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Effect of microencapsulation of *Lactobacillus* sp. 21C2-10 isolated from cassava pulp on physicochemical, sensorial and microbiological characteristics of ice cream

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Abstract

The characteristics of ice cream containing free and microencapsulated *Lactobacillus* sp. 21C2-10 during frozen storage (-20°C) for 180 days were evaluated. *Lactobacillus* sp. 21C2-10 was microencapsulated by emulsion technique using maltodextrin and gelatine as wall materials. Survival rate (%) of *Lactobacillus* sp. 21C2-10 after exposure to the simulated gastro-intestinal condition of ice cream containing encapsulated cells and free cells of *Lactobacillus* sp. 21C2-10 was evaluated after 180 days of frozen storage. Sensory evaluation of the ice cream was conducted after 1 and 180 days of frozen storage. Ice cream containing microencapsulated cells showed significantly higher survival rate, lower acidity and higher pH value as compared to ice cream containing free cells after storage for 180 days. The addition of microencapsulated cells had no significant effect on the sensorial properties of the ice cream. Following the exposure to simulated gastro-intestinal juices for 5 h, the ice cream containing encapsulated cells showed significantly higher survival rate as compared to ice cream containing free cells. Results indicated that microencapsulation of *Lactobacillus* sp. 21C2-10 by emulsion technique protected the microorganisms during 180 days of frozen storage, and after passage through simulated gastro-intestinal conditions there were no significant effects on the sensorial properties of the ice cream.

Introduction

In recent times, the demand for functional food has increased as consumers are becoming increasingly concern about their health (Mohammadi *et al*., 2011). Functional foods can be defined as food products that have the potential to promote beneficial effects for human health (Sousa *et al*., 2012). The market size for functional food products has rapidly expanded, with probiotics now taking a 30% share (Stanton *et al*., 2005). Probiotics are live microorganisms which, when administered in adequate amounts, confer a health benefit on the host (FAO and WHO, 2002). Most probiotics come from the genera *Bifidobacterium* and *Lactobacillus* (Ouwehand, 2002). Some probiotics have been shown to be beneficial through the competitive exclusion of pathogens by adhesion to human gut mucosa, immune modulation and anti-inflammatory potential, induction of apoptosis pathways and anticarcinogenic activity (Shah, 2007; Burgain *et al*., 2011). Recently, *Lactobacillus* sp. has been used in probiotic food products especially dairy products, such as ice cream and yogurt, to provide beneficial effects (Burgain *et al*., 2011). As a guide for positive health, the International Dairy Federation has recommended that the bacteria be active and be present at a quantity of minimally log 7 CFU/g or CFU/mL (Ouwehand and Salminen, 1998). However, the probiotic bacteria must also be able to survive during production, storage and under the gastrointestinal condition (Cruz *et al*., 2010).

Ice cream could be an alternative food vehicle for carrying probiotic bacteria to customers (Kailasapathy and Sultana, 2003). Ice cream has a neutral pH value and this offers the probability to ensure the viability of probiotic bacteria during storage of the food products (Christiansen *et al*., 1996). However, some authors have documented the loss of viable probiotic bacteria due to the effect of freezing injury (Kailasapathy and Sultana, 2003). Therefore, this food vehicle might not be enough to ensure the viability of probiotic bacteria until reaching the target destination. Thus,

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technologies have been researched for the protection of viable probiotic bacteria using microencapsulation. Microencapsulation helps to separate the probiotic bacteria from the harsh environment. Encapsulation is a technology that enhances the protection of probiotic from damages during processing, storage and ultimately, passage through the gastrointestinal tract (Ding and Shah, 2007). Some studies reported that microencapsulation has often been suggested to increase the viable probiotic cells during the freezing process and frozen storage (Sheu *et al*., 1993). Kebary *et al*. (1998) demonstrated that encapsulated probiotic bacteria survived in frozen milk. Shah (2000) suggested that encapsulation improved the number of viable probiotic bacteria in frozen yogurt and might increase the frozen dairy product's shelf-life. To encapsulate, the probiotic bacteria are loaded into capsules consisted of one or various kinds of wall materials such as alginate, starch, soy protein and gelatine using methods such as emulsification and extrusion (Dong *et al*., 2013). The small size of microcapsules (<100 μm) ensures that they will not negatively affect the sensorial properties of the foods. Nawong *et al*. (2016) stated that the encapsulation in maltrodextrin and gelatine which had been cross-linked by transglutaminase below 100 μm in size increased the survival of *Lactobacillus* sp. 21C2–10 during exposure to simulated gastrointestinal tract conditions. Their results indicated that the microcapsules were appropriate to be added in functional foods as a carrier of viable probiotic bacteria. Therefore, the present work evaluated the physicochemical, sensorial and microbial characteristics of probiotic ice cream containing *Lactobacillus* sp. 21C2-10 in free and microencapsulated forms, using emulsion as the microencapsulation technique, and gelatine and maltodextrin as wall materials during frozen storage at -20°C for 180 days. The survival of the free and microencapsulated *Lactobacillus* sp. 21C2-10 during exposure to simulated gastrointestinal tract conditions was also assessed.

Materials and methods

Bacterial strains and culture conditions

Lactobacillus spp. strain 21C2–10 was isolated from cassava pulp in Nakhon Ratchasima province, Thailand. The probiotic bacteria were grown on deMan Rogosa Sharpe (MRS) agar (Himedia Ltd., Bombay, India) at 37°C for 24 h under anaerobic conditions, and identified using the API 50 CHL test kits (BioMérieux Ltd., Marcy-I'Etoile, France) (Nawong *et al*., 2013). The stock culture of the probiotic bacteria was stored at -20°C until further use.

Preparation of activated probiotic bacteria

Firstly, 1 mL stock culture was inoculated into MRS broth (Himedia Ltd., Bombay, India), and incubated at 37°C for 24 h until the cells reached around log 10 CFU/mL. Following incubation, the probiotic bacteria were harvested by centrifugation at 3,578 g at 4°C for 10 min (Thermo scientific Ltd., Asheville, NC, USA), washed twice with 0.1% (w/v) peptone salt solution (Himedia Ltd., Bombay, India) and separated. The cell pellet was later used in the microencapsulation process.

Preparation of microencapsulated probiotic bacteria

The probiotic bacteria were microencapsulated by an emulsion technique following a modified microencapsulation method described by Nawong *et al*. (2016). Briefly, the stock solutions of 24% (w/v) gelatine (Sigma-Aldrich Ltd., Saint Louis, MO, USA) and 24% (w/v) maltodextrin (Sigma-Aldrich Ltd.) were separately added into 0.5% (w/v) NaCl (Carlo Erba, Val de Reuil, France) and stirred at 45°C for 20 min. Next, 1 g cell pellet was added into the 24% (w/v) maltodextrin with the ratio of probiotic bacteria to maltodextrin of 15:1. Then, gelatine was added into the cell-maltodextrin mixture at a ratio of 2:1. 10 units of TGase (Ajinomoto Ltd., Paris, France) per g of gelatine (unit/g) was added into the cell-maltodextrin-gelatine mixture. Then, the mixture was emulsified in oil containing 0.5% (w/w) Span 85 (Sigma-Aldrich Ltd.) (aqueous:oil; 1:5) and stirred at 900 rpm at room temperature (22–23°C) for 18 h using a magnetic stirrer. The microencapsulated probiotic bacteria were washed with 0.85% (w/v) NaCl and 0.5% (w/v) Tween 80 (Sigma-Aldrich Ltd.), respectively. The microencapsulated probiotic bacteria were harvested with centrifugation at 500 × g at room temperature for 1 min and stored at 4°C.

Preparation of ice cream

Ice cream was prepared using a modified method described by Marshall (2003). All ingredients were weighed separately. The skim milk powder (Miky SMP Co., Wellington, New Zealand) was dissolved in water and then heated to 45°C. Dry ingredients such as, sucrose (Mitrphol Ltd., Khonkaen, Thailand) and stabiliser (Palsgaard Ltd., Juelsmide, Denmark) were dissolved in the skim milk powder solution, and the temperature was increased to 75°C. The butter fat (Elle&Vire Ltd., France) was added to the mixture and pasteurised at 75°C for 15 min. The emulsifier (Tween 80, Sigma-Aldrich Ltd.) was then

added into the mixture. The mixture was stirred and homogenised under 2,500 and 5,000 psi (APV Gaulin Co., Inc., Lawrence, USA) and incubated at 4°C in the refrigerator (PTV 19T/43, Montecchio, Italy) for 12 h. There were three treatments for probiotic inoculation: 1) no added probiotic bacteria, 2) added with 1% (w/w) of free *Lactobacillus* sp. 21C2-10, and 3) added with 1% (w/w) of microencapsulated *Lactobacillus* sp. 21C2-10. After that, all treated ice cream samples were mixed with a hand blender (Taylor Ltd., Rockton, Illinois, USA) for 15 min. The ice cream samples were then packed in plastic cups at 100 g per cup. The ice cream samples were hardened, and storage studies were carried out in a freezer (Kendro Laboratory Products Ltd., Asheville, USA) at -20°C for 180 days. The formulation of each treatment is shown in Table 1.

Table 1. The formulations of each treatment.

Composition (g)	T1	T2	T3
Butter fat	300	300	300
Skim milk powder	330	330	330
Sucrose	300	300	300
Stabilizer	10.5	10.5	10.5
Tween 80	4.5	4.5	4.5
Water	2055	2055	2055
Free cells of *Lactobacillus* sp. 21C2-10 (≈log 10 CFU/g)	–	30	–
Microencapsulated cells of *Lactobacillus* sp. 21C2-10 (≈log 10 log CFU/g)	–	–	30

Enumeration of probiotic bacteria in ice cream

The viability of probiotic bacteria was determined in ice cream samples during frozen storage at -20°C for 180 days. The microencapsulated probiotic bacteria in ice cream sample were released from the microcapsules according to the method proposed by Nawong *et al*. (2016). Briefly, 25 g ice cream sample was resuspended in 0.5 mM $CaCl_2$ (Fisher Scientific Ltd., Ottawa, Ontario, Canada) containing 20 unit/mL collagenase (Sigma-Aldrich Ltd.). The pH was adjusted to 7.4 and stirred for 10 s, and then the ice cream sample was incubated at 37°C for 1 h. The probiotic bacteria were incubated on MRS agar at 37°C for 48 h under anaerobic conditions. The survival of viable probiotic bacteria reported as survival rate (%), which were calculated using the following formula:

$$\%\text{Survival rate} = (\log N_t/\log N_0)*100$$

where $\log N_t$ = viability of probiotic bacteria at time t (log CFU/g), and $\log N_0$ = viability of probiotic bacteria at time 0 (log CFU/g).

Proximate analysis

The ice cream samples were analysed for chemical composition after one day of storage (-20 ± 1°C) following the AOAC (2005) official protocols. Moisture content (AOAC, 925.10), ash (AOAC, 900.02A), protein (AOAC, 928.08), fat (AOAC, 963.15), crude fibre (978.10) and carbohydrate were determined by difference.

Determinations of titratable acidity (%) and pH value

The acidity (expressed as % lactic acid) was determined with a standardised solution of 0.01 M NaOH (Carlo Erba Ltd.). Briefly, 10 g ice cream sample was added with 1 mL phenolphthalein indicator and titrated with NaOH until the light pink was durable. The ice cream's pH value was measured using a pH meter (SI Analytics Ltd., Weilheim, Germany).

Microbiological analysis

Total aerobic count, coliform count and *Escherichia coli* count were determined using Petrifilm Aerobic Count Plate™ and Petrifilm EC™ Count Plates (3M Petrifilm Co., Saint Paul, Minnesota, USA), respectively and incubated at 37°C for 24 h. For Yeast and Mould count, the ice cream samples were spread on Potato Dextrose Agar (PDA) (Himedia Ltd.) and incubated at 30°C for 72 h.

Sensory evaluation

The sensory evaluation of the ice cream samples after 1 and 180 days storage at -20°C were conducted using a 9-point hedonic scale for appearance, flavour, texture and total acceptability (1 = extremely dislike and 9 = extremely like). A group of 30 untrained panellists, (age between 18-35 years old) which were students of Food Technology Department, Suranaree University of Technology, Nakhon Ratchasima, Thailand, were involved. The samples were served approximately 25 g of each ice cream treatment and coded with a three-digit randomised number.

Survival of free and microencapsulated probiotic bacteria in ice cream during exposure to gastro-intestinal conditions

Preparation of simulated gastric juice (SGJ) and simulated intestinal juice (SIJ)

Simulated gastric juice (SGJ) was freshly prepared using a method described by Nawong *et al*. (2016). The SGJ was prepared by adding 0.3 g pepsin (Sigma-Aldrich Ltd.) into 0.2% (w/w) NaCl. The volume was adjusted to 1,000 mL. The pH was adjusted to 2.0, and sterilised.

Simulated intestinal juice (SIJ) was freshly prepared following a method described by Huang and Adams (2004). The SIJ was prepared by adding 8 g pancreatin (Sigma-Aldrich Ltd.) and 36 g bile salts (Fisher Scientific Ltd.) into 0.02 M phosphate buffer. The volume was adjusted to 1,000 mL. The pH was adjusted to 7.4, and sterilised.

Survival of free and microencapsulated probiotic bacteria in ice cream during exposure to simulated gastric juice

Ice cream samples (25 g) were added to 225 mL SGJ tempered at 37°C, mixed and sealed with Parafilm, and incubated for 30, 60, 120 min at 37°C. The survival of probiotic bacteria was enumerated on the MRS agar as earlier described in sub-section '*enumeration of probiotic bacteria in ice cream'*. The survival of viable probiotic bacteria was reported as survival rate (%), which was calculated following the formula provided in the sub-section *'enumeration of probiotic bacteria in ice cream'*.

Survival of free and microencapsulated probiotic bacteria in ice cream during sequential exposure to simulated gastro-intestinal juice

Ice cream samples (25 g) were added to 225 mL SGJ tempered at 37°C, mixed and sealed with Parafilm, and incubated for 60 min at 37°C. Next, 25 mL SIJ tempered at 37°C was added into the mixtures, and pH was adjusted to 7.4. The mixture was adjusted to 500 mL with phosphate buffer and sealed with Parafilm, and incubated for 60, 120, 180 and 240 min at 37°C. Survival of probiotic bacteria was enumerated on the MRS agar as described earlier in the sub-section *'enumeration of probiotic bacteria in ice cream'*. The survival of viable probiotic bacteria was reported as survival rate (%), which was calculated following the formula provided in the sub-section *'enumeration of probiotic bacteria in ice cream'*

The morphology of probiotic bacteria in ice cream samples during sequential exposure to simulated gastro-intestinal juices

Briefly, the samples were received from the *in vitro* simulated gastrointestinal condition assay (in sub-section 'sensory evaluation'), for ice cream containing microencapsulated probiotic bacteria was released by collagenase as described in sub-section *'enumeration of probiotic bacteria in ice cream'*, then centrifuged (5,000 g for 5 min) to separate the cell pellet. The cell pellets were re-suspended in 0.85% (w/v) NaCl. The cell suspensions were centrifuged and fixed with 2% (w/v) glutaraldehyde (Carlo Erba Ltd.) for 24 h at 4°C. The samples were washed three times with buffer for 15 min, post-fixed with 2% (v/v) osmium tetroxide (Carlo Erba Ltd.) for 3 h, and then washed three times with distilled water for 15 min. The samples were dehydrated in ethanol solution at concentrations of 50%, 70%, 90% and finally with 99.5% (v/v) ethanol (Carlo Erba Ltd.). The samples were dropped onto cover slide and air-dried at room temperature. The dried cells were placed on aluminium stubs and coated with gold and observed using a scanning electron microscope (Auriga Ltd., Frankfurt, Germany) at 2.5 kV. Sample images were captured at 10,000× magnification.

Statistical analysis

All experiments were conducted in triplicate (n = 3). The results were statistically analysed using SPSS (version 16.0, SPSS Inc., USA). Data were presented as mean ± standard deviation (SD). The group means were compared by Tukey's post hoc test at 5% significance level.

Results and discussion

The chemical composition of ice cream samples is shown in Table 2. The results show that no significant differences ($p > 0.05$) were observed in treatments T1, T2 and T3 for fat, protein, ash, crude fibre and moisture content.

Survival of free and microencapsulated probiotic bacteria in ice cream during storage time at -20°C for 180 days

Figure 1A shows the survival rate (%) of free and microencapsulated probiotic bacteria in the ice cream samples during 180 days of storage at -20°C. The survival rate (%) of the microencapsulated *Lactobacillus* sp. 21C2-10 in ice cream sample (93.858 ± 0.358%) was significantly ($p < 0.05$) higher than the survival rate of free *Lactobacillus* sp.

Table 2. Chemical composition of probiotic ice cream (%wet basis).

Chemical composition	Fat	Protein	Ash	Crude fibre	Total solid
T1	14.055 ± 0.592[a]	6.519 ± 0.504[a]	0.558 ± 0.025[a]	0.054 ± 0.018[a]	36.839 ± 0.488[a]
T2	14.049 ± 0.524[a]	6.561 ± 0.501[a]	0.549 ± 0.024[a]	0.049 ± 0.014[a]	36.848 ± 0.562[a]
T3	14.051 ± 0.156[a]	6.837 ± 0.369[b]	0.557 ± 0.021[a]	0.052 ± 0.221[a]	37.110 ± 0.980[a]

Value are representatives of means ± standard deviation ($n = 3$). Values followed by different superscripts in the same column are significantly ($p < 0.05$) different

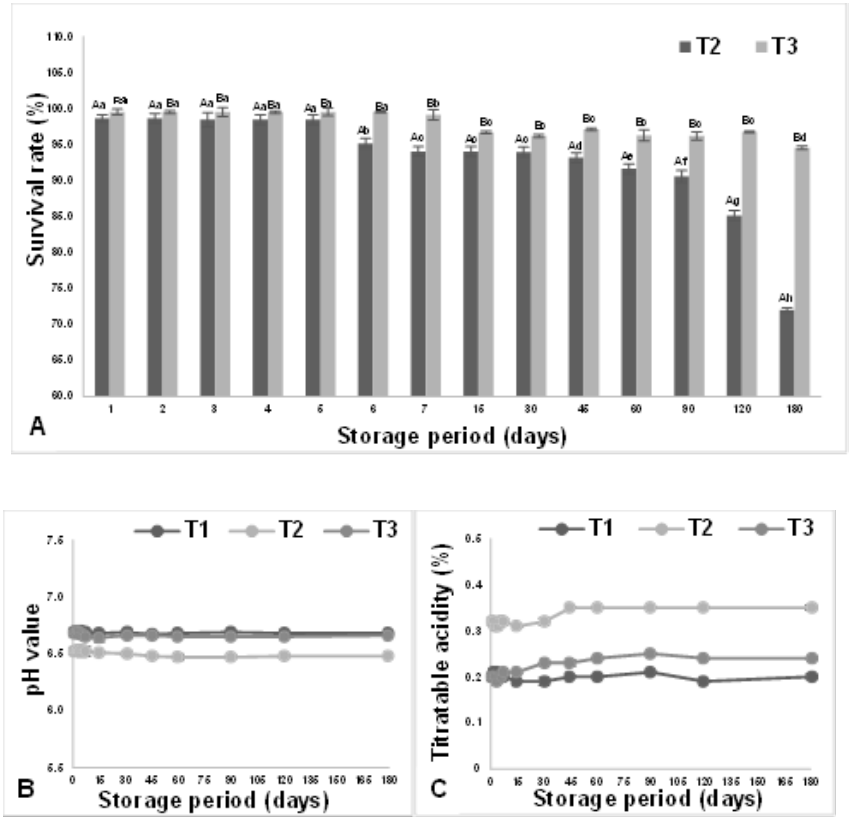


Figure 1. Survival rate (%) (A), pH value (B) and titratable acidity (%) (C) of ice cream without *Lactobacillus* sp. 21C2-10 (T1), ice cream containing free cells of *Lactobacillus* sp. 21C2-10 (T2) and ice cream containing microencapsulated cells of *Lactobacillus* sp. 21C2-10 (T3) during storage time stored at -20ºC. Value are representatives of means ± standard deviation (n = 3). Different superscript capital letters denote significant ($p < 0.05$) differences among ice cream formulations for the same days of storage times. Different superscript small letters denote significant ($p < 0.05$) differences among different days of storage times for the same days of same ice.

21C2-10 in ice cream sample (72.024 ± 0.409%). It was observed that the numbers declined steadily for all the samples as the storage period progressed. The number of free probiotic cells in ice cream dropped substantially (log 0.47 CFU/g) during the first six days of storage. Moreover, the decrease rate was much greater in the free cells, which showed the largest ($p < 0.05$) decrease in their viability after 120 and 180 days (log 1.28, log 2.38 CFU/g, respectively). However, the number of microencapsulated probiotic cells in ice cream dropped substantially (log 0.29 CFU/g) during the first 15 days of storage. The decrease rate was much greater in the free cells which showed the largest ($p < 0.05$) decrease in their viability after 180 days (log 0.53 CFU/g). Therefore, the microencapsulation of probiotic bacteria could increase their viability in ice cream sample. These results are similar to those of other authors. For example, Sheu and Marshall (1993) suggested that entrapped lactobacilli increased the viable probiotic bacteria in ice cream. Homayouni *et al*. (2008) also found that encapsulated probiotic bacteria could enhance the viability in ice cream. Magarinos *et al*. (2007) found that probiotic ice cream containing encapsulated *L. acidophilus* stored at −25°C for 60 days of storage yielded 87% survival rate. Champagne *et al*. (2015) demonstrated that microencapsulated L. rhamnosus had a higher viable count than free cells in ice cream during 210 days of storage. The reduction of probiotic bacteria in ice cream after freezing and during frozen storage might possibly be due to the lethal freezing injury (Kailasapathy and Sultana, 2003). The microcapsules could protect cells from crystallised water thereby providing resistance to freezing damage, and could also protect cells from oxygen toxicity. Microencapsulation helps to separate the probiotic bacteria from a harsh environment (Ding and Shah, 2007). Sheu *et al*. (1993) reported that microencapsulation has often been recommended to increase viable probiotic cells during the freezing process and frozen storage. Some studies demonstrated that microencapsulated probiotic bacteria survived

in frozen milk (Kebary *et al*., 1998). Shah (2000) suggested that encapsulation improved the amount of viable probiotic bacteria in frozen dairy dessert and might increase the frozen dairy products's shelf-life. Consequently, microencapsulation technology could be used to prevent viability loss during the freezing step and during storage periods (Dong *et al*., 2013). However, the neutral pH of ice cream can protect the probiotic bacteria in free forms (Homayouni *et al*., 2008). Further, the number of *Lactobacillus* sp. 21C2-10 (log 8.358 ± 0.214 CFU/g) followed the recommended therapeutic minimum limit of log 7 CFU/g for up to 180 days of storage at -20°C.

pH and titratable acidity (%) value

The results of the pH and titratable acidity (%TA) value for different treatments of ice cream samples during storage time at -20°C are presented in Figures 1B and 1C. The pH and titratable acidity (%) of the ice cream samples were not significantly ($p > 0.05$) different between T1 and T3. However, the pH value of T2 had the lowest value, which was significantly ($p < 0.05$) different as compared to T3 and T1. The titratable acidity (%) (as lactic acid) of T2 had the highest value when compared with T3 and T1. Ice cream is a source of lactose that could produce lactic acid by the probiotic bacteria. Free probiotic bacteria in ice cream during processing could still utilise carbohydrates and produce small amounts of organic acids leading to the lowering of pH of the product during storage (Ding *et al*., 2008). Similarly, the results reported by Kailasapathy and Sultana (2003) suggested that titratable acidity (%) increase after production could be due to the activity of probiotic cells. It is shown that lactic acid bacteria might release enzymes which could convert lactose into lactic acid and increase acidity in dairy food products. However, ice cream containing microencapsulated probiotic bacteria showed minor change. This could be due to the fact that microencapsulation technique with the gel matrix might have reduced the metabolic activity of probiotic in ice cream products thereby no formation of lactic acid occurred as evidenced by the higher pH observed as compared to the pH of the sample with non-encapsulated cells (Akalin *et al*., 2007; Ding *et al*., 2008). However, non-significant changes in pH and titratable acidity of all treatments were observed during storage time (Figure 1B and 1C). Similar results were reported by Moeenf-ard and Tehrani (2008) and Basyigit *et al*. (2006) who found that the titratable acidity of frozen yogurt remained constant during storage at -20°C for 180 days. Alamprese *et al*. (2002) also reported that acidity in ice cream with the addition of microencapsulated *L. johnsonii* stored at -16 and -28°C for 90 days remained unchanged. These studies confirm that adding microencapsulated probiotic bacteria could decrease the activity of probiotic bacteria thereby providing no effect on the total lactic acid of ice cream after production.

Microbiological analysis during storage at -20°C for 180 days

Total aerobic bacterial count, coliform count, *E. coli* count, and yeast and mould count yielded negative results in any of the ice cream samples, therefore no data could be shown.

Sensory evaluation

The sensory scores for ice cream samples after 1 and 180 days of storage are given in Table 3. The sensory scores for T1 and T3 were not significantly ($p > 0.05$) different after 1 and 180 days of storage time. Only T2 showed significantly ($p < 0.05$) lower scores on flavour, taste and total acceptability. This result is similar to Akin (2007) and Fritzen (2013) who reported that frozen dairy dessert containing free probiotic bacteria showed significantly lower scores for flavour and taste, which could be due to low final pH.

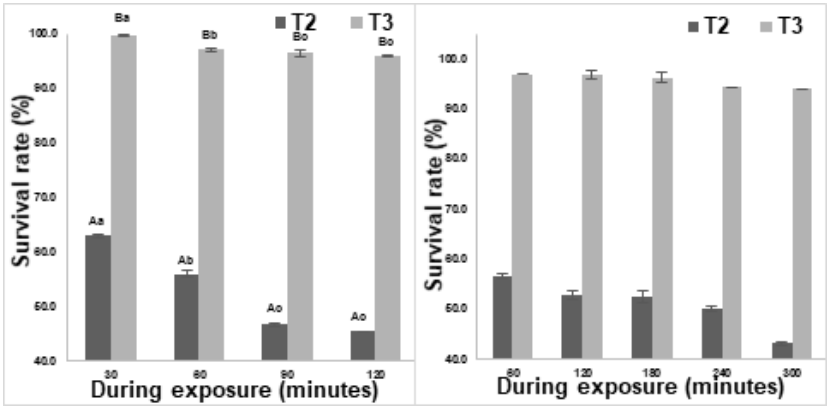
Table 3. Sensory properties of ice creams after 1 and 180 days of frozen storage.

Sensory attributes		During storage time	
		1 day	180 days
Colour and appearance (1-9)	T1	$6.399 \pm 0.061^{a,A}$	$6.378 \pm 0.058^{a,A}$
	T2	$6.375 \pm 0.042^{a,A}$	$6.350 \pm 0.053^{a,A}$
	T3	$6.391 \pm 0.048^{a,A}$	$6.378 \pm 0.057^{a,A}$
Body and texture (1-9)	T1	$6.752 \pm 0.026^{a,A}$	$6.754 \pm 0.023^{a,A}$
	T2	$6.752 \pm 0.034^{a,A}$	$6.739 \pm 0.037^{a,A}$
	T3	$6.739 \pm 0.039^{a,A}$	$6.752 \pm 0.036^{a,A}$
Flavour and taste (1-9)	T1	$6.985 \pm 0.027^{a,A}$	$6.982 \pm 0.028^{a,A}$
	T2	$6.778 \pm 0.035^{b,B}$	$6.793 \pm 0.034^{b,B}$
	T3	$6.970 \pm 0.036^{a,A}$	$6.98 \pm 0.034^{a,A}$
Total acceptability (1-9)	T1	$7.731 \pm 0.075^{a,A}$	$7.718 \pm 0.0277^{a,A}$
	T2	$7.684 \pm 0.034^{b,B}$	$7.673 \pm 0.038^{b,B}$
	T3	$7.720 \pm 0.032^{a,A}$	$7.708 \pm 0.034^{a,A}$

Value are representatives of means ± standard deviation ($n = 30$). Different superscript capital letters denote significant ($p < 0.05$) differences among ice cream formulations for the same days of storage times. Different superscript small letters denote significant ($p < 0.05$) differences among different days of storage times for the same days of same ice cream.

Survival of free and microencapsulated probiotic bacteria in ice cream during sequential exposure to gastro-intestinal juices

The survival of ure 1*Lactobacillus* sp. 21C2-10 during incubation in simulated gastric juices (SGJ)



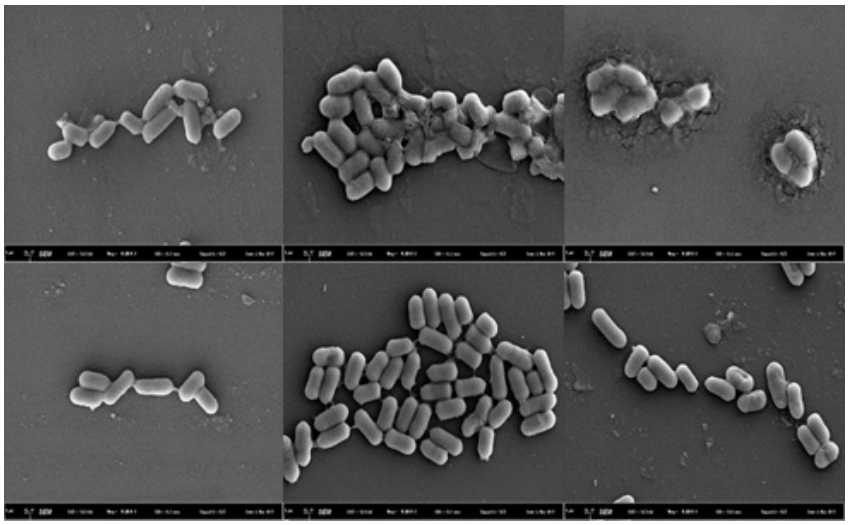


Figure 2. The survival rate (%) of *Lactobacillus* sp. 21C2-10 in ice cream after exposure to the simulated gastric juice at 37°C for 120 minutes under anaerobic conditions (A) and the survival rate (%) of *Lactobacillus* sp. 21C2-10 in ice cream after sequential exposure to the simulated gastric juice for 60 minutes and simulated intestinal juice for 240 minutes at 37°C under anaerobic conditions (B). Scanning electron microscopy showing morphological change of *Lactobacillus* sp. 21C2-10 in ice cream without exposure to the simulated gastric juice (C, F), after exposure to the simulated gastric juice for 60 minutes at 37°C under anaerobic conditions (D, G), after sequential exposure to the simulated gastric juice for 60 minutes and simulated intestinal juice for 240 minutes at 37°C under anaerobic conditions (E, H). Morphological change of *Lactobacillus* sp. 21C2-10 in ice cream containing free cells of *Lactobacillus* sp. 21C2-10 (C, D, E) and ice cream containing microencapsulated cells of *Lactobacillus* sp. 21C2-10 (F, G, H). Ice cream containing free cells of *Lactobacillus* sp. 21C2-10 (T2) and ice cream containing microencapsulated cells of *Lactobacillus* sp. 21C2-10 (T3). Value are representatives of means ± standard deviation (n = 3). Different superscript capital letters denote significant (p < 0.05) differences among ice cream formulations for the same days of storage times. Different superscript small letters denote significant (p < 0.05) differences among different incubation times for the same ice cream.

and under sequential incubation in simulated gastro-intestinal juices are presented in Figures 2A and 2B, respectively. The results showed that the amount of viable *Lactobacillus* sp. 21C2-10 decreased with increasing incubation time. The survival rate (%) of ice cream containing microencapsulated *Lactobacillus* sp. 21C2-10 (95.892 ± 0.198%) was significantly (p < 0.05) higher than ice cream with free *Lactobacillus* sp. 21C2-10 (45.355 ± 0.451%) when exposed to SGJ for 120 min. Figure 2B shows the viability of *Lactobacillus* sp. 21C2-10 in ice cream after exposure to SGJ for 60 min and SGJ for 240 min at 37°C under anaerobic conditions. The results show that the survival rate (%) of ice cream containing encapsulated *Lactobacillus* sp. 21C2-10 (94.018 ± 0.015%) was significantly (p < 0.05) higher than ice cream containing free *Lactobacillus* sp. 21C2-10 (43.201 ± 0.307%) when exposed to SGJ (60 min) and SIJ (240 min). The scanning electron micrographs of *Lactobacillus* sp. 21C2-10 in ice cream samples when exposed to SGJ for 0 min (Figure 2C, 2F), SGJ for 60 min (Figure 2D, 2G) and SGJ/

SIJ for 300 min (Figure 2E, 2H) are presented. These showed morphological changes of the cell surface. Untreated ice cream showed normal morphological structure. For ice cream containing free *Lactobacillus* sp. 21C2-10 during sequential exposure to simulated gastro-intestinal conditions (Figure 2C-E), a lot of the probiotic bacteria had a number of wrinkled structures and shrunken structures on the cell surface, as indicated by black arrows (Figure 2D-E). In contrast, ice cream containing encapsulated probiotics showed the changes of cell surface at the end of the incubation time. Some cells showed shrunken surfaces (Figure 2F-H). The present work has demonstrated that microencapsulation could significantly prevent probiotic bacterial changes in ice cream during exposure to gastrointestinal condition. Ice cream containing free probiotic cells showed the highest loss of probiotic cells during exposure to gastrointestinal condition. Leach *et al*. (1987) suggested that the low pH in SGJ could increase H+ concentration extracellularly. An external pH of close to 2.0 will inhibit enzymes in many kinds of bacteria. Furthermore, bacteria maintain their H+ concentrations using energy (ATP) to actively eliminate protons by way of backward ATPase. This leads to the bacterial cells losing their energy (ATP) source thereby preventing nutrient metabolism, and subsequently death. Zhu *et al*. (2006) suggested that bile salt could be toxic for probiotic bacteria and that many probiotic bacteria has bile salt hydrolases to reduce bile salt (Begley *et al*., 2006). However, microencapsulation helps the survival of *Lactobacillus* sp. 21C2-10 after exposure to SGJ and sequential exposure to SGJ and SIJ. The present work clearly shows that the wall materials prevent the contact of probiotic bacteria with the low pH environment, enzymes and bile salts due to the position of probiotic bacteria inside the microcapsules, the buffering effect of gelatine, and resistance from pepsin by the actions of TGase enzymes that produced iso-peptide control of the enzymatic degradation of gelatine of the microencapsulated cells. However, at SIJ, ice cream containing microencapsulated probiotic SIJ significantly decreased because microcapsule including gelatine precipitated at the pI range 7.0 to 9.0, leading the structure of the microcapsules to be destroyed. Moreover, under longer digestion time, protease could hydrolyse more gelatine. This is similar to Ribeiro *et al*. (2014) who observed that yogurt containing microencapsulated *L. acidophilus* showed more endurance to simulated gastro-intestinal juices than yogurt containing free *L. acidophilus*. Further, Matias *et al*. (2016) reported that ice cream containing free *L. acidophilus* LA-5 showed physiological change when stress was induced on the gastro-intestinal tract (*in vitro* assay). Moreover, Nawong *et al*. (2017) demonstrated that encapsulated probiotic using maltrodextrin and gelatine as wall materials which has been cross-linked by transglutaminase significantly enhanced the viability of *Lactobacillus* spp. after exposure to SGJ and SIJ for 240 min at 37°C. In the present work, the total number of viable probiotic bacteria in the microcapsules (log 8.098 ± 0.124 CFU/g) remained within the values prescribed by the International Dairy Federation.

Conclusion

In conclusion, the present work has indicated that the microencapsulation of *Lactobacillus* sp. 21C2-10 by an emulsion technique using maltrodextrin and gelatine as wall materials did not affect the sensorial properties, pH value and acidity in the ice cream samples, and helped improve the survival of probiotics when exposed to harsh environments as well as effectively protected probiotic bacteria during frozen storage and delivery into the human gastrointestinal tract at higher rate than ice cream containing free probiotic. The number of microencapsulated probiotic bacteria in the ice cream was around log 8.358 ± 0.214 CFU/g at the end of the storage time. This amount of viable probiotic bacteria is higher than the minimum number recommended by the International Dairy Federation (log 7 CFU/g).

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