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Original paper

Effect of Vegetable Milk on Survival of Probiotics in Fermented Ice Cream under Gastrointestinal Conditions

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The effect of the type of milk on *in vitro* gastrointestinal survival of probiotics (*Bifidobacterium animalis* subsp. *lactis* Bb-12 and *Lactobacillus acidophilus* La-05) and organoleptic properties of ice creams were evaluated using ice creams made with cow, soy, coconut, and composite (cow or coconut with soy) milks. Soy milk was found to significantly improve the acid and bile tolerance of the probiotics but it decreased the total acceptability of ice cream. The probiotics in ice creams containing composite milk with cow's milk had higher total acceptability and were more protected against gastrointestinal conditions than ice creams containing coconut milk and also they have than others containing coconut milk. The survival of Bb-12 was also better than La-05 in these conditions. In general, the presence of soy milk in ice creams resulted in a substantial improvement in probiotic tolerance to gastrointestinal conditions.

Keywords: probiotics, vegetable milk, cow's milk, ice cream, gastrointestinal conditions.

Introduction

Probiotics are defined as microorganisms which, when administered in adequate amounts, confer several health benefits to the consumer. These include an improvement in intestinal microflora, a reduction in serum cholesterol, the inhibition of the growth of potential pathogens, and the activation of the immune system (Grajek *et al.,* 2005). In order for probiotics to flourish in the intestine and impart their beneficial effects, they have to be able to survive passage through the host's hostile digestive tract environment (Maragkoudakis *et al.,* 2006). Food generally remains in the stomach for 2 to 4 h, and then it transits through the small intestine over 1 to 4 h. The main factors that are detrimental to the survival of probiotics in the stomach are the low gastric pH and the antimicrobial action of pepsin. The normal pH range of the stomach is 2.5 − 3.5, but it can be as low as pH 1.5 or increase to pH 6 or more, after food intake. Probiotic bacteria may also need to survive the environment in the small intestine where it is exposed to pancreatin and bile salts, with a pH of around 8.0. The tolerance of probiotic bacteria to conditions in the stomach and small intestine is influenced by the carrier. Foods are the most common carriers for probiotics, which may protect the probiotic bacteria from acid conditions and enhance gastric survival (Huang and Adams, 2004). The protection provided by food against gastrointestinal stress is important with respect to (i) increase the pH of the gastric tract, due to food formulations with a high buffering capacity and an appropriate pH of > 5, and (ii) reduce the physical exposure of probiotics to harsh gastrointestinal environments (Ranadheera *et al.*, 2012).

Earlier studies have shown that probiotics with *Lactobacillus* and *Bifidobacterium*, can be protected during passage through the gastrointestinal tract, and hence improve their viability, by incorporating them in appropriate food carier including i) two liquid vegetarian foods: Up and Go® liquid breakfast, and So-Good™ original soy milk (Huang and Adams, 2004), ii) cheese with a high-fat content (Valerio *et al.,* 2006), and iii) ice cream (Ranadheera *et al.,* 2012). Therefore, delivery in a suitable food

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matrix is one of the most appropriate means to maximise probiotic efficacy (Huang and Adams, 2004).

The aim of this study was to evaluate the *in vitro* gastrointestinal tolerance of Bb-12 and La-05 in ice creams made with different milks and their organoleptic properties. It was also to prepare carrier products to promote more active probiotic cultures that protect against gastrointestinal stress with high acceptability in sensory properties.

Materials and Methods

Materials Fresh cow milk, coconut, soybean, 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 diacyl-glycerols of fatty acid, cellulose gum, guar gum, carrageenan were used as stabilizers. *B. animalis* subsp. *lactis* (Bb-12) and *Lactobacillus acidophilus* (La-05) were obtained as pure freeze dried probiotic culture from CHR-Hansen (Horsholm, Denmark). Pepsin (1:10,000, ICN), bile salts and pancreatin (P-1500), sodium chloride, hydrogen chloride and sodium hydroxide were purchased from Sigma Chemical Company (St Louis, MO USA) and maximum recovery diluents (MRD) was purchased from Oxoid company (Australia).

Preparation of starter culture Each strain (1 g) was cultured in 100 mL of sterilized skimmed milk (10 w/v), amplified by the addition of 0.05% (w/v) *L-Cysteine hydrochloride,* 1% (w/v) yeast extract and 2% (w/v) glucose.The incubation was carried out under aerobic condition in a water bath at 42℃ until a pH of 5.0 was reached (Magarinos *et al*., 2007).

Preparation of intermediate culture Inoculation culture for each strain was prepared fresh by adding 4 mL of starter culture into 100 mL of sterilized skimmed milk. Incubation was carried out under anaerobic condition in a water bath at 42℃ until pH reduced to 5.0 (Magarinos *et al*., 2007).

Preparation of soy milk Soybeans (100 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. 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℃ for 10 min and then immediately chilled (4℃) prior to making ice cream (Aboulfazli *et al*., 2014).

Preparation of coconut milk The brown hard coconut shell was cracked open and the white copra was grated 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℃ for 10 min prior to chilling (4℃) and was used within 1 h (Aboulfazli *et al*., 2014).

Preparation of ice cream Ice cream was prepared using various combinations of coconut or cow with soy milk. Ice cream mix was formulated to maintain properties of milks with 43% total solids and 10.5% fat for a total batch of 100 g (Table 1).

The milk or milk combinations with butter were heated to 50℃ before being mixed with the skim milk powder, sugar and water. The mixtures were subjected to two stages of homogenization (Ika Homogenizer T-25 basic Ultra Turrax, Germany) at 16000 rpm for 5 min. The mixtures were then pasteurized at 80℃ for 10 min in a water bath, cooled to 4℃ and aged overnight at 4℃. Then 4% (w/w) fermented milk (inoculation culture) was added to ice cream mixtures and these were incubated in a water bath (42℃) for varying lengths of time until the pH reduced to 5.50. After

Table 1. The content of components used in ice cream mix formulations (percentage by weight)

Sample [A]	Ingredient						
	Milk formula (%)	Butter (%) (Fat = 83.3%)	Skim milk powder (%)	Sugar (%)	Stabilizer–Emulsifier (%)	Vanillin (%)	Water (%)
W	55.4	10.37	7	17	0.6	0.1	9.62
C	55.4	7.31	7	17	0.6	0.1	9.62
S	55.4	10.37	7	17	0.6	0.1	9.62
SW1	55.4	10.37	7	17	0.6	0.1	9.62
SW2	55.4	10.37	7	17	0.6	0.1	9.62
SW3	55.4	10.37	7	17	0.6	0.1	9.62
SC1	55.4	9.6	7	17	0.6	0.1	9.62
SC2	55.4	8.84	7	17	0.6	0.1	9.62
SC3	55.4	8.08	7	17	0.6	0.1	9.62

[A]W: ice cream with 100% cow milk; C: ice cream with 100% coconut milk; S: ice cream with 100% soy milk; SW1: ice cream with 75% soy+25%cow milk; SW2: ice cream with 50% soy+50% cow milk; SW3: ice cream with 25% soy+75%cow milk; SC1: ice cream with 75% soy+25% coconut milk; SC2: ice cream with 50% soy+50% coconut milk; SC3: ice cream with 25% soy+75% coconut milk.

fermentation, the mixtures were cooled to 4℃ in an ice bath followed by freezing in a 1.5 L batch ice cream freezer (Baumatic gelato1ss, UK) and packed in 100 mL plastic cups. The cups were covered using the lids and these were stored at −20℃ in the freezer.

Chemical analysis The pH of ice creams was measured using digital pH meter. Titratable acid (TA) and total solid were determined according to Akin *et al.* (2007). Fat content was calculated by weight after alkaline hydrolysis coupled with soxhlet extraction (petroleum ether) (AOAC, 2005).

Preparation of simulated gastric and intestinal juices Simulated gastric juice (SGJ) was prepared by suspending pepsin (1:10,000, ICN) (Sigma-Aldrich, USA) in sterile filtered 0.5% (w/v) sodium chloride solution to a final concentration of 3 g/L, with the pH adjusted to 2.0 with concentrated hydrogen chloride or sterile 0.1 mol/L sodium hydroxide. Artificial small intestinal juice was prepared by suspending pancreatin USP (P-1500, Sigma-Aldrich, USA) in sterile 0.5% sodium chloride (w/v) solution to a final concentration of 1 g/L, with 0.3% bile salts (Oxoid, Australia) and adjusting pH to 8.00 with sterile 0.1 mol/L sodium hydroxide. Both solutions were filter sterilized through a 0.22 μm membrane (Huang and Adams, 2004).

Cell tolerance to gastrointestinal Fermented ice cream samples (1 g) were transferred into sterile 15 mL falcon tubes containing 9 mL of either artificial gastric or small intestinal juices. The mixture was then homogenized using a vortex mixer (Ratek Instruments Pty Ltd., Australia) at maximum setting for 10 s and incubated at 37℃. Aliquots of 1 mL were removed from tubes (after 1, 30 and 120 min in order to assess acid tolerance and after 1, 60 and 120 min in order to determine bile tolerance) for the determination of total viable cell counts (Huang and Adams, 2004).

Determination of total viable cell counts Aliquots (1 mL) of ice creams subjected to gastrointestinal juices were serially diluted with maximum recovery diluents (MRD) (Oxoid, Australia) and aliquots (1 mL) of the dilutions pour plated in triplicate on MRS agar for *L. acidophilus* and MRS agar supplemented with 0.05% (w/v) *L-Cysteine hydrochloride* (Merck) for *B. lactis*. The plates were incubated at 38 ± 1℃ for 72 h under aerobic condition with 5% CO_2 (v/v) for *L. acidophilus* and under anaerobic condition (Anaerocult A) for *B. animalis* subsp. *lactis*. The bacterial viability was represented as survival rate (Magarinos *et al*., 2007).

Sensory analysis The ice creams were organoleptically evaluated by 42 panelists (25 − 30 year; 22 males, 20 females), using a sensory rating scale of 1 − 10 for taste and flavor, and 1 − 5 for consistency and 1 − 5 for appearance and color. The properties evaluated contained (a) three characteristics for appearance and color (no criticism: 5, dull color: 4 − 1, unnatural color: 3 − 1), (b) seven properties for taste and flavor (no criticism: 10, cooked flavor: 9 − 7, lack of sweetness and too sweet: 9 − 7, lack of flavor: 8 − 6, rancid and oxidized:6 − 1, and other: 5 − 1) and (c) seven terms describing texture and body (no criticism: 5, coarse: 4 − 1, crumbly: 4 − 2, weak: 4 − 1, fluffy: 3 − 1, gummy: 4 − 1, sandy: 2 − 1) (Akin *et al.,* 2007).

Statistics The experiments were assayed in triplicates, and the results were expressed as mean±S.E.M (standard mean error) values. The statistical analysis was carried out using SPSS/PASW statistical software version 17 (SPSS Inc., Chicago, IL, USA). Analysis of variance (ANOVA) and analysis of variance with repeated measures were used in data analyzing with Bonferroni post hoc test for means comparison. The criterion for statistical significance was $p < 0.05$ (Ranadheera *et al.,* 2012).

Result and Discussion

Composition and chemical properties The composition and chemical properties of the ice creams are presented in Table 2. The total solid, fat, pH and titratable acidity were unchanged by the partial replacement of cow's milk with soy, coconut or composite milks.

Simulated gastric and intestinal conditions Each probiotic showed a progressive reduction in viability during a 120 min exposure to gastric juice. Bb-12 showed much greater tolerance to the exposure to gastric juice than La-05, as defined in Table 3. This is in agreement with the results of Grimoud *et al.* (2010), which found that La-05 was more sensitive to high acid conditions, compared to Bb-12. For ice creams made with composite milk, the survival of both Bb-12 and La-05 was higher in samples containing cow's milk, than those containing coconut milk after 120 min. The bacteria survival after 120 min exposure to *in vitro* gastric conditions also increased with a higher soy milk content in the ice cream. The highest tolerance percentage of Bb-12 to gastric juice was found in SW1B, SW2B, and SB ice creams, whereas the lowest tolerance percentage was in SC3B ice cream after 120 min. The highest survival of La-05 during *in vitro* gastric conditions was in SW1L, SW2L, and SL ice creams, whereas the lowest was found in SC3L, SC2L, and SW3L ice creams after 120 min.

The simulated intestinal juice, with 0.3% bile salt, significantly reduced probiotic viability (Table 4). This occurred as early as one minute after exposure to bile salt for both bacteria, whereas Bb-12 showed a higher survival than La-05. Among the ice creams with composite milk, the survival of both probiotics was higher in those containing cow's milk, and their survival increased in ice creams made with composite milk, where the soy milk content was higher after 120 min exposure to bile salt. The highest survival of Bb-12 in the presence of simulated small intestine juice comprising 0.3% bile was noted in SW1B ice cream, whereas the lowest occurred in SC3B ice cream after 120 min. For La-05, the highest survival was in SL and WL ice cream and the lowest was in SC3L ice cream after 120 min.

In the present study, transit time had a significant influence on the bile salt and gastric tolerance of probiotics. When probiotics were exposed to gastric conditions for longer time periods, the loss of probiotic viability increased. In accordance with other research, the survival of both the probiotic strains was progressively reduced during an *in vitro* 120 min gastric and small intestine transit.

Table 2. Composition and chemical properties of experimental ice creams.

Samples [A]	Composition		Chemical properties			
	Total solids (g/100g)[B]	Fat (g/100g)[B]	Titratable acidity (%lactic acid)[B]	pH(value) [B]		
				After fermented	In simulated gastric	In intestinal juices
WL	43.91 ± 0.08[a]	10.5 ± 0.04[a]	0.27 ± 0.006[a]	5.50 ± 0.01[a]	4.46 ± 0.01[a]	5.92 ± 0.01[a]
CL	43.16 ± 0.07[a]	10.4 ± 0.05[a]	0.27 ± 0.004[a]	5.50 ± 0.01[a]	4.45 ± 0.01[a]	5.91 ± 0.01[a]
SL	43.94 ± 0.08[a]	10.5 ± 0.02[a]	0.27 ± 0.003[a]	5.51 ± 0.01[a]	4.37 ± 0.01[a]	5.91 ± 0.01[a]
SW1L	43.23 ± 0.15[a]	10.4 ± 0.04[a]	0.27 ± 0.006[a]	5.50 ± 0.02[a]	4.43 ± 0.02[a]	5.90 ± 0.01[a]
SW2L	43.42 ± 0.17[a]	10.3 ± 0.05[a]	0.36 ± 0.004[a]	5.49 ± 0.01[a]	4.43 ± 0.01[a]	5.92 ± 0.01[a]
SW3L	43.66 ± 0.15[a]	10.5 ± 0.02[a]	0.27 ± 0.003[a]	5.50 ± 0.01[a]	4.45 ± 0.01[a]	5.91 ± 0.01[a]
SC1L	43.62 ± 0.10[a]	10.3 ± 0.02[a]	0.27 ± 0.009[a]	5.50 ± 0.03[a]	4.45 ± 0.03[a]	5.92 ± 0.01[a]
SC2L	42.79 ± 0.12[a]	10.5 ± 0.01[a]	0.27 ± 0.008[a]	5.51 ± 0.01[a]	4.47 ± 0.01[a]	5.90 ± 0.01[a]
SC3L	43.21 ± 0.11[a]	10.4 ± 0.01[a]	0.27 ± 0.005[a]	5.50 ± 0.01[a]	4.45 ± 0.01[a]	5.91 ± 0.01[a]
WB	43.91 ± 0.08[a]	10.5 ± 0.04[a]	0.27 ± 0.006[a]	5.50 ± 0.01[a]	4.46 ± 0.01[a]	5.90 ± 0.01[a]
CB	43.16 ± 0.07[a]	10.4 ± 0.05[a]	0.27 ± 0.004[a]	5.50 ± 0.01[a]	4.47 ± 0.01[a]	5.91 ± 0.01[a]
SB	43.94 ± 0.08[a]	10.5 ± 0.02[a]	0.27 ± 0.003[a]	5.51 ± 0.01[a]	4.45 ± 0.01[a]	5.89 ± 0.01[a]
SW1B	43.23 ± 0.15[a]	10.4 ± 0.04[a]	0.27 ± 0.006[a]	5.50 ± 0.02[a]	4.44 ± 0.02[a]	5.90 ± 0.01[a]
SW2B	43.42 ± 0.17[a]	10.3 ± 0.05[a]	0.27 ± 0.004[a]	5.49 ± 0.01[a]	4.42 ± 0.01[a]	5.91 ± 0.01[a]
SW3B	43.66 ± 0.15[a]	10.5 ± 0.02[a]	0.27 ± 0.003[a]	5.50 ± 0.01[a]	4.44 ± 0.01[a]	5.91 ± 0.01[a]
SC1B	43.62 ± 0.10[a]	10.3 ± 0.02[a]	0.27 ± 0.009[a]	5.52 ± 0.03[a]	4.44 ± 0.03[a]	5.93 ± 0.01[a]
SC2B	42.79 ± 0.12[a]	10.5 ± 0.01[a]	0.27 ± 0.008[a]	5.50 ± 0.01[a]	4.45 ± 0.01[a]	5.91 ± 0.01[a]
SC3B	43.21 ± 0.11[a]	10.4 ± 0.01[a]	0.27 ± 0.005[a]	5.51 ± 0.01[a]	4.43 ± 0.01[a]	5.90 ± 0.01[a]

[A] ice cream 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. ice cream 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.

[a-b] Means in the same column followed by different letters were significantly different ($p < 0.05$).

However, strain-dependent rate variations were apparent in the loss of viability (Mishra and Prasad*.,* 2005). In general, La-05 showed lower bile and acid tolerance than Bb-12 in all ice creams after 120 min. Our finding reaffirm that probiotics have a lower tolerance to bile than to gut acid which is in agreement with in earlier studies (Mishra and Prasad., 2005; Chen *et al.,* 2005).

The results of the present study provide support for a recent clinical study, which indicated that bacterial strains as well as the food matrix, profoundly affect probiotic survival in the presence of simulated gastric and small intestine juices (Ranadheera *et al.,* 2012). Ranadheera *et al*. (2012) showed that the addition of carrier foods containing probiotics increased the pH of the gastric transit test mixture. The pH of the original mixtures was 2.0, 3.0, and 4.0, and these increased to 2.8, 3.9, and 6.3 respectively, in the presence of ice cream, and 2.6, 3.6, and 4.2 respectively, in the presence of plain and fruit yogurts. The survival of the probiotics was improved by an increase in the pH of the gastric content, as a result of the addition of the food matrix, because of the buffering capacity of the food carrier. However, in the present study, all the ice creams had a pH of around 5.5, so there were similar changes to the pH of the combined food and simulated juice mixtures, shown in Table 4. Klingberg and Budde (2006) mentioned that the survival during gastrointestinal transit of *Lactobacillus plantarum* MF 1298 improved in human subjects when administered with fermented sausage, because the sausage could protect the bacteria, for example by a simple physical "encapsulation" within the matrix of sausage meat and fat, or by acting as a buffer. Ranadheera *et al*. (2012) found the survival of probiotics in ice cream was better than in yogurt during gastrointestinal transit in human subjects, because of the higher fat content in ice cream at 10%, rather than 5% in yogurt. In addition, the presence of ingredients in ice creams, such as cocoa powder and stabilisers, such as dextrose and guar gum, may also provide a protective barrier against small intestine and gastric juices. However, in the present study, apart from the types of milk used, the fat content and other ingredients (Table 4) are similar. Thus, the type of milk used could be the determining factor on probiotic viability, during simulated gastric and gastro intestinal transit. In general, the addition of soy milk significantly improved probiotic survival. This could be explained by the ability of soy proteins to form a stable protein network (Akesowan, 2009), and also that soy proteins can 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). Soy proteins may be able to form a stable layer with a

Table 3. Effect of ice creams with different milks on the survival of probiotics during 120 min exposure to simulated gastric juice at pH = 2.0 (n = 3).

Probiotic	Sample	Viable counts (log cfu/g) during simulated gastric transit tolerance				Survival of bacteria after 120 min (%)[A]
		0 min	1min	30 min	120 min	
L. acidophilus (La-5)	SL	7.51 ± 0.05^{d}	7.46 ± 0.04^{d}	7.49 ± 0.07^{c}	$7.31 \pm 0.03^{*c}$	97.34^{a}
	CL	7.77 ± 0.04^{b}	7.71 ± 0.02^{b}	$7.64 \pm 0.05^{*b}$	$7.27 \pm 0.02^{*c}$	93.56^{b}
	WL	7.97 ± 0.04^{a}	$7.88 \pm 0.02^{*a}$	$7.89 \pm 0.04^{*a}$	$6.70 \pm 0.07^{*d}$	84.06^{c}
	SW1L	7.61 ± 0.06^{c}	7.56 ± 0.04^{c}	$7.50 \pm 0.04^{*c}$	$7.49 \pm 0.05^{*b}$	98.42^{a}
	SW2L	7.75 ± 0.07^{b}	7.74 ± 0.06^{b}	7.68 ± 0.03^{b}	$7.56 \pm 0.04^{*a}$	97.55^{a}
	SW3L	7.33 ± 0.03^{e}	$7.16 \pm 0.07^{*e}$	$6.75 \pm 0.02^{*e}$	$5.44 \pm 0.02^{*h}$	74.21^{d}
	SC1L	7.28 ± 0.02^{e}	$6.70 \pm 0.07^{*f}$	$6.66 \pm 0.09^{*e}$	$6.05 \pm 0.08^{*e}$	83.10^{c}
	SC2L	7.27 ± 0.02^{e}	$6.26 \pm 0.02^{*g}$	$6.46 \pm 0.07^{*f}$	$5.75 \pm 0.09^{*f}$	74.48^{d}
	SC3L	7.63 ± 0.05^{c}	$5.,59 \pm 0.01^{*h}$	$5.48 \pm 0.03^{*g}$	$5.56 \pm 0.03^{*g}$	72.87^{d}
B. animalis subsp. *lactis* (Bb-12)	SB	7.40 ± 0.08^{d}	7.30 ± 0.07^{d}	$7.27 \pm 0.04^{*c}$	$7.26 \pm 0.04^{*c}$	98.11^{a}
	CB	7.82 ± 0.08^{a}	$7.51 \pm 0.06^{*c}$	$7.46 \pm 0.05^{*b}$	$7.44 \pm 0.02^{*b}$	95.14^{c}
	WB	7.27 ± 0.06^{d}	$7.01 \pm 0.06^{*e}$	$6.95 \pm 0.05^{*a}$	$6.93 \pm 0.02^{*d}$	95.32^{c}
	SW1B	7.07 ± 0.03^{e}	7.01 ± 0.04^{a}	7.00 ± 0.07^{d}	6.95 ± 0.09^{d}	98.30^{a}
	SW2B	7.38 ± 0.04^{d}	$7.30 \pm 0.03^{*e}$	$7.27 \pm 0.04^{*c}$	$7.25 \pm 0.06^{*c}$	98.24^{a}
	SW3B	7.57 ± 0.05^{c}	$7.31 \pm 0.03^{*e}$	$7.19 \pm 0.09^{*c}$	$7.16 \pm 0.08^{*c}$	94.58^{cd}
	SC1B	7.93 ± 0.07^{a}	7.87 ± 0.04^{a}	7.83 ± 0.07^{a}	$7.76 \pm 0.02^{*a}$	97.86^{b}
	SC2B	7.70 ± 0.04^{b}	7.57 ± 0.07^{a}	$7.47 \pm 0.03^{*b}$	$7.42 \pm 0.01^{*b}$	96.36^{b}
	SC3B	7.96 ± 0.04^{a}	$7.64 \pm 0.04^{*b}$	$7.52 \pm 0.05^{*b}$	$7.39 \pm 0.05^{*b}$	92.84^{d}

Means values ± standard deviation.

[*] In the same row indicates a significant difference of mean viable counts compared to that at 0 min ($p < 0.05$).

[a–h] Values in the same column having different superscripts for mean viable counts for each probiotic differ significantly ($p < 0.05$).

[A] Calculated by subtracting bacteria count at 0 min from bacteria count at 120 min, dividing by bacteria count at 0 min and multiplying by 100.

thickness of between 30 and 40 nm and thus increase physical protection by coating probiotics with these proteins. In the present study both probiotics viability remained significantly higher in gastric and small intestinal juices when fortified with ice cream containing cow milk. Ice cream is an emulsion of oil in water, in which fat droplets in the ice cream mix is stabilized by milk protein and emulsifiers (surfactant adhesion) to the oil/water interface (Ruger *et al*., 2002). Milk protein and emulsifiers covered the oil surface in ice cream (Goff, 2006). Probiotics may also be covered to considerable extent by a layer of protein and emulsifiers. This coating can protect probiotics from gastric conditions, the stability of which may depend on the emulsifying properties of milk proteins (their surface activity) at the outer oil water interface (Pimentel-González *et al.,* 2009). Coconut proteins have lower emulsifying property than cow milk proteins and this can be attributed to the less surface active for coconut proteins than for cow milk proteins (Tangsuphoom and Coupland, 2008). This may imply a protein coverage around probiotics with a lower stable than can cow milk proteins and thus results in faster elimination of the coating surrounding probiotics and the release of probiotics under gastric conditions in ice creams made with coconut milk than in ice creams made with cow milk. This could partially explain the lower survival of probiotics in coconut milk ice creams in contrast with cow milk ice creams under gastric conditions.

Sensory analysis No significant effects ($p > 0.05$) were observed between samples fermented with either with LA-05 and Bb-12 (Tables 5 and 6). The color score decreased with increasing soy milk and close to dull color. The ice creams containing cow milk had a higher color score than ice creams containing coconut milk. The texture score showed little differences among ice creams. There were no significant differences ($p > 0.05$) in sweetness and cooked flavor. However the flavor and taste score decreased with increasing soy milk with the lowest flavor and taste score being seen in SB ice cream. Ice creams containing cow milk had a higher flavor and aroma than ice creams containing coconut milk. In general the highest of total acceptability was seen in WL (16.02 ± 0.07) and WB (16.30 ± 0.05) and lowest in SC1L, SC2L, SL, SC1B, SC2B and SB. The total acceptability was higher in ice creams containing cow than in those containing coconut milk and it decreased with increasing soy milk amount in ice creams (Tables 5 and 6). None of the ice creams were judged to be weak, crumbly, sandy, fluffy, coarse or cooked flavor. All the samples gave a good total impression, were medium sour.

In conclusion, soy milk improved probiotic survival in

Table 4. Effect of ice creams with different milks on the survival of probiotics during 120 min exposure to simulated small intestinal juice pH = 8 (n = 3).

probiotic	Sample[A]	Viable counts (log cfu/g)				Survival of bacteria after 120 min (%) [A]
		0 min	1min	60 min	120min	
L. acidophilus (La-5)	SL	7.45 ± 0.02^{d}	$5.97 \pm 0.05^{*b}$	$5.60 \pm 0.02^{*a}$	$5.23 \pm 0.04^{*a}$	70.20^{a}
	CL	7.10 ± 0.04^{f}	$4.24 \pm 0.03^{*d}$	$3.93 \pm 0.02^{*e}$	$3.90 \pm 0.06^{*e}$	54.93^{bc}
	WL	7.46 ± 0.07^{d}	$6.22 \pm 0.03^{*a}$	$5.64 \pm 0.04^{*a}$	$5.03 \pm 0.06^{*b}$	67.43^{a}
	SW1L	7.21 ± 0.04^{e}	$6.14 \pm 0.08^{*a}$	$5.03 \pm 0.08^{*c}$	$4.18 \pm 0.07^{*d}$	58.00^{b}
	SW2L	7.60 ± 0.05^{c}	$6.18 \pm 0.09^{*a}$	$4.93 \pm 0.07^{*c}$	$4.03 \pm 0.07^{*e}$	53.03^{c}
	SW3L	7.93 ± 0.02^{a}	$5.92 \pm 0.09^{*b}$	$5.06 \pm 0.04^{*c}$	$4.13 \pm 0.09^{*d}$	52.08^{cd}
	SC1L	7.70 ± 0.01^{b}	$6.01 \pm 0.06^{*b}$	$5.60 \pm 0.03^{*a}$	$4.40 \pm 0.08^{*c}$	57.14^{b}
	SC2L	7.65 ± 0.05^{b}	$5.75 \pm 0.06^{*c}$	$4.39 \pm 0.03^{*d}$	$3.75 \pm 0.06^{*f}$	49.02^{d}
	SC3L	7.68 ± 0.03^{b}	$5.43 \pm 0.03^{*c}$	$4.28 \pm 0.04^{*d}$	$3.15 \pm 0.06^{*g}$	41.01^{e}
B. animalis subsp. *lactis* (Bb-12)	SB	7.61 ± 0.08^{b}	$7.16 \pm 0.04^{*a}$	$6.67 \pm 0.09^{*a}$	$6.35 \pm 0.03^{*a}$	83.44^{ab}
	CB	7.83 ± 0.08^{a}	$6.30 \pm 0.04^{*d}$	$5.77 \pm 0.08^{*e}$	$5.54 \pm 0.02^{*d}$	70.75^{f}
	WB	7.40 ± 0.03^{d}	$6.91 \pm 0.05^{*b}$	$6.67 \pm 0.03^{*a}$	$6.16 \pm 0.04^{*b}$	83.24^{ab}
	SW1B	7.20 ± 0.01^{f}	$6.60 \pm 0.07^{*c}$	$6.32 \pm 0.01^{*b}$	$6.18 \pm 0.03^{*b}$	85.83^{a}
	SW2B	7.80 ± 0.07^{a}	$6.36 \pm 0.06^{*d}$	$6.28 \pm 0.04^{*b}$	$6.18 \pm 0.02^{*b}$	79.23^{cd}
	SW3B	7.03 ± 0.05^{g}	$6.53 \pm 0.03^{*c}$	$6.19 \pm 0.02^{*c}$	$5.36 \pm 0.04^{*f}$	76.24^{de}
	SC1B	7.50 ± 0.06^{c}	$7.00 \pm 0.04^{*b}$	$6.65 \pm 0.02^{*a}$	$6.08 \pm 0.03^{*c}$	81.06^{bc}
	SC2B	7.28 ± 0.06^{e}	$6.55 \pm 0.08^{*c}$	$5.95 \pm 0.03^{*d}$	$5.46 \pm 0.02^{*e}$	75.00^{e}
	SC3B	7.68 ± 0.03^{b}	$6.02 \pm 0.08^{*e}$	$5.45 \pm 0.04^{*f}$	$4.80 \pm 0.05^{*g}$	62.50^{g}

Means values ± standard deviation.
[*] In the same row indicates a significant difference of mean viable counts compared to that at 0 min ($p < 0.05$).
[a–g] Values in the same column having different superscripts for mean viable counts for each probiotic differ significantly ($p < 0.05$).
[A] Calculated by subtracting bacteria count at 0 min from bacteria count at 120 min, dividing by bacteria count at 0 min and multiplying by 100.

Table 5. Organoleptic property scores of ice creams with different milks [A]

Samples [B]	Color and Appearance (1–5)	Body and Texture (1–5)	Flavor and Taste (1–10)	Total (1–20)
WL	4.09 ± 0.03^{ab}	4.03 ± 0.04^{a}	7.90 ± 0.05^{ab}	16.02 ± 0.07^{a}
CL	3.20 ± 0.05^{c}	3.20 ± 0.04^{b}	6.40 ± 0.07^{dc}	12.80 ± 0.08^{c}
SL	3.10 ± 0.04^{dc}	3.00 ± 0.05^{bc}	5.07 ± 0.03^{e}	11.17 ± 0.06^{d}
SW1L	3.70 ± 0.04^{b}	3.71 ± 0.04^{a}	6.40 ± 0.05^{a}	13.81 ± 0.06^{dc}
SW2L	4.10 ± 0.07^{a}	4.04 ± 0.03^{a}	7.16 ± 0.03^{dc}	15.30 ± 0.08^{ab}
SW3L	4.16 ± 0.06^{a}	3.89 ± 0.05^{a}	8.43 ± 0.04^{a}	16.48 ± 0.09^{a}
SC1L	2.80 ± 0.07^{d}	2.61 ± 0.07^{c}	5.57 ± 0.04^{de}	10.98 ± 0.08^{d}
SC2L	3.02 ± 0.05^{dc}	2.80 ± 0.05^{bc}	5.30 ± 0.07e	11.12 ± 0.06^{d}
SC3L	3.11 ± 0.04^{dc}	2.73 ± 0.07^{bc}	6.04 ± 0.05de	11.88 ± 0.07^{dc}

[A] Mean values from 42 panelists.
[a–d] Values with different letters in the same column are significantly different ($p < 0.05$) (Tukey test).

simulated gastric and intestinal conditions, The survival of probiotics in ice creams containing coconut milk was lower than those containing cow's milk, in both highly acidic (pH = 2.0) and 0.3% bile conditions. Also total acceptability ice creams containing cow was higher than others.

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Table 6. Organoleptic property scores of ice creams with different milks [A]

Samples [B]	Color and Appearance (1−5)	Body and Texture (1−5)	Flavor and Taste (1−10)	Total (1−20)
WB	4.10 ± 0.05^{ab}	4.10 ± 0.04^{a}	8.10 ± 0.05^{ab}	16.30 ± 0.05^{a}
CB	3.4 ± 0.04^{c}	3.30 ± 0.05^{b}	7.00 ± 0.06^{dc}	13.70 ± 0.03^{c}
SB	3.12 ± 0.04^{dc}	3.00 ± 0.05^{bc}	6.00 ± 0.03^{e}	12.20 ± 0.02^{d}
SW1B	3.75 ± 0.05^{b}	3.74 ± 0.03^{a}	7.00 ± 0.04^{a}	14.49 ± 0.05^{bc}
SW2B	4.20 ± 0.06^{a}	4.10 ± 0.02^{a}	7.30 ± 0.02^{bc}	15.6 ± 0.06^{ab}
SW3B	4.19 ± 0.07^{a}	3.90 ± 0.06^{a}	8.50 ± 0.05^{a}	16.59 ± 0.04^{a}
SC1B	2.80 ± 0.07^{d}	2.60 ± 0.07^{c}	6.00 ± 0.03^{de}	11.40 ± 0.07^{d}
SC2B	3.06 ± 0.06^{dc}	2.82 ± 0.04^{bc}	5.50 ± 0.05^{e}	11.38 ± 0.05^{d}
SC3B	3.14 ± 0.04^{dc}	2.80 ± 0.05^{bc}	6.20 ± 0.03^{de}	12.14 ± 0.05^{dc}

[A] Mean values from 42 panelists.
[a–d] Values with different letters in the same column are significantly different ($p < 0.05$) (Tukey test).

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