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Effects of the replacement of cow milk with vegetable milk on probiotics and nutritional profile of fermented ice cream

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ABSTRACT

In the current study, three types of fermented ice cream with *Lactobacillus acidophilus* (La-05) or *Bifidobacterium bifidum* (Bb-12) were prepared from cow (W), soy (S) or coconut (C) milk as well as the combination of cow milk or coconut milk (1 = 25%, 2 = 50% and 3 = 75%) with soy milk (75%, 50% and 25% respectively). La-05 or Bb-12 was inoculated into ice cream mixture and the time required for probiotic to reduce pH to 5.5 was determined during fermentation. The growth rate of La-05 or Bb-12 in all ice cream samples was evaluated after the freezing. In addition, identification and quantification analysis of sugar and free amino acids contents of ice cream samples were also carried out. Based on the results, the pH declined faster in ice cream samples made from vegetables milk than those made from cows' milk. The replacement of cow milk with soy or coconut milk enhanced ($p < 0.05$) the probiotic growth of Bb-12 (1.2 log10 cfu/g) in fermented ice cream compared to cow milk ice cream (0.84 log10 cfu/g). Similarly, La-5 increased ($p < 0.05$) by 1.29 log10 in fermented soy milk ice cream compared to cow milk ice cream (1.09 log10 cfu/g). The composite milk ice cream (75% soy milk with 25% coconut milk; SC1) increased ($p < 0.05$) the growth rate of La-05 and Bb-12 by 1.55 and 1.07 log10 cfu/g respectively. Both soy and coconut milk ice creams provide a richer growth medium of amino acids and sugar content (particularly lactose and sucrose) for Bb-12 and La-05 than cow's milk ice cream. In conclusion, fermented vegetables milk ice cream could be a good vehicle for the delivery of Bb-12 and La-5.

1. Introduction

The consumption of functional foods (FF) is increasing rapidly worldwide because of increased consumers' awareness about the importance of diet and health (Salem, Fathi, & Awad, 2005). FF are foods considered to provide benefits beyond basic nutrition and may play a role in reducing or minimizing the risk of certain diseases and other health conditions. New food products are being developed to include beneficial components such as probiotics and functional components isolated from plants (Shori & Baba, 2011, 2013, 2014). The dairy industry, in particular, has a vast potential to incorporate probiotic cultures into milk for the purpose of development of new functional products (Baba, Najarian, Shori, Lit, & Keng, 2014; Shori, 2013a, 2013b). Probiotic food is defined as a food product that contains viable probiotic microorganisms in sufficient quantities (Shori, 2015). Some of the main health benefits related to probiotics are prevention and treatment of diarrhea, antimicrobial activity, relief of symptoms caused by lactose intolerance, anti-carcinogenic and anti-mutagenic activities, and stimulation of the immune system (Shori, 2015).

Ice cream is a delicious and nutritious frozen dairy product widely consumed in many parts of the world. Ice cream by virtue of milk as its major ingredient has nutritional properties but owns no health benefits (Pandiyan, Annal Villi, Kumaresan, Murugan, & Gopalakrishnamurthy, 2012; Salem et al., 2005). Recently, the increasing demand from consumers for healthier and functional food has led to produce ice cream containing special ingredients with recognized nutritional and physiological properties such as probiotics (Akın, Akın, & Kırmac, 2007; Alamprese, Foschino, Rossi, Pompei, & Savani, 2002), prebiotics (Akalin & Erisir, 2008; Akın et al., 2007; Leandro, Araújob, Conceicão, Moraesa, & Carvalhoc, 2013) and natural antioxidants (Soukoulis, Lebesi, & Tzia, 2009). Consumption ice cream containing probiotic strains can reduce bacteria levels in the mouth responsible for tooth decay (Çaglar

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et al., 2008), inhibit the growth of potential pathogens (Singh, Damle, & Chawla, 2011), improve the intestinal microflora and activate the immune system (Ranadheera, Evans, Adams, & Baines, 2012).

The main ingredient of ice cream is cow milk and this unfortunately may make dairy ice cream off limits to many consumers who suffer from lactose intolerance. Thus, replacing cow's milk with vegetables milk in general would help address two nutritional issues related to cow's milk: lactose intolerance and high cholesterol content. Several researchers have used vegetable milk such as soy milk to produce probiotic products with nutritional and health properties (Aboulfazli, Baba, & Misran, 2014; Bisla, Archana, & Sharma, 2011; Heenan, Adams, Hosken, & Fleet, 2004; Hermanto & Masdiana, 2011). Soy milk found to be good substrate for commonly used probiotics i.e. *bifidobacterium* and *lactobacillus* species (Farnworth et al., 2007; Wang, Yu, & Chou, 2004). The replacement of cow milk with soybean extract is known to improve the pH of probiotic ice cream for increased survival of probiotics (Heenan et al., 2004). In addition, the lecithin of soybean extract may act as emulsifier and thus provide physical protection against freezing damage and acidic gastric condition by encapsulating probiotics with their lecithin and proteins (Akesowan, 2009). The soy proteins are also able to form a stable network looks like a gel structure (Akesowan, 2009). The raw bean flavour limits the wide consumption of soybean extract and other soybean products (Wang et al., 2004). However, this could be reduced by fermenting soybean extract with *Lactobacillus acidophilus* (Desai, Small, McGill, & Shah, 2002).

Coconut milk is another vegetable extract that may be used to replace cow milk in making probiotic ice cream. It is a popular substitute for cow's milk in the tropics because it is simple to prepare, highly digestible and contains an abundance of nutrients (Wangcharoen, 2012). Coconut milk is rich in minerals (calcium, phosphorus and potassium), vitamins (vitamins C, E and many B vitamins), protein (rich in glutamic acid, aspartic acid, and arginine), lipid and antioxidants (Yuliana, Rangga, & Rakhmia, 2010). The fatty acids (high oleic and lauric acid) in coconut milk are instrumental in preventing arteriosclerosis (Belewu & Belewu, 2007).

Since probiotics generally do not grow rapidly in cow milk, the application of probiotic cultures in fermented ice cream made from cow milk represents a great challenge. Thus, ice creams made with soy and coconut milk can support the growth of probiotics by fulfilling the microbes growth requirement for amino acids and/or carbohydrates (Farnworth et al., 2007). Various species of genera Lactobacillus and Bifidobacterium have been incorporated into dairy and non-dairy products over the years to study the effect of food vehicle on the survivability and functionality of probiotics. The Lactobacillus and Bifidobacterium genera are most commonly studied genera and have played an extensive role as probiotics because of their association with healthy human intestinal tract and specifically in the case of Lactobacillus, due to their association with fermented foods (Shori, 2015). Lactic acid bacteria (LAB) are fastidious microorganisms with regard to nutritional requirements (Guarner et al., 2005). LAB displays a great capacity to reduce the concentration of different carbohydrates and related compounds with the accumulation of lactic acid as the predominant end-product (>50% of sugar carbon). Furthermore, *L. acidophilus* (La-05) possesses a complex system of proteinases and peptidases which increase the availability of peptides and amino acids required for bacterial growth (Donkor, 2007). Since probiotics need to use some amino acids and peptides for their cell growth and survival and hence the total amino acid content in fermented milk reflects the balance between assimilation and proteolysis by bacteria (Donkor, 2007). In the current study, changes in the growth of *L. acidophilus* (La-05) or *Bifidobacterium bifidum* (Bb-12) in three types of non-fermented (pH 7) and fermented (pH 5.5) ice cream made from cow, soy or coconut milk and the combination of cow milk or coconut milk with soy milk were evaluated after the freezing. In addition, identification and quantification of the changes in sugar and free amino acids contents of non-fermented and fermented ice cream samples were also carried out.

2. Materials and methods

2.1. Materials and chemicals

Fresh cow milk, skim milk powder (Dutch lady, Malaysia), soybean, soy oil, coconut fruit, butter, sugar and vanilla were purchased from local market. Cremodan SE 734 veg (Danisco AS, Copenhagen, Denmark, containing mono- and diacyl-glycerols of fatty acid, cellulose gum, guar gum, carrageenan) was used as stabilizer. *B. bifidum* (Bb-12) and *L. acidophilus* (La-05) were obtained as pure freeze-dried probiotic culture from CHR-Hansen (Horsholm, Denmark). 99% amino acid standards (including alanine, arginine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, glycine, histidine, hydroxyproline, leucine, isoleucine, lysine, methionine, phenylalanine, proline, serine, threonine, tyrosine, tryptophan and valine), and sugar standards (including lactose, glucose, fructose, galactose, sucrose, stachyose and raffinose) were all obtained from Sigma Chemical Company (St. Louis, Missouri, USA). Cysteine hydrochloride (L-Cys-HCl), MRS agar, anaerocult A sachets, formic acid (98%) and acetic acid (glacial) were obtained from Merck Company (New Jersey, USA).

2.2. Preparation of soy milk with 12% total solid

Soybeans (10 g) were washed three times using tap water one-time rinsing using de-ionized water followed by soaking in de-ionized water (1 L) for 14 h at room temperature (Wang, Yu, Yang, & Chou, 2003). Excess water was then drained off and the shells were removed. The swollen beans were blended with 250 mL of boiling water in a laboratory blender (Waring, New Hartford, CT, USA) at low speed followed by boiling for 5 min. The blended soybean was then passed through 4 layers of cheesecloth. The soy milk fat content (1.86%) was corrected to 3.4% using 1.54 g soy oil/100 g soy milk. The soy milk was reheated to 80 °C for 10 min and immediately chilled (4 °C) prior to making ice cream.

2.3. Preparation of coconut milk 12% total solid

The brown hard coconut shell was cracked open and the white copra was grated followed by mechanical pressing to obtain the milk (Soler, 2005). To achieve 8% fat coconut milk, 300 g of fresh coconut milk (after sieving with double layers of cheesecloth) was mixed with 700 g of distilled water. The diluted coconut milk was heated at 80 °C for 10 min prior to chilling at 4 °C and was used within 1 h.

2.4. Starter culture

The starter culture was prepared as described by Magarinos, Selaive, Costa, Flores, and Pizarro (2007). Each strain of La-05 or Bb-12 (1 g) was cultured in 100 mL of sterilized skimmed milk (10 w/v). To facilitate the activation of these cultures, 0.05% (wt/vol) L-Cys-HCI was added to the milk in order to diminish the redox potential of the medium and thereby stimulate micro-organism growth. Glucose (2%; wt/vol) and yeast extract (1%; wt/vol) were also added to the mixture. The incubation was carried out under aerobic condition in the water bath (42 °C; Julabo, Model Sw-21c or Haake Model SWD

Table 1
The percentage of milk formula and butter used in ice cream preparation.

Sample	Milk formula (%)	Butter (%)
W	100% cow milk	10.37
C	100% coconut milk	7.31
S	100% soy milk	10.37
SW1	75% soy milk + 25%cow milk	10.37
SW2	50% soy milk + 50% cow milk	10.37
SW3	25% soy milk + 75%cow milk	10.37
SC1	75% soy milk + 25% coconut milk	9.6
SC2	50% soymilk + 50% coconut milk	8.84
SC3	25% soy milk + 75% coconut milk	8.08

20, Germany) until pH was reduced to 5.0. The cultures were kept frozen until required for ice-cream manufacture. Before inoculation to ice-cream mixture, each frozen culture combination (4 mL) had 100 mL of sterilized skimmed milk added prior to incubated at 42 °C until pH reduced to 5.0 (Magarinos et al., 2007).

2.5. Preparation of ice cream

The probiotic ice cream was prepared as described by Akın et al. (2007). Nine ice cream formulations were prepared using cow, soy or coconut milk and combinations of coconut milk or cow milk with soy milk. The nine milk formulations with butter (Table 1) were heated to 50 °C prior to mixing with the skim milk powder (7%), sugar (17%), water (9.62%) and stabilizer (0.6%). The mixtures were homogenized for 10 min at 30,000 g (16,000 rpm, 70 °C; Ika Homogenizer T-25 basic Ultra Turrax, Germany) prior to pasteurization at 80 °C for 10 min and then cooled to 4 °C overnight. Each mixture was inoculated with 4% (w/w) starter culture (La-05 or Bb-12) and vanilla (0.1%) was also added to enhance aroma development followed by thorough gentle mixing. The inoculated ice cream mixture was then equally divided into two portions. The first portion (non-fermented ice cream, pH 7) was immediately frozen in 1.5 L batch ice cream maker (Baumatic gelato1ss, UK; rotor speed 50 round/min, 40 min, −30 °C). The second portion (fermented ice cream; pH 5.5) was incubated in the water bath at 42 °C until pH was reduced to 5.5. After fermentation, the ice cream mixtures were cooled to 4 °C in an ice bath followed by freezing in 1.5 L batch ice cream maker. All fresh ice cream samples were packed in 100 mL plastic cups and covered using the lids before storage at −20 °C in the freezer (Fig. 1). All fresh samples (0 day) were performed in triplicate. The chemical composition of each 100 g of ice cream samples (0 day) contains 43% total solids, ~10% fat and 27% lactic acid (Table 2).

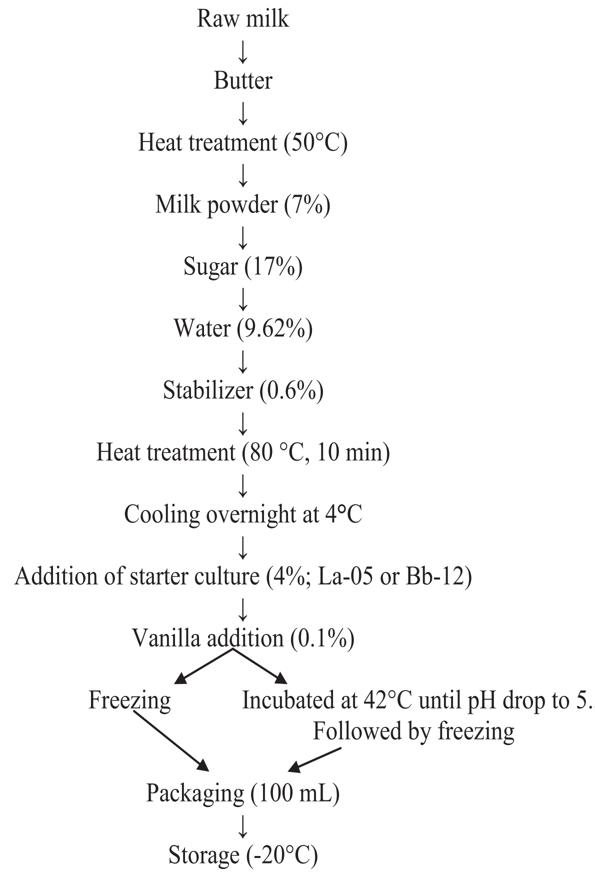

Fig. 1. Production of probiotic ice-cream.

Table 2
Physicochemical properties of fermented ice creams.

Samples[A]	Total solids (g 100 g^{-1})[B]	Fat (g 100 g^{-1})[B]	pH (value)[B]	Titratable acidity (% lactic acid)[B]
WL	43.91 ± 0.08[a]	10.40 ± 0.05[a]	5.50 ± 0.01[a]	0.27 ± 0.006[a]
CL	43.16 ± 0.07[a]	10.40 ± 0.04[a]	5.50 ± 0.01[a]	0.27 ± 0.004[a]
SL	43.94 ± 0.08[a]	10.40 ± 0.03[a]	5.51 ± 0.01[a]	0.27 ± 0.003[a]
SW1L	43.23 ± 0.15[a]	10.50 ± 0.05[a]	5.50 ± 0.02[a]	0.27 ± 0.006[a]
SW2L	43.42 ± 0.17[a]	10.40 ± 0.04[a]	5.49 ± 0.01[a]	0.36 ± 0.004[a]
SW3L	43.66 ± 0.15[a]	10.30 ± 0.05[a]	5.50 ± 0.01[a]	0.27 ± 0.003[a]
SC1L	43.62 ± 0.10[a]	10.40 ± 0.03[a]	5.50 ± 0.03[a]	0.27 ± 0.009[a]
SC2L	42.79 ± 0.12[a]	10.50 ± 0.02[a]	5.51 ± 0.01[c]	0.27 ± 0.008[a]
SC3L	43.21 ± 0.11[a]	10.50 ± 0.02[a]	5.5 ± 0.01[a]	0.27 ± 0.005[a]
WB	43.91 ± 0.08[a]	10.50 ± 0.04[a]	5.50 ± 0.01[a]	0.27 ± 0.006[a]
CB	43.16 ± 0.07[a]	10.40 ± 0.05[a]	5.50 ± 0.01[a]	0.27 ± 0.004[a]
SB	43.94 ± 0.08[a]	10.50 ± 0.02[a]	5.51 ± 0.01	0.27 ± 0.003[a]
SW1B	43.23 ± 0.15[a]	10.40 ± 0.04[a]	5.50 ± 0.02[a]	0.27 ± 0.006[a]
SW2B	43.42 ± 0.17[a]	10.30 ± 0.05[a]	5.49 ± 0.01[a]	0.27 ± 0.004[a]
SW3B	43.66 ± 0.15[a]	10.50 ± 0.02[a]	5.50 ± 0.01[a]	0.27 ± 0.003[a]
SC1B	43.62 ± 0.10[a]	10.30 ± 0.02[a]	5.52 ± 0.03[a]	0.27 ± 0.009[a]
SC2B	42.79 ± 0.12[a]	10.50 ± 0.01[a]	5.50 ± 0.01[a]	0.27 ± 0.008[a]
SC3B	43.21 ± 0.11[a]	10.40 ± 0.01[a]	5.51 ± 0.01[a]	0.27 ± 0.005[a]

[A] Samples inoculated with La-05 and made with 100% cow milk: WL; 100% coconut milk: CL; 100% soy milk: SL; 75% soy + 25% cow milk: SW1L; 50% soy + 50% cow milk: SW2L; 25% soy + 75% cow milk: SW3L; 75% soy + 25% coconut milk: SC1L; 50% soy + 50% coconut milk: SC2L; 25% soy + 75% coconut milk: SC3L.
Samples inoculated with Bb-12 made using 100% cow milk: WB; 100% coconut milk: CB; 100% soy milk: SB; 75% soy + 25% cow milk: SW1B; 50% soy + 50% cow milk: SW2B; 25% soy + 75% cow milk: SW3B; 75% soy + 25% coconut milk: SC1B; 50% soy + 50% coconut milk: SC2B; 25% soy + 75% coconut milk: SC3B.
[B] Means values ± standard deviation.
[a] Means in the same column followed by same letters are not significantly different ($p < 0.05$).

Table 3
Detailed MRM conditions based on retention time compared with reference standard.

Name	Molecular weight	Ionized mass	Specific fragment ions
Sucrose	342.3	341.3	179
Lactose	342.1	341.1	179
Galactose	180.1	179.1	113
Glucose	180.2	179.2	113
Fructose	179.9	178.8	113
Raffinose	594.5	593.5	–
Staychose	666.58	665.58	–

2.6. Bacteriological analysis

The viable cell counts (VCC) of *L. acidophilus* (La-05) and *B. bifidum* (Bb-12) were measured in non-fermented and fermented ice cream. The samples (10 g) were decimally diluted with sterile peptone water (1 g/L). One millilitre aliquot dilutions were pour-plated in triplicate on MRS agar for La-05 and MRS agar supplemented with 0.05% (w/v) L-Cys-HCl for Bb-12. The plates were incubated at 38 ± 1 °C for 72 h under aerobic condition with 5% CO_2 (v/v) for La-05 and anaerobic condition (Anaerocult A) for Bb-12 (Magarinos et al., 2007).

2.7. Sugars content determination

Sugars content in ice cream samples were determined as described by Kumaguai (2001). Ice cream samples were homogenized by ultra turrax and sub-samples of the homogenate were stored at −20 °C in high density polyethylene bottles with plastic screw cap lids. One gram of each homogenized samples were mixed with 10 mL of 0.2 mM acetic acid followed by centrifuging at 10,000 rpm (11,952 g) for 5 min at −5 °C. The clear supernatants were quantitatively transferred into ampoules. The supernatant was filtered through 0.22 μm nylon syringe filter prior to LC/MS analysis. The LC/MS analytical system consists of HPLC system combining an autosampler, temperature-controlled column oven, binary pump and Tandem Mass Spectrometry MS/MS with an electrospray ionisation source (ESI) (Perkin Elmer UHPLC Flexar 15 with AB Sciex QTrap 3200 MS/MS). The analytical separation of samples was performed on Agilent Zorbax RP C18, (150 mm × 4.6 mm, 5um) using a gradient elution of water and acetonitrile with 0.1% formic acid and 5 mM ammonium formate at a flow rate of 0.8 mL/min. Data acquisitions were performed in the multiple reaction monitoring (MRM) mode using the interface parameters. Other Conditions were drying gas (N_2) flow of 40 psi, drying gas and vaporizer temperatures of 500 °C, nebulizer capillary voltage of 4.5 kV, selective collision energy and declustering potential for each sugar compounds. Multiple reactions monitoring (MRM) scan in negative ionisation which is a highly selective and sensitive method was used to analyse each sugar with their mass and fragments. Identified peaks were quantified using authentic standards. In general, the parent masses for all sugar with its respective fragment masses were targeted and the positive identification was resulted in a positive chromatographic peak. Further confirmation is based on retention time compared with reference standard (Table 3).

2.8. Free amino acid determination

The free amino acid content of non-fermented and fermented ice cream was determined. Samples were treated to eliminate proteins and the amino acids were extracted as described by Ozcan and Senyuva (2006). Ice cream samples were homogenized and subsequently 1 g of each homogenized samples were mixed with 10 mL of 0.2 mM acetic acid. The mixtures were centrifuged at 5000 rpm (2810 g) for 10 min at −5 °C and the supernatants were filtered through 0.22 μm-pore diameter filter. The amino acids (99%) used as standards were prepared in the same way as described above. Liquid chromatography/atmospheric pressure chemical ionization mass spectrometry (LC/APCI-MS) analysis was used for the screening and quantification of different free amino acids. For this purpose, the HPLC system combining an autosampler, temperature-controlled column oven and binary pump coupled to MS detector equipped with atmospheric pressure chemical ionization (APCI) was used. The analytical separation of samples was performed on Zorbax Bonus-RP, Narrow Bore (100 mm 2.1 mm, 3.5 mm) using an isocratic mixture of 0.01 mM acetic acid in 0.2% aqueous solution of formic acid at a flow rate of 0.2 mL/min. Data acquisitions were performed in the selected ion monitoring (SIM; positive ion mode) mode using the interface parameters. Other conditions were drying gas (N2) flow of 4 L/

Table 4
The viable cell counts (Log10 cfu/g) of La-05 (L) and Bb-12 (B) in non-fermented and fermented ice cream samples.

Samples	VCC of probiotics in non-fermented ice cream (pH 7) (Log10 cfu/g)[A]	Time required for pH 5.5 (h)[B]	VCC of probiotics in fermented ice cream (pH 5.5) (Log10 cfu/g)[A]	Growth rate (Log10 cfu/g)[C]
SL	7.11 ± 0.08	8.48^{g}	8.40 ± 0.05	1.29^{b}
WL	7.20 ± 0.07	10.18^{e}	8.29 ± 0.04	1.09^{d}
CL	7.16 ± 0.04	6.20^{i}	8.30 ± 0.06	1.14^{c}
SC1L	7.21 ± 0.04	8.20^{g}	8.76 ± 0.07	1.55^{a}
SC2L	7.19 ± 0.06	7.40^{h}	8.18 ± 0.09	0.99^{e}
SC3L	7.27 ± 0.07	6.20^{k}	7.73 ± 0.08	0.46^{i}
SW1L	7.07 ± 0.05	8.48^{i}	8.33 ± 0.09	1.26^{b}
SW2L	7.12 ± 0.03	9.25^{f}	8.04 ± 0.08	0.92^{e}
SW3L	7.26 ± 0.08	9.25^{f}	8.13 ± 0.07	0.87^{f}
SB	7.53 ± 0.09	11.13^{d}	8.76 ± 0.09	1.23^{b}
WB	7.21 ± 0.08	18.10^{a}	8.05 ± 0.10	0.84^{g}
CB	7.45 ± 0.09	10.50^{e}	8.70 ± 0.09	1.25^{b}
SC1B	7.50 ± 0.05	10.50^{e}	8.57 ± 0.06	1.07^{d}
SC2B	7.50 ± 0.02	10.50^{e}	8.57 ± 0.08	1.07^{d}
SC3B	7.47 ± 0.04	10.50^{e}	8.59 ± 0.05	1.12^{c}
SW1B	7.52 ± 0.06	11.30^{d}	8.32 ± 0.08	0.80^{g}
SW2B	7.40 ± 0.09	13.56^{c}	8.19 ± 0.09	0.79^{g}
SW3B	7.46 ± 0.03	16.20^{b}	8.05 ± 0.04	0.59^{h}

[A] Means values ± standard deviation.
[B] Time required for probiotics ice cream to reach pH 5.5 during fermentation.
[C] The difference in the VCC of probiotics between non-fermented and fermented ice cream.
* Means in the same column followed by different letters were significantly different ($p < 0.05$).

Table 5
Sugar contents (mg/mL) in non-fermented ice creams.

Samples	Sugars							Total (mg/mL)
	Raffinose (mg/mL)	Stachyose (mg/mL)	Sucrose (mg/mL)	Lactose (mg/mL)	Galactose (mg/mL)	Glucose (mg/mL)	Fructose (mg/mL)	
S	<LoD	0.192 ± 0.04^{a}	5.70 ± 0.07^{a}	2.37 ± 0.08^{c}	0.026 ± 0.01^{a}	0.018 ± 0.01^{a}	0.034 ± 0.02^{a}	8.34 ± 0.05^{a}
C	<LoD	<LoD	2.80 ± 0.03^{e}	2.38 ± 0.06^{c}	<LoD	0.011 ± 0.01^{a}	<LoD	5.19 ± 0.03^{d}
W	<LoD	<LoD	2.53 ± 0.04^{f}	4.80 ± 0.07^{a}	0.020 ± 0.01^{a}	0.016 ± 0.01^{a}	0.017 ± 0.01^{a}	7.38 ± 0.08^{b}
SC1	<LoD	0.077 ± 0.04^{b}	4.91 ± 0.08^{b}	2.42 ± 0.17^{c}	0.013 ± 0.01^{a}	0.021 ± 0.02^{a}	0.022 ± 0.01^{a}	7.46 ± 0.07^{b}
SC2	<LoD	0.045 ± 0.03^{d}	2.95 ± 0.04^{d}	2.10 ± 0.07^{c}	0.018 ± 0.01^{a}	0.02 ± 0.03^{a}	0.024 ± 0.01^{a}	5.16 ± 0.04^{d}
SC3	<LoD	0.041 ± 0.02^{d}	2.42 ± 0.07	1.94 ± 0.12^{c}	<LoD	0.001 ± 0.01^{a}	<LoD	4.40 ± 0.05^{e}
SW1	<LoD	0.058 ± 0.04^{d}	3.28 ± 0.04^{c}	1.99 ± 0.07^{c}	0.015 ± 0.01^{a}	0.017 ± 0.01^{a}	0.027 ± 0.01^{a}	5.39 ± 0.06^{c}
SW2	<LoD	0.020 ± 0.04^{d}	2.90 ± 0.05^{d}	2.18 ± 0.02^{c}	<LoD	0.022 ± 0.01^{a}	0.027 ± 0.01^{a}	5.15 ± 0.03^{d}
SW3	<LoD	<LoD	1.71 ± 0.02^{g}	3.47 ± 0.07^{b}	<LoD	0.003 ± 0.01^{a}	<LoD	5.18 ± 0.06^{d}

$^{a-g}$ Means in the same column followed by different letters were significantly different($p < 0.05$).

Table 6
Sugar contents (mg/mL) in fermented ice creams.

Samples	Sugars[A]							Total (mg/mL)
	Raffinose (mg/mL)	Stachyose (mg/mL)	Sucrose (mg/mL)	Lactose (mg/mL)	Galactose (mg/mL)	Glucose (mg/mL)	Fructose (mg/mL)	
SB	<LoD	0.122 ± 0.06^{a}	4.88 ± 0.08^{a}	1.22 ± 0.11^{h}	0.159 ± 0.07^{a}	0.174 ± 0.08^{a}	0.171 ± 0.08^{a}	6.73 ± 0.07^{a}
CB	<LoD	<LoD	2.29 ± 0.07^{f}	2.00 ± 0.07^{b}	0.074 ± 0.04^{a}	0.073 ± 0.04^{a}	0.090 ± 0.06^{a}	4.53 ± 0.08^{c}
WB	<LoD	<LoD	2.05 ± 0.04^{g}	2.42 ± 0.09^{a}	0.078 ± 0.03^{a}	0.089 ± 0.06^{a}	0.111 ± 0.07^{a}	4.75 ± 0.07^{c}
SC1B	<LoD	0.027 ± 0.01^{b}	3.11 ± 0.09^{c}	1.19 ± 0.08^{h}	0.023 ± 0.02^{b}	0.031 ± 0.02^{a}	0.034 ± 0.03^{a}	4.41 ± 0.09^{c}
SC2B	<LoD	0.015 ± 0.01^{b}	1.17 ± 0.17^{k}	1.70 ± 0.06^{d}	0.030 ± 0.01^{a}	0.036 ± 0.02^{a}	0.034 ± 0.0^{2a}	2.99 ± 0.06^{e}
SC3B	<LoD	0.021 ± 0.02^{b}	1.92 ± 0.11^{h}	1.62 ± 0.09^{e}	0.060 ± 0.02^{a}	0.080 ± 0.04^{a}	0.083 ± 0.06^{a}	2.78 ± 0.05^{e}
SW1B	<LoD	0.018 ± 0.01^{b}	2.03 ± 0.04^{g}	1.59 ± 0.07^{e}	0.021 ± 0.01^{b}	0.030 ± 0.01^{a}	0.032 ± 0.02^{a}	3.72 ± 0.08^{d}
SW2B	<LoD	0.004 ± 0.00^{c}	1.38 ± 0.06^{j}	1.83 ± 0.07^{c}	0.023 ± 0.01^{b}	0.026 ± 0.01^{b}	0.032 ± 0.01^{a}	3.29 ± 0.09^{d}
SW3B	<LoD	<LoD	0.88 ± 0.08^{k}	2.42 ± 0.08^{a}	0.029 ± 0.01^{b}	0.038 ± 0.02^{a}	0.045 ± 0.03^{a}	3.41 ± 0.08^{d}
SL	<LoD	0.172 ± 0.07^{a}	3.71 ± 0.07^{b}	1.46 ± 0.17^{f}	0.079 ± 0.03^{a}	0.081 ± 0.04^{a}	0.087 ± 0.04^{a}	5.59 ± 0.07^{b}
CL	<LoD	<LoD	2.64 ± 0.08^{d}	1.57 ± 0.07^{e}	0.015 ± 0.01^{a}	0.024 ± 0.01^{b}	0.031 ± 0.02^{a}	4.28 ± 0.06^{c}
WL	<LoD	<LoD	2.46 ± 0.07^{e}	2.02 ± 0.07^{b}	0.025 ± 0.01^{a}	0.036 ± 0.01^{a}	0.038 ± 0.03^{a}	4.58 ± 0.07^{c}
SC1L	<LoD	0.047 ± 0.02^{b}	2.17 ± 0.06^{f}	1.34 ± 0.07^{g}	0.093 ± 0.06^{a}	0.104 ± 0.06^{a}	0.106 ± 0.08^{a}	3.86 ± 0.07^{d}
SC2L	<LoD	0.015 ± 0.01^{b}	1.90 ± 0.05^{h}	1.10 ± 0.07^{i}	0.085 ± 0.05^{a}	0.101 ± 0.06^{a}	0.111 ± 0.07^{a}	3.31 ± 0.06^{d}
SC3L	<LoD	0.038 ± 0.01^{b}	1.70 ± 0.07^{i}	1.87 ± 0.07^{c}	0.069 ± 0.04^{a}	0.082 ± 0.05^{a}	0.091 ± 0.06^{a}	3.85 ± 0.07^{d}
SW1L	<LoD	0.038 ± 0.01^{b}	2.66 ± 0.08^{d}	1.85 ± 0.07^{c}	0.143 ± 0.07^{a}	0.093 ± 0.04^{a}	0.163 ± 0.07^{a}	4.95 ± 0.09^{c}
SW2L	<LoD	0.016 ± 0.01^{b}	2.87 ± 0.09^{c}	1.53 ± 0.04^{e}	0.082 ± 0.05^{a}	0.148 ± 0.07^{a}	0.097 ± 0.06^{a}	4.74 ± 0.08^{c}
SW3L	<LoD	<LoD	1.70 ± 0.06^{i}	2.37 ± 0.07^{a}	0.010 ± 0.01^{b}	0.017 ± 0.07^{b}	0.018 ± 0.01^{a}	4.11 ± 0.10^{c}

$^{a-j}$ Means in the same column followed by different letters were significantly different ($P < 0.05$).
LoD = limit of detection.

Table 7
Changes in sugar content (mg/mL) in fermented ice cream with La-05 (L) and Bb-12 (B).

Samples	Changes of sugars							Total (mg/mL)
	Raffinose (mg/mL)	Stachyose (mg/mL)	Sucrose (mg/mL)	Lactose (mg/mL)	Galactose (mg/mL)	Glucose (mg/mL)	Fructose (mg/mL)	
SB	na	0.070^{a}	0.82^{a}	1.15^{d}	-0.133^{a}	-0.156^{a}	-0.137^{a}	1.61^{f}
CB	na	na	0.51^{a}	0.38^{g}	-0.074^{a}	-0.062^{a}	-0.090^{a}	0.66^{h}
WB	na	na	0.48^{a}	2.38^{a}	-0.058^{a}	-0.073^{a}	-0.094^{a}	2.63^{c}
SC1B	na	0.050^{a}	1.80^{a}	1.23^{c}	-0.010^{a}	-0.010^{a}	-0.012^{a}	3.05^{a}
SC2B	na	0.030^{a}	1.78^{a}	0.40^{g}	-0.011^{a}	-0.016^{a}	-0.010^{a}	2.17^{d}
SC3B	na	0.020^{a}	0.50^{a}	1.32^{b}	-0.060^{a}	-0.079^{a}	-0.080^{a}	1.62^{f}
SW1B	na	0.040^{a}	1.25^{a}	0.40^{g}	-0.007^{a}	-0.013^{a}	-0.005^{a}	1.66^{f}
SW2B	na	0.020^{a}	1.52^{a}	0.35^{g}	-0.023^{a}	-0.004^{a}	-0.005^{a}	1.86^{d}
SW3B	na	na	0.83^{a}	1.05^{e}	-0.029^{a}	-0.035^{a}	-0.045^{a}	1.77^{e}
SL	na	0.020^{a}	1.99^{a}	0.91^{f}	-0.053^{a}	-0.062^{a}	-0.053^{a}	2.75^{b}
CL	na	na	0.16^{a}	0.81^{f}	-0.015^{a}	-0.013^{a}	-0.031^{a}	0.91^{a}
WL	na	na	0.07^{a}	2.78^{a}	-0.005^{a}	-0.019^{a}	-0.020^{a}	2.80^{b}
SC1L	na	0.030^{a}	2.74^{a}	1.08^{e}	-0.072^{a}	-0.084^{a}	-0.084^{a}	3.61^{a}
SC2L	na	0.030^{a}	1.05^{a}	1.00^{e}	-0.075^{a}	-0.081^{a}	-0.088^{a}	1.84^{d}
SC3L	na	0.003^{b}	0.72^{a}	0.07^{i}	-0.069^{a}	-0.081^{a}	-0.091^{a}	0.55^{h}
SW1L	na	0.020^{a}	0.62^{a}	0.14^{g}	-0.128^{a}	-0.131^{a}	-0.137^{a}	0.38^{h}
SW2L	na	0.010^{a}	0.03^{a}	0.65^{f}	-0.082^{a}	-0.071^{a}	-0.071^{a}	0.47^{h}
SW3L	na	na	0.01^{a}	1.10^{d}	-0.010^{a}	-0.014^{a}	-0.018^{a}	1.07^{g}

$^{a-i}$ Means in the same column followed by different letters were significantly different ($p < 0.05$).
na = not applicable.

min, drying gas and vaporizer temperatures of 320 °C, nebulizer capillary voltage of 3 kV, corona current of 8 mA, fragmentor voltage of 55 eV and pressure of 55 psig. Full scan analyses were performed in the mass range of 50–500 Da for the spectral identification of amino acids and sample co-extractives respectively.

2.9. Statistical analysis

All experiments were performed in three separate batches. Results for each analysis were expressed as mean ± standard deviation. The statistical analysis was performed using SAS statistical software, Version 6.12 edition (15) followed by Duncan's post hoc test to provide significance levels for the difference between the treatments. The criterion for statistical significance was $p < 0.05$.

3. Results and discussion

3.1. The growth of probiotics in ice cream

There was no significant difference in La-05 counts among the three types of non-fermented ice cream made from cow, soy or coconut milk (Table 4). However, Bb-12 had higher ability ($p < 0.05$) to grow in non-fermented soy and coconut ice creams (7.5 log10 cfu/g) than cow milk (7.21 log10 cfu/g). The highest growth of La-05 in non-fermented composite milk ice creams was shown in SC3 and SW3 (7.3 log10 cfu/g). Similar level of Bb-12 cell counts was almost found in all non-fermented composite milk ice cream samples (7.5 log10 cfu/g).

The time required for Bb-12 to reduce pH to 5.5 in ice cream samples during fermentation was longer (10.5 h–8.10 h) than La-05 (6.20 h–9.25 h; Table 4). In addition, pH declined faster in ice cream samples made from vegetables milk than those made from cows' milk. Fermented soy milk ice cream showed the highest growth rate of La-05 (1.29 log10 cfu/g; $p < 0.05$) followed by fermented coconut milk and cow milk ice creams (1.14 and 1.09 log10 cfu/g respectively; Table 4). The viable cell counts of Bb-12 increased ($p < 0.05$) to similar level (1.2 log10 cfu/g) in fermented ice cream made from vegetables milk compared to fermented cow milk ice cream that showed only 0.84 log10 cfu/g. In addition, the growth rate of La-05 increased ($p < 0.05$) with soy milk concentrations increase in fermented composite milk ice creams (Table 4). However, there were no significant differences ($p > 0.05$) in cell counts of Bb-12 in fermented ice cream SC1, SC2 and SC3 (1.1 log10 cfu/g). Both fermented ice cream SW1 and SW2 had similar growth level of 0.80 log10 cfu/g.

Nowadays, consumers are increasingly demanding for products fortified with probiotic bacteria (Stanton, Ross, Fitzgerald, & Van Sinderen, 2005). The strains of *L. acidophilus* and *B. bifidum* have been widely used in ice cream manufacturing (Akalin & Erisir, 2008; Akın et al., 2007; Ranadheeraa, Evansa, Adamsa, & Bainesc, 2013). An ice cream is called probiotic if live bacteria are present in the final product. The viable number of probiotics in the final product was suggested to be at least 10^6–10^7 cfu/g to be considered in the promotion of health and well-being for human (Madureira, Amorim, Gomes, Pintado, & Malcata, 2011). The viability of the probiotic bacteria in ice cream is an important parameter to be determined to ensure compliance with the food industry standards and meeting consumer expectation. Thus, it is importance to reduce cell death during the ice cream manufacture. Previous study found that the viable counts of La-5 and Bb-12 in probiotic ice cream were significantly reduced during production (Ranadheeraa et al., 2013). Earlier study demonstrated the ability of La-5 and Bb-12 to grow in non-fermented vegetable milk ice cream (soy or coconut milk) and survive over 30 days of storage at −20 °C (Aboulfazli et al., 2014). Based on the present study, the fermentation of vegetable milk ice cream mixture before freezing process increased ($p < 0.05$) the counts of La-5 and Bb-12 compared to non-fermented ice cream. Favaro-Trindade, Bernardi, Bodini, De Carvalho, and De Almeida (2006) noted a similar observation on fermented yellow mombin (*Spondias mombin* L) ice cream with *L. acidophilus* strains. Probiotics use free amino acids during their growth. The growth of probiotic bacteria in cow milk is slow because of lack of proteolytic activity. However, higher growth of La-5 and Bb-12 was found in vegetable milk ice cream than cow milk ice cream (Table 4) which probably due to the high nutrients in soy and coconut milk (Akesowan, 2009; Yuliana et al., 2010). In addition, phytochemical compounds present in soy and coconut milk could affect the growth of probiotics (Abdullah et al., 2003; Bisla et al., 2011; Shori, 2013a, 2013b; Wangcharoen, 2012). Heenan et al. (2004) reported that frozen soy dessert can be used as a suitable food for the delivery of probiotic bacterial strains with excellent viability and acceptable sensory characteristics. Other study found that soy extract powder as prebiotic could promote the growth of yogurt bacteria in the frozen product (Hermanto & Masdiana, 2011). Yuliana et al. (2010) stated that *L. acidophilus* can grow well in coco milk drink prepared from mixture of coconut water and coconut milk combination and maintain high viability even at low pH 3.5. The high growth of Bb-12 and La-5 in composite milk (cow or coconut milk) containing 75% of soy milk could be attributed to soy milk proteins that provide physical protection

Table 8
The free amino acid concentration in non-fermented ice creams.

Amino acids (mg/mL)	Samples								
	S	C	W	SC1	SC2	SC3	SW1	SW2	SW3
Alanine	1.16 ± 0.08^{c}	1.53 ± 0.11^{a}	1.32 ± 0.06^{b}	1.29 ± 0.08^{b}	1.06 ± 0.05^{d}	1.03 ± 0.09^{d}	1.30 ± 0.09^{b}	1.18 ± 0.04^{c}	1.04 ± 0.06^{d}
Arginine	4.76 ± 0.21^{a}	1.32 ± 0.31^{c}	<LoD	2.69 ± 0.29^{b}	2.08 ± 0.09^{c}	1.48 ± 0.09^{c}	2.43 ± 0.35^{b}	2.44 ± 0.31^{b}	1.67 ± 0.09^{c}
Aspartic acid	<LoD	0.32 ± 0.09^{a}	<LoD	<LoD	<LoD	0.18 ± 0.08^{a}	<LoD	<LoD	<LoD
Cytsine	<LoD	<LoD	<LoD	<LoD	<LoD	<LoD	<LoD	<LoD	<LoD
Glutamic acid	<LoD	1.2 ± 0.08^{a}	0.08 ± 0.03^{c}	<LoD	0.29 ± 0.09^{b}	0.91 ± 0.09^{a}	<LoD	<LoD	0.06 ± 0.04^{c}
Histidine	0.06 ± 0.03^{a}	<LoD	<LoD	<LoD	<LoD	<LoD	<LoD	<LoD	<LoD
Leucine	3.47 ± 0.21^{c}	4.20 ± 0.18^{b}	0.36 ± 0.05^{d}	4.29 ± 0.41^{b}	4.37 ± 0.24^{b}	5.15 ± 0.09^{a}	4.64 ± 0.34^{b}	4.35 ± 0.09^{b}	3.42 ± 0.18^{c}
Isoleucine	2.99 ± 0.51^{b}	4.05 ± 0.09^{a}	<LoD	2.64 ± 0.49^{b}	2.25 ± 0.63^{b}	2.98 ± 0.71^{b}	2.47 ± 0.56^{b}	2.30 ± 0.49^{b}	1.67 ± 0.08^{c}
Lysine	0.56 ± 0.10^{b}	0.87 ± 0.09^{a}	0.61 ± 0.11^{b}	0.59 ± 0.10^{b}	0.59 ± 0.08^{b}	0.62 ± 0.07^{b}	0.54 ± 0.11^{b}	0.39 ± 0.05^{c}	0.32 ± 0.06^{c}
Methionine	1.91 ± 0.09^{d}	2.66 ± 0.41^{c}	<LoD	4.25 ± 0.20^{a}	3.05 ± 0.09^{b}	3.44 ± 0.50^{b}	2.34 ± 0.34^{c}	2.36 ± 0.35^{c}	1.06 ± 0.09^{d}
Proline	0.28 ± 0.19^{b}	0.83 ± 0.09^{a}	<LoD	0.45 ± 0.39^{b}	0.26 ± 0.15^{b}	1.11 ± 0.09^{a}	0.04 ± 0.02^{c}	0.09 ± 0.05^{c}	<LoD
Serine	<LoD	0.21 ± 0.09^{a}	<LoD	<LoD	<LoD	0.03 ± 0.01^{b}	<LoD	<LoD	<LoD
Threonine	9.00 ± 0.76^{a}	5.00 ± 0.65^{c}	4.00 ± 0.09^{d}	10.2 ± 0.89^{a}	9.55 ± 0.72^{a}	5.75 ± 0.80^{c}	7.60 ± 0.78^{b}	7.60 ± 0.63^{b}	4.05 ± 0.08^{d}
Tyrosine	8.20 ± 0.66^{a}	5.00 ± 0.09^{d}	5.00 ± 0.07^{d}	8.70 ± 0.51^{a}	7.40 ± 0.32^{b}	5.90 ± 0.09^{c}	7.25 ± 0.41^{b}	7.20 ± 0.29^{b}	4.65 ± 0.18^{e}
Valine	5.45 ± 0.42^{b}	6.25 ± 0.29^{a}	0.83 ± 0.08^{e}	5.70 ± 0.53^{b}	6.45 ± 0.32^{a}	6.60 ± 0.41^{a}	4.08 ± 0.53^{c}	3.85 ± 0.48^{c}	2.57 ± 0.46^{d}
Phenylalanine	10.00 ± 0.89^{a}	3.80 ± 0.31^{f}	2.50 ± 0.11^{g}	9.65 ± 0.92^{a}	7.95 ± 0.72^{c}	5.80 ± 0.09^{e}	6.95 ± 0.47^{d}	8.15 ± 0.08^{b}	3.63 ± 0.22^{f}
Total	47.85 ± 0.44^{b}	37.25 ± 0.28^{e}	14.71 ± 0.19^{g}	50.45 ± 0.24^{a}	45.31 ± 0.63^{c}	40.99 ± 0.96^{d}	39.65 ± 0.86^{d}	39.92 ± 0.76^{d}	24.15 ± 0.09^{f}

[a–g] Values with different letters in the same row are significantly different ($P < 0.05$) (Tukey test).
LoD = limit of detection.

Table 9
The free amino acids concentration (mg/mL) in fermented ice cream with La-05 (L).

Changes of amino acids (mg/mL)	Samples								
	SL	CL	WL	SC1L	SC2L	SC3L	SW1L	SW2L	SW3L
Alanine	2.25 ± 0.19^{b}	3.46 ± 0.11^{a}	1.58 ± 0.21^{c}	1.61 ± 0.08^{c}	1.89 ± 0.11^{c}	2.22 ± 0.09^{b}	1.95 ± 0.09^{c}	1.66 ± 0.32^{c}	1.82 ± 0.42^{c}
Arginine	7.30 ± 0.51^{a}	1.31 ± 0.41^{e}	<LoD	4.23 ± 0.23^{b}	3.13 ± 0.11^{c}	1.8 ± 0.76^{e}	3.38 ± 0.53^{c}	2.31 ± 0.10^{d}	1.18 ± 0.11^{e}
Aspartic acid	<LoD	<LoD	<LoD	<LoD	<LoD	<LoD	<LoD	<LoD	<LoD
Cytsine	<LoD	<LoD	<LoD	<LoD	<LoD	<LoD	<LoD	<LoD	<LoD
Glutamic acid	<LoD	1.02 ± 0.20^{a}	<LoD	<LoD	0.38 ± 0.09^{b}	1.21 ± 0.11^{a}	<LoD	<LoD	<LoD
Histidine	0.04 ± 0.02^{a}	<LoD	<LoD	<LoD	<LoD	<LoD	<LoD	<LoD	<LoD
Leucine	8.60 ± 0.39^{b}	11.20 ± 0.72^{a}	7.75 ± 0.63^{c}	6.25 ± 0.42^{d}	6.19 ± 0.33^{d}	6.70 ± 0.56^{d}	2.38 ± 0.11^{g}	3.41 ± 0.32^{f}	5.20 ± 0.23^{e}
Isoleucine	8.55 ± 0.09^{b}	11.45 ± 0.13^{a}	<LoD	2.66 ± 0.09^{e}	2.81 ± 0.09^{e}	5.40 ± 0.09^{c}	4.96 ± 0.09^{d}	2.70 ± 0.09^{e}	1.05 ± 0.09^{f}
Lysine	1.24 ± 0.11^{a}	1.05 ± 0.09^{a}	1.03 ± 0.04^{a}	1.14 ± 0.11^{a}	0.75 ± 0.20^{b}	0.5 ± 0.14^{c}	0.55 ± 0.16^{c}	0.58 ± 0.11^{c}	0.80 ± 0.09^{b}
Methionine	2.15 ± 0.054^{a}	0.94 ± 0.31^{b}	<LoD	<LoD	<LoD	1.00 ± 0.24^{b}	0.13 ± 0.35^{c}	0.12 ± 0.29^{c}	<LoD
Proline	1.31 ± 0.76^{b}	2.58 ± 0.47^{a}	<LoD	0.88 ± 0.44^{c}	0.92 ± 0.84^{c}	2.24 ± 0.53^{a}	0.80 ± 0.64^{c}	0.73 ± 0.36^{c}	<LoD
Serine	<LoD	<LoD	<LoD	<LoD	<LoD	<LoD	<LoD	<LoD	<LoD
Threonine	3.11 ± 0.65^{a}	2.71 ± 0.49^{a}	0.96 ± 0.09^{b}	2.70 ± 0.72^{a}	2.64 ± 0.48^{a}	1.11 ± 0.55^{b}	0.13 ± 0.22^{d}	0.17 ± 0.07^{d}	0.69 ± 0.09^{c}
Tyrosine	2.28 ± 0.11^{a}	0.47 ± 0.09^{c}	1.06 ± 0.23^{b}	1.12 ± 0.31^{b}	1.00 ± 0.46^{b}	<LoD	<LoD	1.09 ± 0.26^{b}	<LoD
Valine	1.40 ± 0.22^{b}	2.20 ± 0.11^{a}	0.71 ± 0.08^{c}	0.89 ± 0.07^{c}	2.25 ± 0.65^{a}	2.69 ± 0.42^{a}	1.09 ± 0.19^{b}	1.50 ± 0.64^{b}	1.62 ± 0.25^{b}
Phenylalanine	3.00 ± 0.09^{a}	2.34 ± 0.23^{b}	0.32 ± 0.66^{d}	0.69 ± 0.72^{d}	0.48 ± 0.84^{d}	0.71 ± 0.23^{c}	1.89 ± 0.10^{c}	3.29 ± 0.44^{a}	0.53 ± 0.10^{d}
Total	41.235 ± 0.89^{a}	40.74 ± 0.79^{a}	13.41 ± 0.09^{d}	22.175 ± 0.96^{b}	22.438 ± 0.68^{b}	23.36 ± 0.81^{b}	17.26 ± 0.32^{c}	17.56 ± 0.46^{c}	12.89 ± 0.21^{d}

$^{a-f}$ Means with different letters in the same row are significantly different ($P < 0.05$).
LoD = limit of detection.

Table 10
The free amino acids concentration (mg/mL) in fermented ice cream with Bb-12 (B).

Changes of amino acids (mg/mL)	Samples								
	SB	CB	WB	SC1B	SC2B	SC3B	SW1B	SW2B	SW3B
Alanine	1.42 ± 0.22^{d}	2.00 ± 0.20^{a}	1.62 ± 0.18^{d}	1.42 ± 0.26^{b}	1.70 ± 0.32^{c}	1.80 ± 0.35^{b}	1.41 ± 0.42^{d}	1.29 ± 0.34^{d}	1.40 ± 0.21^{d}
Arginine	5.15 ± 0.23^{a}	1.41 ± 0.65^{d}	<LoD	3.39 ± 0.32^{b}	2.35 ± 0.11^{c}	2.51 ± 0.09^{c}	3.56 ± 0.29^{b}	1.52 ± 0.46^{d}	0.78 ± 0.27^{e}
Aspartic acid	<LoD	<LoD	<LoD	<LoD	<LoD	<LoD	<LoD	<LoD	<LoD
Cytsine	<LoD	<LoD	<LoD	<LoD	<LoD	<LoD	<LoD	<LoD	<LoD
Glutamic acid	<LoD	<LoD	<LoD	<LoD	<LoD	<LoD	<LoD	0.12 ± 0.04^{a}	<LoD
Histidine	<LoD	<LoD	<LoD	<LoD	<LoD	<LoD	<LoD	<LoD	<LoD
Leucine	3.30 ± 0.24^{a}	3.10 ± 0.08^{a}	0.31 ± 0.33^{d}	1.42 ± 0.08^{c}	2.95 ± 0.09^{b}	2.13 ± 0.46^{b}	1.84 ± 0.22^{c}	1.72 ± 0.41^{c}	0.58 ± 0.10^{d}
Isoleucine	0.62 ± 0.09^{d}	2.44 ± 0.09^{a}	<LoD	1.50 ± 0.20^{b}	1.30 ± 0.32^{b}	1.83 ± 0.19^{b}	1.17 ± 0.09^{c}	1.07 ± 0.09^{c}	0.80 ± 0.09^{d}
Lysine	0.65 ± 0.20^{c}	1.02 ± 0.11^{a}	1.00 ± 0.09^{a}	0.80 ± 0.21^{b}	0.49 ± 0.31^{c}	0.92 ± 0.39^{b}	0.58 ± 0.21^{c}	0.52 ± 0.27^{c}	0.31 ± 0.10^{c}
Methionine	<LoD	<LoD	0.004 ± 0.00^{a}	<LoD	<LoD	<LoD	0.02 ± 0.01^{a}	0.06 ± 0.02^{a}	<LoD
Proline	0.54 ± 0.32^{b}	0.93 ± 0.31^{a}	0.11 ± 0.23^{d}	0.53 ± 0.19^{b}	0.61 ± 0.18^{b}	0.99 ± 0.21^{a}	0.49 ± 0.18^{b}	0.36 ± 0.17^{c}	0.27 ± 0.17^{c}
Serine	<LoD	<LoD	<LoD	<LoD	<LoD	<LoD	<LoD	<LoD	<LoD
Threonine	5.30 ± 0.52^{a}	1.12 ± 0.11^{c}	0.82 ± 0.31^{d}	1.18 ± 0.17^{b}	1.31 ± 0.32^{b}	1.14 ± 0.21^{b}	1.70 ± 0.41^{b}	1.70 ± 0.10^{b}	0.97 ± 0.09^{d}
Tyrosine	6.15 ± 0.67^{a}	0.71 ± 0.09^{d}	0.00 ± 0.00^{e}	1.41 ± 0.10^{b}	1.31 ± 0.13^{b}	1.03 ± 0.08^{c}	1.60 ± 0.07^{b}	1.35 ± 0.34^{b}	0.95 ± 0.21^{d}
Valine	1.28 ± 0.26^{a}	1.08 ± 0.04^{b}	0.19 ± 0.10^{c}	0.42 ± 0.02^{c}	0.49 ± 0.21^{c}	0.27 ± 0.11^{c}	1.25 ± 0.10^{a}	1.05 ± 0.42^{b}	0.23 ± 0.14^{c}
Phenylalanine	5.25 ± 0.57^{a}	1.05 ± 0.23^{d}	0.29 ± 0.11^{e}	1.48 ± 0.24^{d}	1.32 ± 0.26^{d}	1.15 ± 0.31^{d}	2.36 ± 0.25^{c}	4.89 ± 0.32^{b}	0.82 ± 0.14^{e}
Total	$29.66 \pm 0.22a$	14.86 ± 0.34^{c}	$4.34 \pm 0.21f$	16.33 ± 0.39^{b}	13.83 ± 0.32^{d}	13.77 ± 0.41^{d}	15.98 ± 0.45^{b}	15.53 ± 0.52^{b}	7.11 ± 0.61^{e}

$^{a-e}$ Means with different letters in the same row are significantly different ($P < 0.05$).
LoD = limit of detection.

against freezing damage through encapsulating probiotics with stable network looks like a gel structure (Batista, Portugal, Sousa, Crespo, & Raymundo, 2005). The time required for Bb-12 or La-05 to reduce pH to 5.5 in ice cream samples made from soy or coconut milk was faster than cow milk ice cream (Table 4). This could be related to the buffering capacity that was different between treated ice creams, further study is required in order to determine the buffering capacities of ice cream samples.

To promote their beneficial effects in the host, probiotics must survive transit through the harsh acidic conditions of gastric environment and being capable of reaching the large intestine in adequate amounts to enable colonization and proliferation (Shori & Baba, 2015). Thus, further studies are needed to study the survival of Bb-12 or La-05 in all ice cream samples during gastrointestinal digestion.

3.2. Changes of sugar content in ice cream

The content of sugars in non-fermented ice creams and fermented ice creams by La- 05 and Bb-12 are presented in Tables 5 and 6 respectively. In composite milk ice cream, the presence of 50% of soy milk increased ($p < 0.05$) the stachyose and sucrose contents in both non-fermented and fermented ice creams. However, the lactose content increased ($p < 0.05$) with decreasing soy milk proportion in SW3, SW3L and SW3B (Tables 5 and 6). Table 7 shows the changes of sugar content in ice cream samples fermented with La-05 (L) or Bb-12 (B). Lactose and sucrose were the primary sugars consumed by the probiotics during fermentation. Both types of probiotics in soy milk ice cream were found to metabolize sucrose to the highest extent (0.82 mg/mL and 1.99 mg/mL for SB and SL respectively; $p < 0.05$). On the other hand, lactose was highly consumed by probiotics in cow milk ice cream (2.38 mg/mL and 2.78 mg/mL for WB and WL respectively). In composite milk ice cream, Bb-12 showed the greatest ($p < 0.05$) level of reduction in sucrose content of SC1 and SC2 (1.8 mg/mL) followed by SW1 and SW2 (1.25 mg/mL). In addition, La-05 was consumed higher ($p < 0.05$) amount of sucrose in SC1 (2.74 mg/mL) than SW1 (0.62 mg/mL). The highest ($p < 0.05$) quantities of lactose were

Table 11
Changes in free amino acids concentration (mg/mL) in fermented ice cream with La-05 (L).

Changes of amino acids (mg/mL)	Samples								
	SL	CL	WL	SC1L	SC2L	SC3L	SW1L	SW2L	SW3L
Alanine	−1.09^{c}	−1.93^{d}	−0.26^{a}	−0.32^{a}	−0.83^{a}	−1.19^{c}	−0.65^{b}	−0.48^{a}	−0.78^{b}
Arginine	−2.54^{f}	0.005^{b}	nd	−1.54^{e}	−1.05^{d}	−0.32^{c}	−0.95^{d}	0.13^{b}	0.49^{a}
Aspartic acid	na	na	na	na	na	na	na	na	na
Cytsine	na	na	na	na	na	na	na	na	na
Glutamic acid	na	0.18^{a}	na	na	−0.088^{b}	−0.30^{c}	na	na	na
Histidine	0.02^{a}	na	na	na	na	na	na	na	na
Leucine	−5.13^{d}	−7^{e}	−7.39^{e}	−1.96^{c}	−1.82^{c}	−1.55^{c}	2.26^{c}	0.94^{b}	−1.78^{c}
Isoleucine	−5.56^{e}	−7.4^{f}	na	−0.02^{b}	−0.56^{c}	−2.42^{d}	−2.49^{d}	−0.40^{c}	0.61^{a}
Lysine	−0.68^{c}	−0.18^{b}	−0.42^{a}	−0.55^{c}	−0.16^{b}	0.12^{b}	−0.01^{a}	−0.19^{b}	−0.48^{c}
Methionine	−0.24^{c}	1.72^{b}	na	na	na	2.44^{a}	2.21^{a}	2.24^{a}	na
Proline	−1.03^{b}	−1.75^{c}	na	−0.43^{a}	−0.66^{a}	−1.13^{b}	−0.76^{a}	−0.64^{a}	na
Serine	na	na	na	na	na	na	na	na	na
Threonine	5.89^{c}	2.29^{e}	3.04^{d}	7.5^{a}	6.91^{b}	6.86^{b}	7.47^{a}	7.43^{a}	3.36^{d}
Tyrosine	5.92^{c}	4.53^{d}	3.94^{e}	7.58^{a}	6.4^{b}	5.90^{c}	7.25^{a}	6.11^{b}	4.65^{d}
Valine	4.05^{a}	4.05^{a}	0.12^{a}	4.81^{a}	4.2^{a}	3.91^{c}	2.99^{c}	2.35^{c}	0.95^{c}
Phenylalanine	7.00^{b}	1.46^{f}	2.18^{e}	8.96^{a}	7.47^{b}	5.09^{c}	5.06^{c}	4.86^{c}	3.10^{d}
Total	6.61^{f}	−4.02^{h}	1.21^{g}	24.02^{a}	19.81^{c}	17.41^{d}	22.38^{b}	22.35^{b}	10.12^{e}

$^{a-f}$ Means with different letters in the same row are significantly different ($P < 0.05$).
na = not applicable.

Table 12
Changes in free amino acids concentration (mg/mL) in fermented ice cream with Bb-12 (B).

Changes of Amino acids	Samples								
	SB	CB	W	SC1B	SC2B	SC3B	SW1B	SW2B	SW3B
Alanine	−0.26^{b}	−0.47^{b}	−0.30^{b}	−0.13^{b}	−0.34^{b}	−0.47^{b}	−1.11^{c}	0.08^{a}	−0.36^{b}
Arginine	−0.39^{c}	−0.09^{d}	nd	−0.87^{c}	−0.44^{c}	0.70^{a}	−0.96^{c}	0.09^{b}	−0.84^{c}
Aspartic acid	na	na	na	na	na	na	na	na	na
Cytsine	na	na	na	na	na	na	na	na	na
Glutamic acid	na	na	na	na	na	na	na	na	na
Histidine	na	na	na	na	na	na	na	na	na
Leucine	−0.67^{d}	1.09^{c}	−3.95^{f}	2.45^{a}	1.45^{b}	1.57^{b}	1.69^{b}	−1.05^{e}	1.28^{b}
Isoleucine	2.37^{c}	1.60^{c}	na	0.14^{c}	−0.05^{c}	0.14^{c}	−0.50^{c}	1.73^{c}	1.58^{c}
Lysine	−0.09^{c}	−0.15^{c}	−0.87^{e}	−0.21^{c}	0.10^{a}	−0.30^{d}	−0.04^{c}	−0.13^{c}	0.01^{b}
Methionine	na	na	na	na	na	na	2.32^{a}	2.30^{a}	na
Proline	−0.26^{c}	−0.10^{b}	na	−0.08^{b}	−0.35^{c}	0.11^{a}	−0.45^{c}	−0.13^{b}	na
Serine	na	na	na	na	na	na	na	na	na
Threonine	3.70^{e}	3.87^{e}	3.57^{e}	9.01^{a}	8.24^{b}	4.60^{d}	5.84^{c}	5.33^{c}	3.07^{e}
Tyrosine	2.05^{f}	4.29^{d}	5.00^{c}	7.29^{a}	6.09^{b}	4.87^{d}	6.30^{b}	5.59^{c}	3.29^{e}
Valine	4.16^{c}	6.17^{a}	−0.36^{c}	5.28^{b}	5.95^{b}	6.33^{a}	3.63^{d}	2.59^{e}	2.34^{e}
Phenylalanine	4.75^{d}	2.74^{e}	2.20^{e}	8.32^{a}	6.46^{b}	4.64^{d}	5.06^{c}	5.78^{c}	2.80^{e}
Total	17.34^{f}	23.36^{d}	0.84^{h}	35.45^{a}	30.45^{b}	26.77^{c}	21.78^{e}	22.08^{e}	14.05^{g}

$^{a-h}$ Means with different letters in the same row are significantly different ($p < 0.05$).
na = not applicable.

consumed by Bb-12 in SC3 (1.32 mg/mL). No significant difference in lactose consummation by La-05 between SC1 and SC2 (1.0 mg/mL) whereas SW3 had the highest lactose reduction among others (SW1 and SW2; Table 7). In general, Bb-12 and La-05 in coconut milk ice cream had the lowest changes of total sugar content (Table 7). The highest reduction of total sugar content by Bb-12 and La-05 was seen in SC1 (3.05 mg/mL and 3.61 mg/mL respectively).

According to sugar analysis, the two probiotic bacteria tested showed ability to metabolize sucrose as sources of energy in soy milk ice cream which improved cell growth during fermentation (Tables 6 and 7). This observed phenomenon was in agreement with the report of Wang et al. (2003) on fermented soy milk with probiotics. Since lactose is the main carbohydrate in cow milk, this explain the highly consumed of lactose sugar by Bb-12 and La-5 in cow milk ice cream (Tables 6 and 7). It can be demonstrated that composite milk (coconut or cow milk) with soy milk increased ($p < 0.05$) the availability of lactose and sucrose sugars for probiotics growth. In addition, ice cream combination of coconut milk and soy milk could be suitable as sugars source for Bb-12 and La-5 growth (Tables 6 and 7).

3.3. Changes of amino acids content in ice cream

Soy milk ice cream showed the highest ($p < 0.05$) amino acids (47.85 ± 0.44 mg/mL) whereas cow milk ice cream contains the lowest (14.71 ± 0.19 mg/mL; Table 8). Coconut milk ice cream showed higher amino acid concentrations of glutamic acid, aspartic acid, alanine, serine, proline, isoleucine, leucine, valine, lysine and methionine than soy milk and cow milk ice creams. On the other hand, soy milk ice cream had higher arginine, cytsine, histidine, lysine, threonine, tyrosine and phenylalanine than coconut milk and cow milk ice creams (Table 8). Tables 9 and 10 present the concentration of free amino acids contents (mg/mL) in fermented ice creams with La-05 or Bb-12 respectively. Regardless of the probiotic used, all types of amino acids were higher in ice creams containing 100% of soy or coconut milk than ice cream containing cow milk (Tables 9 and 10). The changes in the concentration of amino acids in fermented ice cream with La-05 or Bb-12 are presented in Tables 11 and 12 respectively. This results indicated higher ($p < 0.05$) utilization of amino acids by both probiotics in fermented ice cream containing vegetables milk than in those containing cows' milk (Tables 11 and 12). Both La-05 and Bb-12 decreased ($p < 0.05$) amino acids content in all fermented ice cream samples except coconut milk ice cream fermented with La-05 (Tables 11 and 12). The highest ($p < 0.05$) utilization of amino acids by La-05 and Bb-12 was shown in SC1 (24.02 mg/mL and 35.45 mg/mL respectively). The most types of amino acids utilized by both probiotic strains were threonine, tyrosine, valine and phenylalanine in all fermented ice cream samples. In addition, Bb-12 had higher ($p < 0.05$) utilization of isoleucine, leucine, threonine, tyrosine, valine and phenylalanine than La-05 in all fermented ice cream samples (Tables 11 and 12).

Previous study reported that *L. acidophilus* and *Bifidobacterium lactis* can increase the release of amino acids groups and peptides during fermentation of milk and the cell growth of these probiotics reflects the balance between proteolysis and assimilation of amino acids (Donkor, 2007). Probiotics need free amino acids such as glutamic acid, arginine, leucine, isoleucine, cysteine, valine, tryptophan and tyrosine essential for their growth (Gomes, Xavier Malcata, & Klaver, 1998). Cow's milk may not be able to support extensive growth of probiotics due to the lack of some essential free amino acids and peptides (Tables 8–10). In fact, several amino acids in cow milk are generally available in insufficient amounts either unbound or as low molecular mass peptides (Gomes et al., 1998). However, most of the proteins (80%) in the coconut milk can be classified as globulins and albumins (Yuliana et al., 2010) with relatively high levels of glutamic acid, aspartic acid, arginine, alanine, serine, proline, isoleucine, leucine, valine, lysine and methionine. Soy milk also contains high levels of arginine, cytsine, histidine, alanine, lysine, threonine, tyrosine and phenylalanine. Hence, both soy and coconut milk ice creams provide a richer growth medium of amino acids for Bb-12 and La-05 than cow's milk ice cream and possess high capacity to support faster microbial growth and metabolism resulting in a shorter time of pH decline in ice cream containing vegetable milk (Table 4).

4. Conclusion

Preparation of fermented ice cream has a promising potential for utilization as functional product. The replacement of cow milk with soy or coconut milk improved ($p < 0.05$) the probiotic growth of Bb-12 by 1.2 log10 cfu/g in fermented ice cream compared to cow milk ice cream 0.84 log10 cfu/g. Similarly, La-5 increased ($p < 0.05$) by 1.29 log10 in fermented soy milk ice cream compared to cow milk ice cream (1.09 log10 cfu/g). The growth rate of La-05 increased ($p < 0.05$) with soy milk concentrations increase in fermented composite milk ice creams. Both soy and coconut milk ice creams provide a richer growth medium of amino acids and sugar content (particularly lactose and sucrose) for Bb-12 and La-05 than cow's milk ice cream. Therefore, fermented vegetables milk ice cream could be a good vehicle for the delivery of Bb-12 and La-5 and can be used as new functional food. Further study is needed to evaluate the viability of Bb-12 and La-5 in vegetables milk ice cream during freezing storage.

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References

Abdullah, M., Rehman, S., Zubair, H., Saeed, H., Kousar, S., & Shahid, M. (2003). Effect of skim milk in soymilk blend on the quality of ice cream. *Pakistan Journal of Nutrition, 2*, 305–311.

Aboulfazli, F., Baba, A. S., & Misran, M. (2014). Effects of vegetable milk on survival of probiotics and rheological and physicochemical properties of bio-ice cream. In *International conference on biological and medical sciences (ICBMS'2014) Jan. 15–16, 2014 Kuala Lumpur (Malaysia)*.

Akalin, A. S., & Erisir, D. (2008). Effects of inulin and oligofructose on the rheological characteristics and probiotic culture survival in low-fat probiotic ice cream. *Journal of Food Science, 73*, 184–188.

Akesowan, A. (2009). Influence of soy protein isolate on physical and sensory properties of ice cream. *Thai Journal of Agricultural Science, 42*, 1–6.

Akın, M., Akın, M., & Kırmac, Z. (2007). Effects of inulin and sugar levels on the viability of yogurt and probiotic bacteria and the physical and sensory characteristics in probiotic ice-cream. *Food Chemistry, 104*, 93–99.

Alamprese, C., Foschino, R., Rossi, M., Pompei, C., & Savani, L. (2002). Survival of *Lactobacillus johnsonii* La1 and influence of its addition in retail-manufactured ice cream produced with different sugar and fat concentrations. *International Dairy Journal, 12*, 201–208.

Baba, A. S., Najarian, A., Shori, A. B., Lit, K. W., & Keng, G. A. (2014). *In vitro* inhibition of key enzymes related to diabetes and hypertension in *Lycium barbarum*-yogurt. *The Arabian Journal for Science and Engineering, 39*(7), 5355–5362.

Batista, A. P., Portugal, C. A., Sousa, I., Crespo, J. G., & Raymundo, A. (2005). Accessing gelling ability of vegetable proteins using rheological and fluorescence techniques. *International Journal of Biological Macromolecules, 36*(3), 135–143.

Belewu, M., & Belewu, K. (2007). Comparative physico-chemical evaluation of tigernut, soybean and coconut milk sources. *International Journal of Agriculture and Biology, 5*, 785–787.

Bisla, G., Archana, P. V., & Sharma, S. (2011). Development of ice creams from soybean milk & watermelon seeds milk and evaluation of their acceptability and nourishing potential. *Research Journal of Dairy Sciences, 5*, 4–8.

Çaglar, E., Kuscu, O. O., Kuvvetli, S. S., Cildir, S. K., Sandalli, N., & Twetman, S. (2008). Short-term effect of ice-cream containing Bifidobacterium lactis Bb-12 on the number of salivary mutans streptococci and lactobacilli. *Acta Odontologica Scandinavica, 66*(3), 154–158.

Desai, A., Small, D., McGill, A. E. J., & Shah, N. P. (2002). Metabolism of raffinose and stachyose in reconstituted skim milk and of n-hexanal and pentanal in soymilk by bifidobacteria. *Bioscience Microflora, 21*, 245–250.

Donkor, O. N. (2007). *Influence of probiotic organisms on release of bioactive compounds in yoghurt and soy yoghurt*. PhD thesis. Melbourne, Australia: Victoria University.

Farnworth, E., Mainville, I., Desjardins, M. P., Gardner, N., Fliss, I., & Champagne, C. (2007). Growth of probiotic bacteria and bifidobacteria in a soy yogurt formulation. *International Journal of Food Microbiology, 116*, 174–181.

Favaro-Trindade, C. S. S., Bernardi, R. B., Bodini, J. C., De Carvalho, B., & De Almeida, E. (2006). Sensory acceptability and stability of probiotic microorganisms and vitamin c in fermented acerola (Malpighia emarginata DC.) ice cream. *Journal of Food Science, 71*, S492–S495.

Gomes, A. M. P., Xavier Malcata, F., & Klaver, F. A. M. (1998). Growth enhancement of *Bifidobacterium lactis* Bo and *Lactobacillus acidophilus* Ki by milk hydrolyzates. *Journal of Dairy Science, 81*, 2817–2825.

Guarner, F., Perdigon, G., Corthier, G., Salminen, S., Koletzko, B., & Morelli, L. (2005). Should yogurt cultures be considered probiotic? *British Journal of Nutrition, 93*, 783–786.

Heenan, C., Adams, M., Hosken, R., & Fleet, G. (2004). Survival and sensory acceptability of probiotic microorganisms in a nonfermented frozen vegetarian dessert. *LWT – Food Science and Technology, 37*, 461–466.

Hermanto, M. P., & Masdiana, P. (2011). Fate of yoghurt bacteria in functional ice cream in the presence of soy extract powder as prebiotic. In *The 12th asean food conference, Thailand, 16–18 June* (pp. 263–269). Bangna, Bangkok, Thailand: BITEC.

Kumaguai, H. (2001). Application of liquid chromatography/mass spectrometry to the analysis of sugars and sugar-alcohol. *Application Note*, 5988–4236EN. Agilent Technolgies, 200-203.

Leandro, E. D. S., Araújob, E. A. D., Conceicão, L. L. D., Moraesa, C. A. D., & Carvalhoc, A. F. D. (2013). Survival of Lactobacillus delbrueckii UFV H2b20 in ice cream produced with different fat levels and after submission to stress acid and bile salts. *Journal of Functional Foods, 5*(1), 503–507.

Madureira, A. R., Amorim, M., Gomes, A. M., Pintado, M. E., & Malcata, F. X. (2011). Protective effect of whey cheese matrix on probiotic strains exposed to simulated gastrointestinal conditions. *Food Research International, 44*, 465–470.

Magarinos, H., Selaive, S., Costa, M., Flores, M., & Pizarro, O. (2007). Viability of probiotic micro-organisms (*Lactobacillus acidophilus* La-5 and *Bifidobacterium animalis* subsp. Lactis Bb-12) in ice cream. *International Journal of Dairy Technology, 60*, 128–134.

Ozcan, S., & Senyuva, H. Z. (2006). Improved and simplified liquid chromatography/atmospheric pressure chemical ionization mass spectrometry method for the analysis of underivatized free amino acids in various foods. *Journal of Chromatography A, 1135*, 179–185.

Pandiyan, C., Annal Villi, R., Kumaresan, G., Murugan, B., & Gopalakrishnamurthy, T. R. (2012). Development of synbiotic ice cream incorporating *Lactobacillus acidophilus* and *Saccharomyces boulardii*. *International Food Research Journal, 19*(3), 1233–1239.

Ranadheeraa, C. S., Evansa, C. A., Adamsa, M., & Bainesc, S. K. (2013). Production of probiotic ice cream from goat's milk and effect of packaging materials on product quality. *Small Ruminant Research, 112*, 174–180.

Ranadheera, C. S., Evans, C. A., Adams, M. C., & Baines, S. K. (2012). In vitro analysis of gastrointestinal tolerance and intestinal cell adhesion of probiotics in goat's milk ice cream and yogurt. *Food Research International, 49*, 619–625.

Salem, M. M., Fathi, F. A., & Awad, R. (2005). Production of probiotic ice cream. *Polish Journal of Food and Nutrition Sciences, 14*, 267–271.

Shori, A. B. (2013a). Antioxidant activity and viability of lactic acid bacteria in soybean-yogurt made from cow and camel milk. *Journal of Taibah University for Science, 7*, 202–208.

Shori, A. B. (2013b). Nutritional and therapeutical values of chickpea water extract enriched yogurt made from cow and camel milk. *American Journal of Drug Discovery and Development, 3*(2), 47–59.

Shori, A. B. (2015). The potential applications of probiotics on dairy and non-dairy foods and their viability during storage. *Biocatalysis and Agricultural Biotechnology, 4*(4), 423–431.

Shori, A. B., & Baba, A. S. (2011). *Cinnamomum verum* improved the functional properties of bio-yogurts made from camel and cow milks. *Journal of the Saudi Society of Agricultural Sciences, 10*(2), 101–107.

Shori, A. B., & Baba, A. S. (2013). Antioxidant activity and inhibition of key enzymes linked to type-2diabetes and hypertension by *Azadirachta indica*-yogurt. *Journal of Saudi Chemical Society, 17*(3), 295–301.

Shori, A. B., & Baba, A. S. (2014). Comparative antioxidant activity, proteolysis and in vitro α-amylase and α-glucosidase inhibition of *Allium sativum*-yogurts made from cow and camel milk. *Journal of Saudi Chemical Society, 18*(5), 456–463.

Shori, A. B., & Baba, A. S. (2015). Survival of *Bifidobacterium bifidum* in cow- and camel- milk yogurts fortified with *Cinnamomum verum* and *Allium sativum*. *Journal of the Association of Arab Universities for Basic and Applied Sciences, 18*, 7–11.

Singh, R. P., Damle, S. G., & Chawla, A. (2011). Salivary mutans streptococci and lactobacilli modulations in young children on consumption of probiotic ice-cream containing *Bifidobacterium lactis* Bb12 and *Lactobacillus acidophilus* La5. *Acta Odontologica Scandinavica, 69*(6), 389–394.

Soler, L. (2005). *Development of non-dairy frozen emulsion containing soy protein andcoconut milk*. Lousiana: (Doctor in Food Science)-Louisiana State University and Agricultural and Mechanical College, Department of Food Science.

Soukoulis, C., Lebesi, D., & Tzia, C. (2009). Enrichment of ice cream with dietary fibre: effects on rheological properties, ice crystallisation and glass transition phenomena. *Food Chemistry, 115*, 665–671.

Stanton, C., Ross, R. P., Fitzgerald, G. F., & Van Sinderen, D. (2005). Fermented functional foods based on probiotics and their biogenic metabolites. *Current Opinion in Biotechnology, 16*, 198–203.

Wangcharoen, W. (2012). Development of ginger-flavoured soya milk ice cream: comparison of data analysis methods. *Maejo International Journal of Science and Technology, 6*, 505–513.

Wang, Y. C., Yu, R. C., & Chou, C. C. (2004). Viability of lactic acid bacteria and bifidobacteria in fermented soymilk after drying, subsequent rehydration and storage. *International Journal of Food Microbiology, 93*, 209–217.

Wang, W.-C., Yu, R.-C., Yang, H.-Y., & Chou, C.-C. (2003). Sugar and acid contents in soymilk fermented with lactic acid bacteria alone or simultaneously with bifidobacteria. *Food Microbiology, 20*, 333–338.

Yuliana, N., Rangga, A., & Rakhmia. (2010). Manufacture of fermented coco milk-drink containing lactic acid bacteria cultures. *African Journal of Food Science, 4*, 558–562.