU/g compared to that of the free cell samples [12]. This showed that encapsulation technique significantly improved the viability of probiotic bacteria. Besides emulsion, extrusion, and spray drying methods, encapsulation into yeast cells also shows promised potential. However, studies on the effect of yeast cells on the viability of probiotic bacteria in muffins under artificial digestion conditions have not been fully published. **Figure 3** showed that, although the *L. plantarum* viability was no significant difference among samples, the significant difference was recorded in SGF and SIF tests after 3 days of storage (**Table 2**). This showed that the yeast cell could help *L. plantarum* against the diffusion of H^+ ions in SGF conditions.

The results also indicated that the addition of stevia during muffin making improved the *L. plantarum* viability under SGF conditions (**Table 2**). Besides studies on wall materials, the research for supplement components improving probiotic viability in which prebiotic was received interest. Rad *et al*. (2012) suggested that prebiotics enhance the activity and promote the growth of many microorganisms in the gut, mainly *Lactobacillus* và *Bifidobaterium* [41]. One of the important prebiotics is FOS, which stimulate the growth of intestinal microflora, prevent harmful bacteria, and improves the immune system [42]. Lopes *et al.* (2016) suggested that FOS isolating from the roots of Stevia rebaudiana stimulated the growth of *Lactobacillus* and *Bifidobacterium* [10]. Similarly, FOS isolated from *S. rebaudiana* was fermented by Bifidobacteria or Lactobacillus, showing the probiotic growth was 2.28 and 1.7 times higher than that of FOS isolated from chicory [43]. In addition, Geuns *et al.* (2007) found that compounds in stevia were either not absorbed or extremely low absorption in the gastrointestinal tract [44]. The *L. plantarum* viability in muffin after baking was not reached the probiotic product request (**Figure 3**). However, the result showed that the *L. plantarum* viability in MSE samples could overcome the simulated digestive fluid (**Table 2**). These showed that the addition of stevia into muffin has both positive efficiencies. Firstly, improved the *L. plantarum* in simulated gastric fluid; secondly, made muffin with low calorie.

Sensory evaluation

The sensory evaluation of comparison of the muffin-stevia with and without *L. plantarum* (MSF or MSE samples). The Chi-square value of MSF and MSE samples were 1.29 and 1.22, respectively, that indicated that the difference between these samples was not significant ($p > 0.05$). Food sensory has a significant influence on customers' purchase decisions. The addition of encapsulated particles without affecting the sensory properties of the original product would be easily accepted by customers. The encapsulated particles size is always an issue to consider when added to food. The particles made by the extrusion method with large particle size showed the effect on organoleptic. The addition of encapsulated particles by extrusion method with big particles size would affect the organoleptic. The sensory analysis of blackberry-flavored yogurts revealed that samples containing encapsulated bifidobacteria had a grainy texture [45]. Meanwhile, the encapsulated particles by emulsion method with smaller particles size were not significantly different compared to that of the free cell when added to the cupcake [12]. Similarly, the cream-filled cakes containing encapsulated *Lactobacillus casei* (average size was 280 µm), which was not shown a significant difference in the body and texture compared to that of free cells [30]. However, the emulsion process requires adding oil, which is not suitable for products intended for dieters. The result obtained in the present study showed that there was no significant difference between the samples containing probiotics (free cell or encapsulated cell) and the samples without probiotics. In contrast to other wall materials, encapsulation of *L. plantarum* by *S. cerevisiae* cells allows achieving a small size that easily surpasses the perception of consumers. The results indicated that adding encapsulated *L. plantarum* by *S. cerevisiae* would not affect the sensory of the muffin as well as ensuring the viability of probiotics during baking, storing, and under simulated digestive fluid.

Conclusions

The ultrasonic treatment on *S. cerevisiae* was necessary to encapsulate *L. plantarum* and improve their viability in SGF conditions. In muffin baking, stevia was related to positive protection on the probiotic from high temperatures. This could help to improve the *L. plantarum* viability encapsulating by *S. cerevisiae* in the MS samples, which supplement stevia during muffin making. The *L. plantarum* viability in all muffin samples tended to increase slightly after a day of storage and then decrease gradually in the following days of storage. Although the *L. plantarum* viability was no significant difference among samples, the significant difference was recorded in SGF and SIF tests after 3 days of storage. The MSE muffin samples containing stevia, and encapsulated *L. plantarum* by yeast cells showed the best result. The result also showed that there was no significant difference in sensory property between the samples with and without probiotic supplements. The addition of stevia into muffin containing encapsulated *L. plantarum*

has both positive efficiencies. Firstly, improved the *L. plantarum* viability in the simulated gastric fluid after 3 days of muffin storage; secondly, made muffins with low calorie by replacing part of the sugar. The results suggested the promised potential of yeast cells as wall material for *L. plantarum* encapsulation, and stevia acts as a sugar substitute with the improved capability of probiotic viability.

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