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ORIGINAL RESEARCH

Replacement of bovine milk with vegetable milk: Effects on the survival of probiotics and rheological and physicochemical properties of frozen fermented dessert

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In this study, frozen fermented desserts containing Lactobacillus acidophilus (La-05; L) and Bifidobacterium bifidum (Bb-12; B), were made from bovine (W), soya (S), coconut (C) and composite (i.e. combinations of coconut or bovine milks with soya milk) milks. The changes in frozen dessert eating qualities and the survival of added microbes were evaluated. The highest viscosity and melting resistance, and the lowest total sensory scores, were found in the products made using soya milk. After 90-day storage at −20 °C, the highest survival percentage of La-05 was found in the products made using coconut milk (CL); and for Bb-12, it was found in the products made using soya milk, coconut milk and a 25:75 blend of soya milk and coconut milk respectively (samples SB, CB and SC3B) SB, SCB and CB samples.

Keywords Frozen fermented dessert, Soya milk, Coconut milk, Probiotic bacteria.

INTRODUCTION

The current tendency for consuming functional foods is increasing rapidly worldwide, mostly due to consumer awareness about the relationship between diet and health. Functional foods are thought to provide benefits beyond basic nutrition and may play a role in reducing or minimising the risk of certain diseases and other health conditions (Pinto *et al.* 2012).

Ice cream and frozen dairy desserts are delicious, wholesome and nutritious products that are widely consumed in different parts of the world. Despite having nutritional significance, they encompass no therapeutic properties (Salem *et al.* 2005). Current industrial dessert products are mainly milk based, which may lead to constraints in product use, due to their cholesterol and lactose contents. Soya- and coconut-based products are suitable dairy product substitutes for lactose-intolerant or vegetarian individuals (Granato *et al.* 2010). Soya milk is regarded as a suitable bovine milk replacement in ice cream because of its high nutritional quality, especially with respect to protein content and balanced amino acids (Gandhi *et al.* 2001). Frequent consumption of soya products offers health benefits, such as lowering the risk of getting cancers, cardiovascular-associated diseases, hypercholesterolaemia, diabetes, and bone and kidney diseases (Dervisoglu *et al.* 2005;). Coconut milk's suitability to replace bovine milk stems from its easy digestibility and its abundant mineral (particularly calcium, phosphorus and potassium) and vitamin (B, C and E vitamins) contents, and antioxidant activities. The high oleic and lauric acid content of coconut milk helps in preventing arteriosclerosis and related illnesses (Belewu and Belewu 2007).

The general acceptance of vegetable milks in food preparation can be associated with their favourable vegetable protein effects on improving the physical properties of foods. For instance, the viscosity, melting time and hardness of ice cream samples increased by substituting skimmed milk powder with soya protein isolate (Akesowan 2009). The fortification of yoghurt ice cream with soya protein improved

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the texture, firmness and viscosity of the product (Mahdian *et al.* 2012). Samoto *et al.* (2007) noted that soya lecithin acts as an emulsifier and helps to increase the viscosity, stability, texture and melting time of ice cream.

Probiotic bacteria may be added to frozen dairy desserts to produce a functional frozen dairy dessert. Probiotics are defined as live micro-organisms which, when administered in adequate amounts, confer several health benefits to their consumers. They improve intestinal microflora, activate the immune system, reduce serum cholesterol and inhibit the growth of potential pathogens (Grajek *et al.* 2005). It is possible to produce ice cream-type frozen yoghurt using different ratios of fermented mixes (Alamprese *et al.* 2002). Fermented ice cream is a complex fermented frozen dairy dessert that combines the physical characteristics of ice cream with the sensory and nutritional properties of fermented milk products (Pinto *et al.* 2012). It emphasises the ways of avoiding or masking too strong a yoghurt flavour, and the use of other cultured milk products, as a base for healthy ice cream products (Salem *et al.* 2005).

Challenges to the making of functional frozen dessert, based on probiotic and vegetable milk, include the following: probiotic bacteria do not generally grow rapidly in bovine milk and some consumers avoid using soya products, because of their undesirable off-flavours. However, soya and coconut milks are very rich mediums for probiotic bacteria (Farnworth *et al.* 2007; Yuliana *et al.* 2010), and any soya milk beany flavour may be significantly reduced by fermenting with some strains of *Bifidobacterium* (Donkor 2007). The addition of probiotics into frozen desserts containing vegetable milk may therefore improve growth and the survival of probiotics, and the product's sensory properties. This study was carried out to investigate the effect of replacing bovine milk with soya, coconut or composite milks, on the survival of probiotics, and the physical and organoleptic properties of frozen fermented desserts during storage.

MATERIALS AND METHODS

Fresh bovine milk, coconut, soya bean, soya oil, butter and skim milk powder (Dutch lady, Malaysia), sugar and vanilla were purchased from local grocery. Cremodan SE 734 veg (Danisco AS, Copenhagen, Denmark) containing mono- and diacylglycerols of fatty acid, cellulose gum, guar gum and carrageenan were used as stabilisers. *Bifidobacterium bifidum* (Bb-12) and *Lactobacillus acidophilus* (La-05) were obtained as pure freeze-dried probiotic culture from CHR Hansen (Horsholm, Denmark).

Preparation of culture

Starter culture

Each strain (1 g) was cultured in 100 mL of sterilised skimmed milk (10w/v), supplemented by the addition of 0.05% (w/v) L-Cys-HCI, 1% (w/v) yeast extract and 2% (w/v) glucose. The incubation was carried out under aerobic conditions, in a water bath at 42 °C, until a pH of 5.0 was achieved (Magarinos *et al.* 2007).

Culture for inoculation

Inoculation culture for each strain was freshly prepared by adding 4 mL of starter culture into 100 mL of sterilised skimmed milk. Incubation was carried out under anaerobic conditions, in a water bath at 42 °C, until the pH reduced to 5.0 (Magarinos *et al.* 2007).

Preparation of soya milk with 12% total solid

Soya beans (100 g) were washed three times using tap water, rinsed once using deionised water and soaked in deionised water (1 L) for 14 h at room temperature. 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 soya bean was then passed through four layers of cheesecloth. The soya milk fat content (1.86%) was corrected to 3.4% using 1.54 g soya oil/100 g soya milk. The soya milk was reheated to 80 °C for 10 min and then immediately chilled (to 4 °C) prior to making frozen dessert.

Preparation of coconut milk with 12% total solid

The hard brown coconut shell was cracked open, and the white copra was grated. This was followed by mechanical pressing to obtain the milk. 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 (to 4 °C) and then used within 1 h.

Preparation of frozen dessert

Frozen aerated dessert was prepared using various combinations of coconut or bovine milks with soya milk. To achieve frozen desserts with 43% total solids and 10.5% fat, for a total batch of 100 g, frozen dessert mixes were prepared using the formula shown in Table 1.

The milk or milk combinations with butter were heated to 50 °C prior to mixing with the skimmed milk powder, sugar, water and stabiliser. The mixtures were subjected to two stages of homogenisation (16 000 rpm, 70 °C, 5 min; Ika Homogenizer T-25 basic Ultra-Turrax, Staufen im Breisgau, Baden-Württemberg, Germany). The mixtures were pasteurised at 80 °C for 10 min in a water bath and then cooled to 4 °C and aged overnight at 4 °C. Next, 4% (w/w) fermented milk (intermediate culture) was added to the mixtures followed by incubation in a water bath at 42 °C, for varying lengths of time, until the pH reduced to 5.5. After fermentation, the mixtures were cooled to 4 °C, followed by

Table 1 The content of components used in frozen desserts mix formulations (percentage by weight)

	Ingredient						
Sample[a]	*Milk (%)*	*Butter% (Fat = 83.3%)*	*Skim milk powder (%)*	*Sugar (%)*	*Stabiliser–emulsifier (%)*	*Vanillin (%)*	*Water (%)*
W	55.4	10.375	7	17	0.6	0.1	9.625
C	55.4	7.31	7	17	0.6	0.1	9.625
S	55.4	10.375	7	17	0.6	0.1	9.625
SW1	55.4	10.375	7	17	0.6	0.1	9.625
SW2	55.4	10.375	7	17	0.6	0.1	9.625
SW3	55.4	10.375	7	17	0.6	0.1	9.625
SC1	55.4	9.6	7	17	0.6	0.1	9.625
SC2	55.4	8.84	7	17	0.6	0.1	9.625
SC3	55.4	8.08	7	17	0.6	0.1	9.625

[a]Samples made with 100% bovine milk, W; 100% coconut milk, C; 100% soya milk, S; 75% soya + 25% bovine milk, SW1; 50% soya + 50% bovine milk, SW2; 25% soya + 75% bovine milk, SW3; 75% soya + 25% coconut milk, SC1; 50% soya + 50% coconut milk, SC2; 25% soya + 75% coconut milk, SC3.

freezing in a 1.5-L ice cream maker (Baumatic gelato1ss, Baumatic Ltd., Reading, Berkshire, UK; rotor speed 50 round/min, 40 min, −30 °C). Each frozen dessert batch was drawn, packed in 100-mL plastic cups and stored at −20 °C in a freezer. Three separate batches of frozen dessert were made for each treatment.

Chemical analysis

The pH of the frozen desserts was measured using a digital pH meter. Meanwhile, the titratable acidity (TA) was determined by titrating samples (10 g) with NaOH (0.1 N), using phenolphthalein (1% w/v) as an indicator. The total solids were measured by drying samples at 100 ± 1 °C for 3.5 h using an air oven (Akin *et al.* 2007). Fat content was calculated by weight after alkaline hydrolysis coupled with Soxhlet extraction (petroleum ether) (AOAC 2005). All measurements were made three times.

Physical analysis

The overrun was calculated using the following formula (Eqn (1); Akin *et al.* 2007):

$$\text{Overrun} = \frac{\text{weight of unit mix} - \text{weight of equal volume of frozen dessert} \times 100}{\text{weight of equal volume of frozen dessert}} \quad (1)$$

The frozen dessert melting rate was determined as described by Mahdian *et al.* (2012). Tempered frozen desserts (−20 °C, 30 g) were placed on a 0.2-cm wire mesh screen above a beaker at room temperature (25 °C). The weight of the melted material was measured after 20 min and declared as percentage weight melted.

Size and zeta potential

The average particle size and zeta potential of the fat globules of ice cream mixes were determined using Malvern Zetasizer Nano Series (Malvern Instruments, UK) at a constant temperature of 25 °C. Measurements were made with the ice cream mixes diluted to approximately 1:10 000 with deionised water. Samples were placed into a cuvette instead of the zetasizer. The zeta potential and size of the mixes were monitored after the ageing step (Aboulfazli *et al.* 2014).

Rheological measurements

The rheological measurements of melted frozen desserts were determined using a Physica MCR 301 rheometer (Anton Paar GmbH, Graz, Austria), with a concentric cylinder geometry, coupled with a circulating cooling bath at 4.0 ± 0.1 °C. Melted samples (of approximately 20 g) were left to equilibrate at 4 °C for 15 min. The samples flow behaviour was generated by linearly increasing the shear rate from 19.6 to 67.3/s over 20 min, followed by returning to 19.6/s over a further 20 min.

The consistency index and the flow behaviour were explained by the power law model ($\sigma = K(\gamma)^n$). The apparent viscosity of the frozen desserts was estimated as a function of time under a constant shear rate of 20/s.

where σ = the shear stress (Pa); K = consistency index (Pa s^n); γ = the shear rate (s); and n = the flow behaviour index (Rossa *et al.* 2012).

Bacteriological analysis

The viability of probiotics was measured immediately after inoculating the probiotic cultures and again after 1, 30, 60 and 90 days of frozen storage at −20 °C. The samples (10 g) were decimally diluted with sterile peptone water (1 g/L; Merck). 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 (Merck) 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. The bacterial viability was represented as survival rate (Magarinos *et al.* 2007).

Sensory analysis

The frozen fermented desserts were organoleptically evaluated by sixteen consumer panellists (25–30 year; eight males, eight females), using a sensory rating scale of 1–10 for taste and flavour, 1–5 for consistency and 1–5 for appearance and colour (Akin *et al.* 2007).

The defect properties that were evaluated are as follows: (a) four attributes for flavour and taste (i.e. cooked flavour, sweetness, lack of flavour and acidic/sour); (b) six characteristics of body and texture (i.e. crumbly, coarse, weak, gummy, fluffy and sandy); and (c) two terms describing colour and appearance (dull colour or unnatural colour). For each criterion, samples were ranked from 1 to 10 as follows: 1–2 = low intensity, 5–6 = moderate intensity and 9–10 = high intensity (Lin 2012).

Statistics

The viability of the probiotic micro-organisms was evaluated three times during storage (0, 1, 30, 60 and 90 days) in triplicates, and the results were expressed as mean ± SEM (standard mean error) values. Statistical analysis was performed using SAS statistical software, version 6.12 (SAS 1996), followed by Duncan's multiple range method for mean comparison. The criterion for statistical significance was $P < 0.05$ (Homayouni *et al.* 2008). Principal component analysis (PCA) was performed using XLSTAT software, version 2014, on the covariance matrix for all sensory attributes.

RESULTS AND DISCUSSION

Physicochemical properties

The physicochemical compositions of the frozen desserts are presented in Table 2. Total solid, fat, pH, titratable acid-

Table 2 Physicochemical properties of the frozen fermented desserts

Samples ^{A}	*Total solids(g 100/g)* ^{B}	*pH (value)* ^{B}	*Titratable acidity (% lactic acid)* ^{B}	*Fat (g 100/g)* ^{B}	*Overrun (%)*	*Melting rate (% melted after 20 min)* ^{B}
WL	43.91 ± 0.08^{a}	5.50 ± 0.01^{a}	0.27 ± 0.006^{a}	10.50 ± 0.04^{a}	29 ± 2^{a}	30.51 ± 0.06^{b}
CL	43.16 ± 0.07^{a}	5.50 ± 0.01^{a}	0.27 ± 0.004^{a}	10.40 ± 0.05^{a}	28 ± 1^{a}	27.82 ± 0.02^{d}
SL	43.94 ± 0.08^{a}	5.51 ± 0.01^{a}	0.27 ± 0.003^{a}	10.50 ± 0.02^{a}	26 ± 4^{a}	0.00 ± 0.07^{j}
SW1L	43.23 ± 0.15^{a}	5.50 ± 0.02^{a}	0.27 ± 0.006^{a}	10.40 ± 0.04^{a}	27 ± 4^{a}	0.23 ± 0.03^{j}
SW2L	43.42 ± 0.17^{a}	5.49 ± 0.01^{a}	0.36 ± 0.004^{a}	10.30 ± 0.05^{a}	28 ± 1^{a}	18.32 ± 0.04^{f}
SW3L	43.66 ± 0.15^{a}	5.50 ± 0.01^{a}	0.27 ± 0.003^{a}	10.50 ± 0.02^{a}	29 ± 1^{a}	28.78 ± 0.02^{dc}
SC1L	43.62 ± 0.10^{a}	5.50 ± 0.03^{a}	0.27 ± 0.009^{a}	10.30 ± 0.02^{a}	27 ± 5^{a}	0.10 ± 0.04^{j}
SC2L	42.79 ± 0.12^{a}	5.51 ± 0.01^{c}	0.27 ± 0.008^{a}	10.50 ± 0.01^{a}	27 ± 3^{a}	9.14 ± 0.01^{i}
SC3L	43.21 ± 0.11^{a}	5.50 ± 0.01^{a}	0.27 ± 0.005^{a}	10.40 ± 0.01^{a}	28 ± 3^{a}	12.99 ± 0.08^{h}
WB	43.91 ± 0.08^{a}	5.50 ± 0.01^{a}	0.27 ± 0.006^{a}	10.50 ± 0.04^{a}	29 ± 2^{a}	35.51 ± 0.04^{a}
CB	43.16 ± 0.07^{a}	5.50 ± 0.01^{a}	0.27 ± 0.004^{a}	10.40 ± 0.05^{a}	28 ± 2^{a}	29.82 ± 0.03^{bc}
SB	43.94 ± 0.08^{a}	5.51 ± 0.01	0.27 ± 0.003^{a}	10.50 ± 0.02^{a}	26 ± 4^{a}	0.00 ± 0.07^{j}
SW1B	43.23 ± 0.15^{a}	5.50 ± 0.02^{a}	0.27 ± 0.006^{a}	10.40 ± 0.04^{a}	27 ± 1^{a}	0.53 ± 0.03^{j}
SW2B	43.42 ± 0.17^{a}	5.49 ± 0.01^{a}	0.27 ± 0.004^{a}	10.30 ± 0.05^{a}	28 ± 4^{a}	21.32 ± 0.04^{e}
SW3B	43.66 ± 0.15^{a}	5.50 ± 0.01^{a}	0.27 ± 0.003^{a}	10.50 ± 0.02^{a}	28 ± 3^{a}	30.78 ± 0.02^{b}
SC1B	43.62 ± 0.10^{a}	5.52 ± 0.03^{a}	0.27 ± 0.009^{a}	10.30 ± 0.02^{a}	27 ± 4^{a}	0.30 ± 0.06^{j}
SC2B	42.79 ± 0.12^{a}	5.50 ± 0.01^{a}	0.27 ± 0.008^{a}	10.50 ± 0.01^{a}	27 ± 2^{a}	10.14 ± 0.05^{i}
SC3B	43.21 ± 0.11^{a}	5.51 ± 0.01^{a}	0.27 ± 0.005^{a}	10.40 ± 0.01^{a}	28 ± 2^{a}	15.99 ± 0.02^{g}

[A]Samples inoculated with La-05 and made with 100% bovine milk: WL; 100% coconut milk: CL; 100% soya milk: SL; 75% soya + 25% bovine milk: SW1L; 50% soya + 50% bovine milk: SW2L; 25% soya + 75% bovine milk: SW3L; 75% soya + 25% coconut milk: SC1L; 50% soya + 50% coconut milk: SC2L; 25% soya + 75% coconut milk: SC3L. Samples inoculated with Bb-12 made using 100% bovine milk: WB; 100% coconut milk: CB; 100% soya milk: SB; 75% soya + 25% bovine milk: SW1B; 50% soya + 50% bovine milk: SW2B; 25% soya + 75% bovine milk: SW3B; 75% soya + 25% coconut milk: SC1B; 50% soya + 50% coconut milk: SC2B; 25% soya + 75% coconut milk: SC3B.
[B]Means values ± standard deviation. [a–j]Means in the same column followed by different superscript letters are significantly different ($P < 0.05$).

ity (TA) and overrun were unchanged by the partial replacement of bovine milk with soya or coconut milks. However, melting rate did change with milk replacement.

Frozen desserts showed change in melting behaviour as a function of milk replacement. All vegetable and composite milk frozen desserts showed slower melting rates (0–30.78%) than the W sample (35.51%) ($P < 0.05$). The melting rate decreased with increasing soya milk content in frozen desserts, and the S sample showed the slowest melting rate among all treatments. This can be explained by the emulsifying properties of soya lecithin (Salem *et al.* 2005), which provides protection for the membrane proteins against damage due to freezing (Samoto *et al.* 2007). The proteins of soya milk also have the ability to bind with water molecules, which consequently prevents their free movement among other molecules of the mixture, by forming a stable gel network (Akesowan 2009). The contents of butter, which are used to balance the fat (10.5%), were less in coconut frozen desserts (7.31 g vs. 10.37 g for samples containing bovine milk and the sample with 100% soya milk). This is regarded as having a minor effect on melting behaviour. Hyvönen *et al.* (2003), for instance, reported that different types of fat (i.e. dairy and vegetable) had no significant effect on the perceived melting of ice creams, although the amount of fat did affect the melting rate of ice creams.

Frozen desserts containing bovine milk had a higher melting rate than those containing coconut milk. The melting rates of samples made with La-05 were lower than those made with Bb-12. This may be attributed to the differences in freezing points and recipe viscosities (Salem *et al.* 2005).

Effect of milk replacement on droplets suspension

Measurement of zeta potential (the electrical charge of droplets) together with particle size can be used to predict the stability of ice cream emulsions. Theoretically, a high negative zeta potential prevents the aggregation of emulsion droplets and increases stability through electrostatic repulsion (Achouri *et al.* 2012). The effect of fermentation and different milks on the microscopic structure of ice cream showed that ice creams containing coconut milk had larger fat globule sizes than others (Table 3). The coconut proteins have less surface activity than whey protein isolate. Because this gives rise to poor emulsifying, they are therefore not particularly effective at either creating small droplets or preventing droplet aggregation in ice cream mixes (Onsaard *et al.* 2006). In the results of fermentation, samples with higher particle sizes made a gel structure with larger aggregates instead of compact structures, which resulted in a higher firmness, and subsequently, an increase in the apparent viscosity of the fermented ice creams (Amatayakul *et al.* 2006).

Rheological measurement

The apparent viscosity, consistency index and flow behaviour index of the frozen fermented desserts produced with different milks are shown in Tables 4 and 5. All frozen desserts demonstrated non-Newtonian behaviour, that is their viscosity decreased with increasing shear rate (Figure 1). This viscosity decrease is partly due to the aggregation of fat globules, which decrease in size during shearing, and thus reduce the viscosity of the samples (Rossa *et al.* 2012). Such a decrease in apparent viscosity with the increase in shear rate is a common factor of milk products of similar texture, such as frozen yoghurt (Pinto *et al.* 2012). Samples made with La-05 tended to have a higher apparent viscosity than those made with Bb-12. Samples of CL, CB, WL and WB had a lower apparent viscosity than those containing soya milk. This could be explained by the ability of soya proteins to form a stable network that looks like a gel structure, because of their molecular properties, which readily bind with water molecules (Akesowan 2009), and hence

Table 3 Effect of mix formulation on zeta potential and particle diameter (D_m) of fat globules of frozen fermented desserts before and after fermentation (±SE, $n = 3$)

	Non fermented		*Fermented by La-05*		*Fermented by Bb-12*	
Samples	*Particle size (μm)*	*Zeta potential (mV)*	*Particle size (μm)*	*Zeta potential (mV)*	*Particle size (μm)*	*Zeta potential (mV)*
W	0.91 ± 0.08^{e}	-36.56 ± 0.80^{d}	4.86 ± 0.11^{e}	-35.02 ± 0.56^{b}	4.09 ± 0.09^{e}	-36.33 ± 0.40^{d}
C	1.74 ± 0.03^{b}	-30.70 ± 0.60^{b}	5.29 ± 0.06^{d}	-35.73 ± 0.62^{b}	4.68 ± 0.06^{d}	-37.40 ± 0.70^{d}
S	1.60 ± 0.10^{c}	-35.50 ± 0.70^{cd}	7.05 ± 0.08^{b}	-37.93 ± 0.71^{c}	7.70 ± 0.30^{b}	-37.67 ± 0.90^{d}
SW1	0.81 ± 0.03^{e}	-36.87 ± 0.90^{d}	6.84 ± 0.06^{c}	-37.57 ± 0.62^{c}	6.19 ± 0.50^{c}	-31.20 ± 0.60^{a}
SW2	0.82 ± 0.05^{e}	-37.60 ± 1.08^{d}	6.56 ± 0.10^{c}	-36.60 ± 0.41^{bc}	6.09 ± 0.21^{c}	-32.67 ± 0.50^{b}
SW3	0.83 ± 0.04^{e}	-26.40 ± 0.78^{a}	4.20 ± 0.08^{e}	-31.73 ± 1.30^{a}	4.07 ± 0.11^{e}	-30.80 ± 0.48^{a}
SC1	1.57 ± 0.06^{c}	-33.20 ± 0.65^{bc}	8.13 ± 0.04^{a}	-36.43 ± 0.54^{bc}	8.09 ± 0.30^{a}	-37.33 ± 0.80^{d}
SC2	1.68 ± 0.07^{c}	-34.30 ± 0.08^{cd}	8.86 ± 0.05^{a}	-38.13 ± 1.10^{c}	8.42 ± 0.30^{a}	-34.87 ± 1.04^{c}
SC3	2.54 ± 0.11^{a}	-26.70 ± 1.20^{a}	8.15 ± 0.02^{a}	-37.77 ± 0.72^{c}	8.07 ± 0.20^{a}	$-35.17 \pm 0.\ 80^{c}$

[a–j]Means in the same column followed by different letters are significantly different ($P < 0.05$).

Table 4 Rheological parameters of the frozen fermented desserts inoculated with Bb-12 obtained using the power law model

Samples	*Apparent viscosity (mPa s)*[A]	*K (Pa s^n)*[A]	*n^A*	*R^{2B}*
Upward curves				
WB	323 ± 1.02[g]	0.71 ± 0.02[g]	0.72 ± 0.01[ab]	0.998
CB	179 ± 1.10[h]	0.29 ± 0.02[h]	0.83 ± 0.02[a]	0.999
SB	2860 ± 0.91[a]	28.54 ± 0.02[a]	0.16 ± 0.02[f]	0.509
SW1B	1680 ± 1.02[b]	11.74 ± 0.02[b]	0.29 ± 0.02[ef]	0.796
SW2B	697 ± 0.88[e]	3.00 ± 0.00[e]	0.47 ± 0.02[dc]	0.954
SW3B	330 ± 0.91[g]	0.70 ± 0.02[g]	0.75 ± 0.02[a]	0.999
SC1B	1520 ± 1.03[c]	10.37 ± 0.01[c]	0.33 ± 0.02[df]	0.945
SC2B	1050 ± 0.78[d]	6.61 ± 0.02[d]	0.35 ± 0.02[de]	0.947
SC3B	537 ± 2.01[f]	1.94 ± 0.01[f]	0.56 ± 0.02[bc]	0.996
Downward curves				
WB	233 ± 1.03[g]	0.25 ± 0.02[h]	0.96 ± 0.01[a]	0.998
CB	182 ± 0.97[h]	0.33 ± 0.02[gh]	0.79 ± 0.02[ac]	0.996
SB	1430 ± 2.01[a]	4.48 ± 0.02[a]	0.60 ± 0.01[c]	0.990
SW1B	968 ± 1.76[b]	2.47 ± 0.01[b]	0.67 ± 0.00[dc]	0.985
SW2B	479 ± 1.43[d]	1.14 ± 0.02[e]	0.70 ± 0.02[bc]	0.993
SW3B	322 ± 1.02[f]	0.49 ± 0.02[g]	0.84 ± 0.01[ab]	0.984
SC1B	965 ± 2.10[b]	2.71 ± 0.01[c]	0.64 ± 0.01[dc]	0.991
SC2B	655 ± 1.50[c]	1.58 ± 0.02[d]	0.69 ± 0.02[bc]	0.991
SC3B	427 ± 1.43[e]	0.94 ± 0.02[f]	0.72 ± 0.02[bc]	0.994

[A]Mean values ± standard deviation. Values with different letters in the same column are significantly different ($P < 0.05$) (Tukey's test). [B]Coefficient of determination. *K*, consistency index; *n*, flow behaviour index.

Table 5 Rheological parameters of the frozen fermented desserts inoculated with La-05 obtained using the power law model

Samples	*Apparent viscosity (mPa s)*[A]	*K* (Pa s^n)[A]	n^A	R^{2B}
Upward curves				
WL	450 ± 2.01[h]	0.90 ± 0.01[i]	0.76 ± 0.01[a]	0.998
CL	420 ± 1.76[i]	1.09 ± 0.02[h]	0.66 ± 0.02[a]	0.990
SL	3770 ± 0.89[a]	43.12 ± 0.02[a]	0.11 ± 0.01[c]	0.600
SW1L	2080 ± 1.02[g]	12.61 ± 0.01[d]	0.35 ± 0.02[b]	0.920
SW2L	1680 ± 1.66[c]	9.56 ± 0.02[e]	0.38 ± 0.01[b]	0.950
SW3L	818 ± 1.32[f]	2.32 ± 0.02[f]	0.64 ± 0.02[a]	0.990
SC1L	3440 ± 1.1[b]	36.41 ± 0.01[b]	0.14 ± 0.02[c]	0.420
SC2L	1990 ± 1.32[d]	17.25 ± 0.01[c]	0.22 ± 0.02[bc]	0.740
SC3L	556 ± 1.03[g]	1.95 ± 0.02[g]	0.59 ± 0.01[a]	0.990
Downward curves				
WL	437 ± 1.52[h]	0.93 ± 0.02[g]	0.74 ± 0.01[a]	0.996
CL	373 ± 0.83[i]	0.87 ± 0.02[g]	0.71 ± 0.01[a]	0.997
SL	1720 ± 1.07[a]	5.24 ± 0.02[a]	0.61 ± 0.02[a]	0.990
SW1L	1370 ± 1.32[c]	4.33 ± 0.02[b]	0.61 ± 0.02[a]	0.994
SW2L	1120 ± 0.94[d]	3.01 ± 0.02[c]	0.66 ± 0.01[b]	0.993
SW3L	760 ± 0.87[f]	2.07 ± 0.02[e]	0.66 ± 0.02[a]	0.997
SC1L	1550 ± 2.01[b]	4.16 ± 0.02[b]	0.66 ± 0.02[a]	0.990
SC2L	990 ± 1.43[e]	2.43 ± 0.02[d]	0.69 ± 0.02[a]	0.996
SC3L	500 ± 1.62[g]	1.17 ± 0.02[f]	0.70 ± 0.01[a]	0.995

[A]Mean values ± standard deviation. Values with different letters in the same column are significantly different ($P < 0.05$) (Tukey's test). [B]Coefficient of determination. *K*, consistency index; *n*, flow behaviour index.

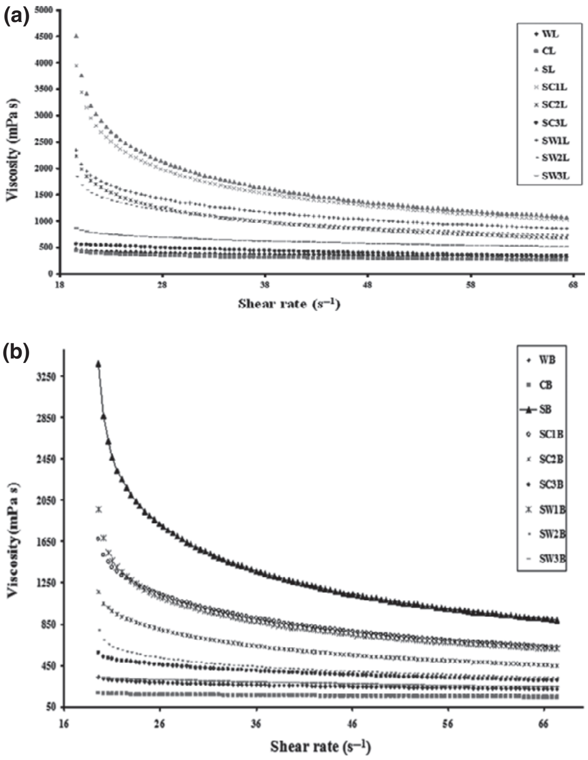

Figure 1 Effect of shear rate on the apparent viscosity of frozen fermented desserts inoculated with La-05 (a) and Bb-12 (b). Samples inoculated with La-05 and made with 100% bovine milk: WL; 100% coconut milk: CL; 100% soya milk: SL; 75% soya + 25 % bovine milk: SW1L; 50% soya + 50% bovine milk: SW2L; 25% soya + 75% bovine milk: SW3L; 75% soya + 25% coconut milk: SC1L; 50% soya + 50% coconut milk: SC2L; 25% soya + 75% coconut milk: SC3L. Samples inoculated with Bb-12 made using 100% bovine milk: WB; 100% coconut milk: CB; 100% soya milk: SB; 75% soya + 25% bovine milk: SW1B; 50% soya + 50% bovine milk: SW2B; 25% soya + 75% bovine milk: SW3B; 75% soya + 25% coconut milk: SC1B; 50% soya + 50% coconut milk: SC2B; 25% soya + 75% coconut milk: SC3.

present greater resistance to flow. The formation of gel by globular proteins is a complex process that may involve reactions, such as molecular unfolding, dissociation–association and aggregation (Batista *et al.* 2005). The soya proteins were shown to adsorb at the interface of oil droplets, with surface loads varying between 2 and 4 mg/m^2 and a layer thickness of between 30 and 40 nm (Keerati-u-rai and Corredig 2011). Hence, this could explain the highest apparent viscosity shown in S sample, followed by SC1 and SW1 samples (Tables 4 and 5). Among frozen desserts with composite milks, samples containing coconut milk had higher apparent viscosities than those containing bovine milk. This may be attributed to the higher particle size of frozen desserts containing coconut milk, due to the poor emulsifying properties of coconut proteins. This resulted in an ineffective prevention of droplet aggregation and the creation of small droplets during or after homogenisation (Onsaard *et al.* 2006). This is in contrast to the effects of the milk protein concentrates in ice cream, which increase viscosities, due to the increased voluminosity of the dispersed particles (as described by the Eilers equation) (Alvarez *et al.* 2005).

The frozen dessert's rheological behaviour after the reduction of shear rate (i.e. downward curves) is shown in Tables 4 and 5. The consistency index (K) varied from 0.25 to 43.12 Pa/s ($P < 0.05$) (Tables 4 and 5). SL and SB samples had the highest consistency index. The highest K values were related to samples containing soya milk that also increased with increasing soya milk content. This further demonstrated that the addition of soya milk increased the resistance of structural breakdown due to the aggregation of soya proteins resulting in gel formation and subsequent increase in water retention (Zayas 1997). The flow behaviour index (n), which reflects the degree of pseudoplasticity characteristics of a fluid, ranged from 0.11 to 0.96 ($n = 1$) ($P < 0.05$). Furthermore, the n values of upward curve were less than those of the downward curve, indicating a decrease in the pseudoplastic properties as the shear rate decreased. The decrease in K and the increase in n can be ascribed to the structural rupture of the frozen dessert protein network, because of shearing, which favours this behaviour (Rossa *et al.* 2012).

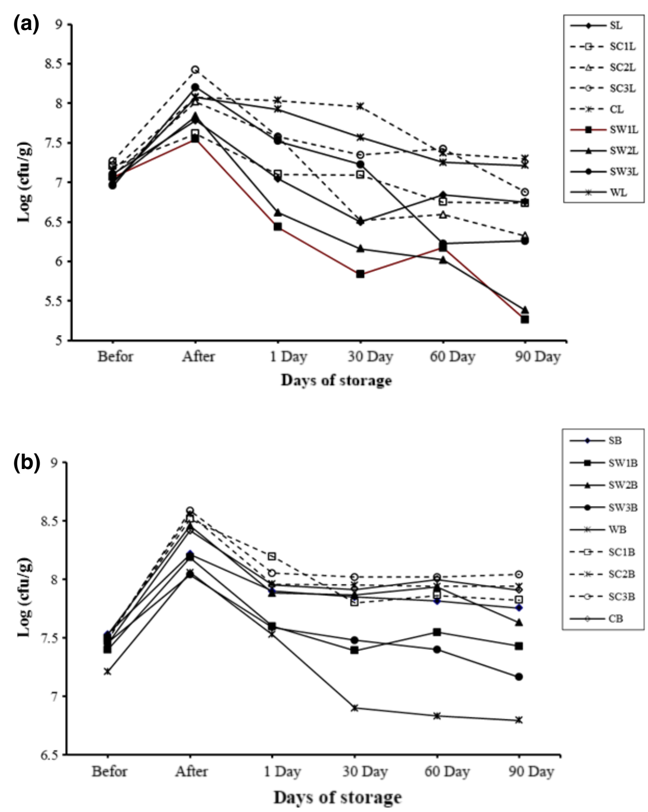

Figure 2 Effect of mix formulation on the viable counts of La-05 (a) and Bb-12 (b) in frozen fermented desserts during 90-day storage at −20 °C.

Viability of probiotic bacteria in frozen fermented desserts

Figure 2 shows the change in bacterial counts in frozen fermented desserts made using bovine or vegetable milks. The survival rate of probiotic bacteria in samples after 90 days tended to be higher in Bb-12 than in the presence of La-05 (Table 6). The reduction in viable bacterial counts, which occurred during freezing, is associated with the freeze injury of cells. Furthermore, the mechanical stresses associated with the mixing and freezing processes, which incorporate oxygen into the mixture, may be responsible for the further bacterial count decline (Haynes and Playne 2002). *Bifidobacterium* tended to have a better survival rate than *Lactobacillus* in all samples. This is similar to the findings reported by Akin *et al.* (2007). The survival of both probiotics in samples increased slightly ($P < 0.05$) in the presence of both soya and coconut milks. The survival rates of Bb-12 after 90 days were 94.42, 91.88, 92.78, 93.66, 93.95, 90.73, 90.22, 89.11 and 84.32% in S, SC1, SC2, SC3, CB, SW1, SW2, SW3 and W samples, respectively. The highest survival rate of Bb-12 occurred in S, C and SC3 samples, and the lowest occurred in the WB sample (Table 6). Among the composite milk samples, those containing coconut milk had a higher survival rate percentage than those containing bovine milk. The survival rate of Bb-12 increased with increasing soya milk content in samples containing bovine milk, but decreased with increasing soya milk content in samples containing coconut milk. Bovine milk frequently does not support the extensive growth of bacteria, due to a lack of the free amino acids that are essential for the growth of *bifidobacteria,* such as glutamic acid, arginine, leucine, isoleucine, cysteine, valine, tryptophan and tyrosine. However, most of the proteins (80%) contained within coconut milk can be classified as globulins and albumins, which contain relatively high levels of glutamic acid, aspartic acid and arginine. Hence, coconut milk is a richer medium than bovine milk (Yuliana *et al.* 2010). Moreover, under comparable conditions *Bifidobacterium* can grow more extensively in soya than in cow milk (Farnworth *et al.* 2007). This is probably because soya milk contains oligosaccharides (raffinose and stachyose), which may be utilised by *bifidobacteria* (Farnworth *et al.* 2007). Throughout the 90-day storage at −20 °C, the survival rate percentage of La-05 was 86.74, 88.46, 78.85, 81.62, 90.42, 69.69, 68.65, 76.26 and 89.30 in S, SC1, SC2, SC3, C, SW1, SW2, SW3 and W samples, respectively. The highest survival rate of La-05 was achieved by sample C (Table 6). Among the composite milk samples, those containing coconut milk had a higher survival rate than those containing bovine milk, which may have been due to a lack of free amino acids in bovine milk (Magarinos *et al.* 2007). The viability of La-05 increased with decreasing soya milk content in frozen desserts containing coconut milk (probably because the main disaccharide in coconut milk is sucrose, which La-05 is able to utilise) (Yuliana *et al.* 2010), but decreased with increasing soya milk content in ice creams containing bovine milk, because the original medium of La-05 is dairy milk, and soya milk has no nutritionally fastidious La-05 (Yuliana *et al.* 2010). Furthermore, Table 6 shows that the survival rate of La-05 in sample S is lower than that of the C and W samples.

Sensory evaluation

The replacement of dairy milk by vegetable milks decreased the body–texture, colour and taste of frozen fermented desserts (Figure 3). Frozen desserts containing higher amounts of soya milk showed lower total sensory scores, due to a decrease in their colour and taste scores that are associated with soya milk's unnatural colour and woody or beany off-flavours (Donkor 2007), and in the texture of the samples, as a result of the emulsifying properties of proteins and lecithin in soya milk (Salem *et al.* 2005). Among composite milk samples, those containing bovine milk had a higher

Table 6 Counts of probiotic bacteria in mixtures and frozen fermented desserts

	Fermented by La-05			*Fermented by Bb-12*		
Samples	*Mixture after fermentation (Log10 cfu/g)*	*Frozen dessert after 90 days (Log10 cfu/g)*	*Survival rate (%)*	*Mixture after fermentation (Log10 cfu/g)*	*Frozen dessert after 90 days (Log10 cfu/g)*	*Survival rate (%)*
W	8.08 ± 0.04	7.21 ± 0.08	89.30^{ab}	8.06 ± 0.09	6.79 ± 0.07	84.32^{e}
C	8.07 ± 0.04	7.3 ± 0.08	90.42^{a}	8.42 ± 0.07	7.91 ± 0.1	93.95^{a}
S	7.78 ± 0.08	6.75 ± 0.04	86.74^{c}	8.21 ± 0.05	7.76 ± 0.07	94.42^{a}
SC1	7.61 ± 0.07	6.74 ± 0.07	88.46^{b}	8.51 ± 0.08	7.82 ± 0.10	91.88^{bc}
SC2	8.02 ± 0.03	6.32 ± 0.05	78.85^{e}	8.56 ± 0.10	7.94 ± 0.07	92.78^{ab}
SC3	8.42 ± 0.04	6.87 ± 0.05	81.62^{d}	8.59 ± 0.07	8.04 ± 0.06	93.66^{a}
SW1	7.55 ± 0.03	5.26 ± 0.11	69.69^{g}	8.19 ± 0.05	7.43 ± 0.04	90.73^{cd}
SW2	7.84 ± 0.06	5.38 ± 0.09	68.65^{g}	8.46 ± 0.02	7.63 ± 0.02	90.22^{cd}
SW3	8.20 ± 0.07	6.26 ± 0.08	76.26^{f}	8.04 ± 0.07	7.16 ± 0.07	89.11^{d}

$^{a–j}$Means in the same column followed by different letters are significantly different ($P < 0.05$).

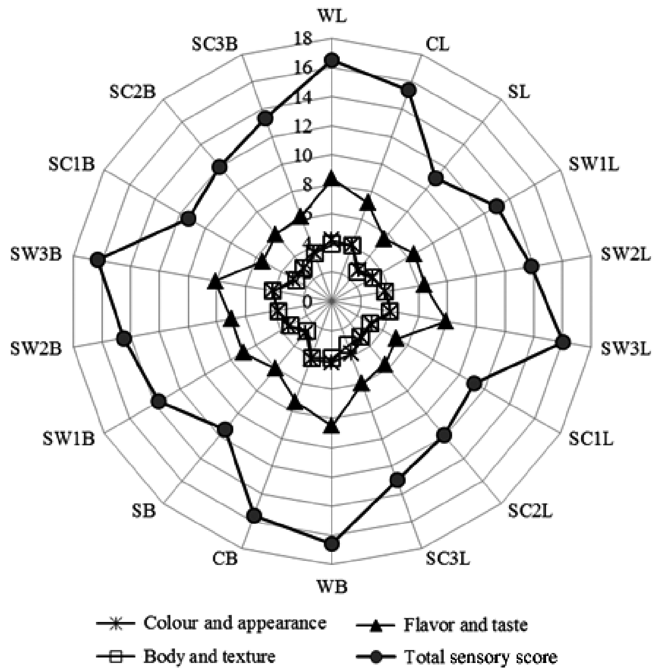

Figure 3 Effect of mix formulation on sensory evaluation of frozen fermented desserts ($P < 0.05$). Note: Sample inoculated with La-05 and made with 100% bovine milk: WL; 100% coconut milk: CL; 100% soya milk: SL; 75% soya + 25 % bovine milk: SW1L; 50% soya + 50% bovine milk: SW2L; 25% soya + 75% bovine milk: SW3L; 75% soya + 25% coconut milk: SC1L; 50% soya + 50% coconut milk: SC2L; 25% soya + 75% coconut milk: SC3L. Sample inoculated with Bb-12 made using 100% bovine milk: WB; 100% coconut milk: CB; 100% soya milk: SB; 75% soya + 25% bovine milk: SW1B; 50% soya + 50% bovine milk: SW2B; 25% soya + 75% bovine milk: SW3B; 75% soya + 25% coconut milk: SC1B; 50% soya + 50% coconut milk: SC2B; 25% soya + 75% coconut milk: SC3B.

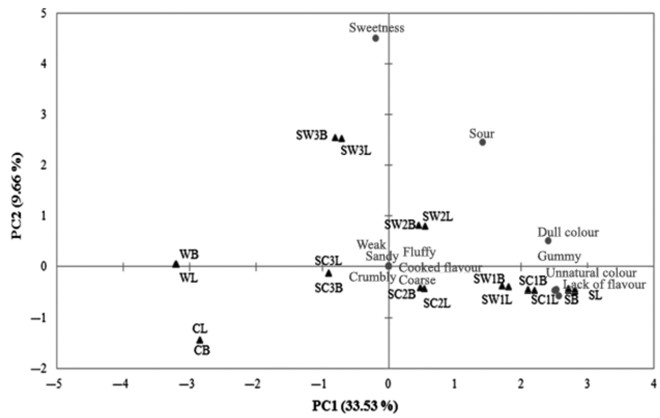

Figure 4 Principal component analysis (PCA) of frozen fermented desserts with sensory attributes on PC1 and PC2 ($P < 0.05$). Note: Sample inoculated with La-05 and made with 100% bovine milk: WL; 100% coconut milk: CL; 100% soya milk: SL; 75% soya + 25% bovine milk: SW1L; 50% soya + 50% bovine milk: SW2L; 25% soya + 75% bovine milk: SW3L; 75% soya + 25% coconut milk: SC1L; 50% soya + 50% coconut milk: SC2L; 25% soya + 75% coconut milk: SC3L. Sample inoculated with Bb-12 made using 100% bovine milk: WB; 100% coconut milk: CB; 100% soya milk: SB; 75% soya + 25% bovine milk: SW1B; 50% soya + 50% bovine milk: SW2B; 25% soya + 75% bovine milk: SW3B; 75% soya + 25% coconut milk: SC1B; 50% soya + 50% coconut milk: SC2B; 25% soya + 75% coconut milk: SC3B.

total sensory scores than those containing coconut milk. The highest total sensory scores was achieved by the sample made with 100% bovine milk, whereas the lowest was seen in sample made with 100% soya milk (Figure 3).

The PCA was carried out on two principal components (Figure 4). The first axis (PC1) explained 33.53% of the total variation in the data set and was dominated by lack of flavour, gummy, unnatural colour and dull colour attributes. The second axis (PC2) explained 9.66% of the total variation and was controlled by sweetness. According to the values obtained by the PCA, some of the descriptors could be correlated. For example, dull colour, unnatural colour, lack of flavour and gummy were positively correlated with one another. Moreover, all of the flavour attributes were positively correlated with the colour attributes. W (WL and WB) and C (CL and CB) ice creams had similar minimum defects properties. However, composite milk ice creams containing higher amounts of soya milk or those containing coconut milk were more unnatural, dull in colour and appearance, gummier in texture and poor in flavour. No significant difference between samples can be attributed to the type of probiotic (i.e. La-05 and Bb-12) used. None of the ice creams were judged to be weak, crumbly, sandy, fluffy, coarse, or have a cooked flavour. All samples gave a good total impression, that is scored as medium sour.

CONCLUSION

The use of different types of milk changed the physical and sensory properties of frozen fermented dessert and the survival of probiotics. Vegetable milks increased the values of consistency index, apparent viscosity and melting resistance. The survival of Bb-12 and La-05 was increased by replacing bovine milk with vegetable milks. However, replacing bovine milk with vegetable milks decreased the total sensory scores of frozen desserts, particularly with increasing soya milk content. This study demonstrates that while replacement of bovine milk with vegetable milks improves rheology and physical properties, and the survival of probiotics, they still have less desirable affects in the organoleptic properties of frozen fermented dessert.

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