Effects of Vegetable Milk on Survival of Probiotics and Rheological and Physicochemical Properties of Bio-Ice Cream

Fatemeh. Aboulfazli, Ahmad salhin. Baba, and Misni. Misran

Abstract — In this study, non fermented probiotic ice cream made using soy and coconut milk. The changes in ice cream eating qualities and survival of added microbes were evaluated. The highest viscosity and melting resistance and lowest total acceptability and particle size were found in ice creams containing soy milk. The viable of Lactobacillus acidophilus reduced by 0.204 and 0.251 log cycles in soy and coconut milk ice creams throughout 30 days storage respectively. The counts of Bifidobacterium lactis decreased by 0.01 and 0.091 log cycles in soy and coconut milk ice creams respectively. The produce of ice cream with vegetable milks developed the growth and viability of B. lactis and L. acidophilus.

Keywords — Ice cream, Soy milk, Coconut milk, Probiotic bacteria

I. INTRODUCTION

Increasing consumers’ interest in functional foods became evident in recent years because these foods offer additional health benefits beyond adequate nutrition. Functional food that contains viable probiotics in particular addresses the improvement in intestinal micro flora activation of the immune system, reduction in serum cholesterol and inhibition of potential pathogens [1]. However, these probiotics must be present in adequate amount (10^5–10^7 cfu/g) at the time of consumption in order to render it being effective [2]. Fermented yogurt and milk can effectively be used to deliver probiotic bacteria. The former unfortunately may cause loss of viable probiotic due to pH reduction and accumulation of organic acids as a result of fermentation [3]. In this regard ice cream, due to its neutral pH, may be used to deliver the probiotics [4]. Partial replacement cow milk with vegetable milks such as soy milk could improve the pH of probiotic ice cream resulting increased the survival of probiotics [5]. On the other hand, Ice cream is a delicious, wholesome, nutritious frozen dairy product, which is widely consumed in different parts of the world. Ice cream has nutritional significance but encompasses no therapeutic properties [6]. Ice cream is traditionally made from cow’s milk and thus contains about 15 – 17% (w/w) lactose [7]. The demand for alternatives to cows’ milk is growing due to problems with its fat, cholesterol and lactose (allergenicity) contents and desire for vegetarian alternatives. In this regard, soy milk has been recognized as being nutritionally helpful [8]. Ice cream can be made a functional food by adding fruits, protein rich ingredients and also using vegetable milk such as coconut and soy milks and the addition of probiotics. Moreover, the coconut and soy milks contain unique nutrient compositions in coconut and soy milks can support the growth and survival of the lactic acid bacteria in ice cream and thus could improve the health benefits and nutritional components and health benefits of probiotic ice creams. This is because soy milk contains protein and indigestible fiber and polysaccharide, amino acids, lecithin and unsaturated fat, minerals and vitamins, as well as bioactive polyphenols such as isoflavones, phenolic acids, saponins and tannins [9]. Frequent consumption of soy products also offers health benefit such as lowering the risk of getting cancers, diseases associated with cardiovascular, hypercholesterolemia, diabetes as well as bone and kidney diseases [10]. On the other hand, coconut (Cocos nucifera) milk is easy to digest and contains an abundance of nutrients (particularly calcium, phosphorus and potassium). The coconut milk content Vitamins (C, E and many B vitamins) and antioxidant activities. The high oleic and lauric acid content of coconut milk help in preventing arteriosclerosis and related illness [11]. Partial replacement of cow milk with vegetable milks may affect the physical properties of ice creams. Fortification of yoghurt ice cream with soy protein has been shown to improve the textural quality of the product including firmness and viscosity [12]. Lecithin in the soy also acts as emulsifiers which increase the viscosity and stability of ice cream, refined texture and extend the melting time [13]. Abdullah et al. [14] improved the quality of ice cream by using different ratios of skim milk in soymilk blend and found that large quantity of skim milk in soymilk decline beany flavour of soy beans and increased quality of ice cream. Kerdhouay and Surapat [15]
studied the physical and sensory properties of low fat coconut milk ice cream by adding whey protein concentrate (WPC) and sugar contents and found the addition of sugar and replacing skim milk powder with WPC in low fat coconut milk ice cream increased ice cream mix viscosity and reduced melting rate of ice cream. The present study was carried out to investigate the effect of soy and coconut milks on the survival of probiotic in ice cream during storage and the rheological and physicochemical properties of non fermented bio-ice cream.

II. MATERIAL AND METHODS

A. Bacteria strain and preparation of cultures

Bifidobacterium bifidum (Bb-12) and Lactobacillus acidophilus (La-05) were obtained as pure freeze-dried probiotic culture from CHR-Hansen (Horsholm, Denmark).

B. Starter culture

Each strain (1 g) was cultured in 100 mL of sterilized skimmed milk (10 w/v), simplified by the addition of 0.05% (w/v) L-Cys-HCl, 1% (w/v) yeast extract and 2% (w/v) glucose. The incubation was carried out under aerobic condition in a water bath (42°C) until pH was reached to 5.0 [16].

C. Culture for inoculation

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 (42°C) until pH reduced to 5.0 [16].

D. Ice cream production

Fresh pasteurized and homogenized milks (soy and coconut milk), butter, soy oil and skim milk powder (Dutch lady, Malaysia), sugar and vanilla was purchased from local grocery stores. 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. Ice cream mix formulated according to some properties of milks (TABLE I) such that it had 38–40% total solids and 10.5% fat for a total batch of 100kg (TABLE II).

The milk (soy or coconut milk) was initially heated to 50°C followed by the addition of soy oil respectively, and other additives. The mixture was mixed at 65°C in two stages by a homogenizer with 16000 rpm for 5 min (IkaHomogenizer T-25 basic Ultra Turrax) followed by pasteurization (10 min heating at 80°C followed by cooling to 4°C). The mixture was aged overnight at 4°C prior to the addition of each probiotic culture (4% w/w) followed by immediate freezing in batch ice cream maker (1.5 L, Baumatic Gelato1SS). The frozen mixture was packed in 100 mL plastic cups and then stored in a freezer (−20°C). Three different batches of ice cream per treatment were manufactured.

E. Chemical analysis

Milk and ice creams were analyzed for pH by a digital pH-meter and for titratable acid (TA) by titrating a sample (10 g) with NaOH (0.1 N) using phenolphthalein (0.1 w/v) as an indicator. The dry matters were measured by drying samples at 100±1°C for 3.5 h using an air oven [4]. The fat and protein contents of milk and ice creams were measured by the Soxhlet and Kjeldahl methods respectively [17]. All chemical analyses were carried out in triplicate.

F. Physical analysis

The overrun was calculated using the following formula (equation (1)) [4]:

\[
\text{Overrun} = \frac{\text{Weight of unit mix} - \text{weight of equal volume of ice cream} \times 100}{\text{weight of equal volume of ice cream}}
\]  

(1)

Samples melting rate were determined as described by Mahdian et al. [12]. Tempered samples (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.

G. Rheological measurements

Rheological measurements of melted ice cream samples were determined using a Physica MCR 301 rheometer (Anton-Paar GmbH, Graz, Austria) with concentric cylinder geometry and coupled with a circulating cooling bath at 4.0±0.1°C. Ice creams were left to equilibrate at 4.0°C for 15 min. The samples flow behavior was generated by a linearly increased shear rate from 19.6 to 67.3 s⁻¹ in 20 min and returning to 19.6 s⁻¹ over a further 20 min.

The flow behavior and the consistency index were explained by the Power Law model (equation (2)) of the samples. Apparent viscosity of samples was estimated as a function of time under a constant shear rate of 20 s⁻¹.

\[
\sigma = K\gamma^n
\]  

(2)
Where: $\sigma =$ shear stress (Pa); $K =$ consistency index (Pa s$^n$); $\gamma =$ the shear rate ($s^{-1}$); and $n =$ the flow behavior index [18].

**H. Size and zeta potential**

The size and zeta potential of the ice creams were evaluated using a Malvern Nano Series Zeta Sizer (Malvern, UK) at a constant temperature of 25 °C. The zeta potential and size of ice cream mixes were monitored after aging [19].

**I. Optical polarizing microscope imaging (OPM)**

The OPM micrographs of ice cream mixes were obtained using a Leica Polarizing Microscope equipped with a Leica QWin software. All measurements were carried out at room temperature (25°C) [19].

**J. Bacteriological analysis**

The viability of probiotics was measured immediately after inoculating the probiotic cultures and after 1 and 30 days of frozen storage at −20°C. The samples (10 g) were decimally diluted with sterile peptone water (1 g L$^{-1}$; Merck). One mL aliquot dilutions were pour plated in triplicate on MRS agar for *L. acidophilus* and MRS agar supplemented with 0.05% (w/v) L-Cys-HCl (Merck) for *B. lactis*. The plates were incubated at 38±1°C for 72 h under aerobic condition with 5% CO$_2$ (v/v) for *L. acidophilus* and anaerobic condition (Anaerocult A) for *B. lactis*. The bacterial viability was represented as survival rate [16].

**K. Sensory analysis**

The ice creams were organoleptically evaluated by sixteen panelists, using a sensory rating scale of 1–10 for taste and flavor, and 1–5 for consistency and 1–5 for appearance and colour [4]. The properties evaluated contained (a) three characteristics for appearance and colour (no criticism: 5, dull colour: 4–1, unnatural colour: 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, fluffly: 3–1, gummy: 4–1, sandy: 2–1).

**L. Statistics**

The viability of the probiotic micro-organisms were evaluated at three times of storage (0, 1 and 30 days), in triplicates and the results were expressed as mean±S.E.M (standard mean error) values. The statistical analysis was performed using SAS statistical software, Version 6.12 edition (standard mean error) values. The statistical analysis was performed using SAS statistical software, Version 6.12 edition [20] followed by Duncan’s multiple range method for mean comparison. The mean values and the standard error were calculated from the data obtained with triplicate trials. The criterion for statistical significance was p < 0.05 [21].

### III. RESULTS AND DISCUSSION

**A. Physicochemical properties**

The physicochemical compositions of the probiotic ice creams, soy and coconut milks are shown in TABLE III. The highest pH and lowest TA and overrun values were in ice creams containing soy milk. The melting behavior was a more function of type of milk used than type of probiotic added. Soy milk based ice creams showed slower melting time than ice cream made with coconut milk. Soy protein, which contains lecithin acts as an emulsifier and thus provides protection for the membrane proteins against damage due to freezing. In addition lecithin also assists in good air distribution and fat structure in the ice cream thereby increasing the time of melting of the ice cream [22]. The lower melting time of coconut milk ice creams was due to the poor emulsifying properties of the coconut proteins. The differences in melting resistance of ice cream treatments may also be influenced amongst others by the difference in freezing points and viscosity [6].

**B. Rheological measurements**

The apparent viscosity, consistency index and flow behavior index of the probiotic ice cream produced with different milks are shown in TABLE IV. The apparent viscosity of the ice cream decreased with an increase in the shear rate, as illustrated by the non-Newtonian fluid behavior (Fig 1). This decrease in viscosity is partly because of the aggregation of fat globules, which decrease in size during shearing, thus the reduction in the viscosity of ice cream [18]. Such a decrease in the apparent viscosity with the increase in shear rate is a common factor of milk products of similar texture such as frozen yogurt [23]. Ice cream made with soy milk tended to have a higher apparent viscosity than ice cream made with coconut milk (TABLE IV). The differences can be attributed to the type of their proteins [24]. Soy proteins are able to form a stable network like a gel structure because of their molecular properties which can bind with water molecules [25], which present greater resistance to flow. The formation of gel by globular proteins is a complex process, and it may involve reactions such as molecular unfolding, dissociation–

### TABLE III

<table>
<thead>
<tr>
<th>Ice Cream</th>
<th>Total Solids (g 100 g$^{-1}$)$^{a}$</th>
<th>pH$^{b}$</th>
<th>Titratable Acidity (% Lactic acid)$^{b}$</th>
<th>Fat (g 100 g$^{-1}$)$^{b}$</th>
<th>Protein (g 100 g$^{-1}$)$^{b}$</th>
<th>Overrun</th>
<th>Melting Rate (%) (melted after 20 min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S</td>
<td>11.0±0.07</td>
<td>7.0±0.1</td>
<td>0.072±0.001</td>
<td>1.4±0.01</td>
<td>2.2±0.02</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C</td>
<td>12.0±0.08</td>
<td>6.76±0.01</td>
<td>0.162±0.004</td>
<td>3.4±0.02</td>
<td>3.4±0.02</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>SL</td>
<td>39.2±0.12$^{a}$</td>
<td>7.14±0.02$^{a}$</td>
<td>0.072±0.006$^{a}$</td>
<td>10.4±0.04$^{a}$</td>
<td>2.47±0.03$^{a}$</td>
<td>11.57±1.67$^{d}$</td>
<td>60.68±8.1$^{b}$</td>
</tr>
<tr>
<td>SB</td>
<td>40.8±0.11$^{a}$</td>
<td>7.15±0.01$^{a}$</td>
<td>0.072±0.004$^{a}$</td>
<td>10.3±0.05$^{a}$</td>
<td>2.51±0.02$^{a}$</td>
<td>12.5±0.1$^{e}$</td>
<td>61.31±2.10$^{b}$</td>
</tr>
<tr>
<td>CL</td>
<td>39.9±0.12$^{a}$</td>
<td>6.71±0.01$^{b}$</td>
<td>0.117±0.003$^{b}$</td>
<td>10.3±0.02$^{a}$</td>
<td>2.13±0.02$^{a}$</td>
<td>17.7±1.03$^{b}$</td>
<td>81.02±9.3$^{a}$</td>
</tr>
<tr>
<td>CB</td>
<td>38.8±0.10$^{a}$</td>
<td>6.72±0.03$^{b}$</td>
<td>0.126±0.009$^{a}$</td>
<td>10.4±0.02$^{a}$</td>
<td>2.32±0.04$^{a}$</td>
<td>18.36±1.56$^{a}$</td>
<td>83.44±11.4$^{a}$</td>
</tr>
</tbody>
</table>

$^{a}$ S: soy milk; C: coconut milk. Ice creams inoculated with *Lactobacillus acidophilus* (La-05) made using soy milk (SL) or coconut milk (CL); ice creams inoculated with *Bifidobacterium bifidum* (Bb-12) made using soy milk (SB) or coconut milk (CB).

$^{b}$ Means values ± standard deviation.

$^{a}$ Values in the same column followed by different letters were significantly different (p < 0.05).
association, and aggregation [26].

The lower apparent viscosity of ice cream made using coconut milk was due to the poor emulsifying properties of coconut proteins [27]. No significant differences in the apparent viscosity were among ice creams incubated with La-05 and Bb-12. The Power Law model was used to establish consistency index (K) and flow behavior index (n) (TABLE IV). The flow behavior index (n) showed a shear thinning behavior (n<1) ranging from 0.604 to 0.647 (TABLE IV) which is in accordance with several other studies [18, 23]. Soy milk gives ice cream the highest consistency index (K) and lowest flow behavior index (n) which again demonstrates that the soy milk increased the resistance to structural breakdown due to aggregation of soy proteins which resulted in gel formation and subsequent increase in water retention [22].

E. Effect of milk replacement on droplets suspension

The microscopic structure of ice cream showed that ice creams containing coconut milk had larger fat globule sizes than others. The coconut proteins have less surface active [27] and this could be a factor on the tendency to create small droplets or to prevent droplet aggregation during or after homogenization[28]. As a result the reduction in the fat particle diameters is an increase in K value and thus develope the product stability [29]. The micrographs provide further evidence to support finding from the rheological studies that increased viscosity of ice creams containing soy milk was caused by the change in microstructure. This is reflected by the largest size for ice creams containing coconut milk in contrast to the smallest droplet size for ice creams containing soy milk (TABLE V). Taken as a whole, samples containing coconut milk showed larger droplet sizes than others because of poor emulsifying properties of coconut proteins adsorbed at the oil–water interface [27]. No significant differences in the apparent viscosity were among ice creams incubated with La-05 and Bb-12.

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**TABLE IV**

RHEOLOGICAL PARAMETERS OF PROBIOTIC ICE CREAMS MADE USING SOY OR COCONUT MILKs OBTAINED USING LAW MODEL

<table>
<thead>
<tr>
<th>Ice creams</th>
<th>Apparent viscosity (mPa s)</th>
<th>K(Pa sn)</th>
<th>n</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>SL</td>
<td>351±0.89</td>
<td>1.099±0.01</td>
<td>0.612±0.01</td>
<td>0.998</td>
</tr>
<tr>
<td>SB</td>
<td>385±1.04</td>
<td>1.182±0.01</td>
<td>0.604±0.01</td>
<td>0.997</td>
</tr>
<tr>
<td>CL</td>
<td>315±1.05</td>
<td>0.817±0.03</td>
<td>0.647±0.02</td>
<td>0.993</td>
</tr>
<tr>
<td>CB</td>
<td>293±1.12</td>
<td>0.785±0.02</td>
<td>0.633±0.01</td>
<td>0.994</td>
</tr>
</tbody>
</table>

---

**TABLE V**

ZETA POTENTIAL AND FAT PARTICLE DIAMETER (Dₐ) OF ICE CREAMS MADE USING VEGETABLE MILKS

<table>
<thead>
<tr>
<th>Samples</th>
<th>Particle size (nm)</th>
<th>Zeta potential (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SL</td>
<td>3797 b</td>
<td>-38.5</td>
</tr>
<tr>
<td>SB</td>
<td>3680 b</td>
<td>-38.27 a</td>
</tr>
<tr>
<td>CL</td>
<td>5335 a</td>
<td>-42.47 b</td>
</tr>
<tr>
<td>CB</td>
<td>5963 a</td>
<td>-41.14 b</td>
</tr>
</tbody>
</table>

---

**Fig.1.** Effect of shear rate on the apparent viscosity of soy or coconut milk ice creams. Note: ice cream (La-05) made with soy milk (SL), with coconut milk (CL); ice cream (Bb-12) made with soy milk (SB), with coconut milk (CB).

**Fig.2.** Micrographs (×50 magnification) of ice cream samples with different milk. Ice creams inoculated with *Lactobacillus acidophilus* (La-05) made using soy milk (SL) or coconut milk (CL); ice creams inoculated with *Bifidobacterium bifidum* (Bb-12) made using soy milk (SB) or coconut milk (CB).

**Fig 3.** Shows the changes in bacterial counts in non-fermented ice creams made using vegetable milks. The survival rate of probiotic bacteria in ice creams after 30 days tend to be higher in Bb-12 (7.767 and 7.371 log cfu g⁻¹ for soy and coconut ice cream respectively) than in the presence of La-05 (7.847 and 6.870 log cfu g⁻¹ for soy and coconut ice cream respectively) (TABLE VI).

**D. Viability of probiotic bacteria in ice cream**

Fig 3 shows the changes in bacterial counts in non-fermented ice creams made using vegetable milks. The survival rate of probiotic bacteria in ice creams after 30 days tend to be higher in Bb-12 (7.767 and 7.371 log cfu g⁻¹ for soy and coconut ice cream respectively) than in the presence of La-05 (7.847 and 6.870 log cfu g⁻¹ for soy and coconut ice cream respectively) (TABLE VI). However, the reduction in viable bacterial counts due to freezing is associated with freeze injury on these cells. In addition the mechanical stresses
associated with the mixing and freezing process which incorporate oxygen into the mixture may be responsible in further reduction in bacterial count [16]. The survival of both probiotics in ice cream was high (p<0.01) in the presence of soy and coconut milks. This could be explained by the high pH of soy and coconut ice creams which is known to be conducive to probiotic survival since these organisms are susceptible to inactivation when stored in acidic conditions [30]. Heenan et al. [5] demonstrated that the survival of probiotics increased in the frozen soy dessert due to the prevailing neutral pH. The highest survival of both probiotics was in soy milk ice cream. It is probably due to the soy milk proteins which provide physical protection against freezing damage by encapsulating probiotics by form a stable network looks like a gel structure [25]. Keerati-u-rai and Corredig [11] mentioned soy proteins adsorb at the interface of oil droplets, with surface loads varying between 2 and 4 mg m⁻² and a layer thickness between 30 and 40 nm. So probably, these proteins can cover probiotics as a capsule. The high survival rate of L. Acidophilus cells during the frozen storage in other studies was attributed to the protection provided to the cells by the solid ingredients and the high fat content of the ice cream in the form of emulsion [31].

**E. Sensory evaluation**

TABLE VII shows the organoleptic property scores in non-fermented ice creams made using vegetable milks (TABLE VII). The lowest total acceptability was seen in soy milk ice cream which could be explained by the soy milk woody or beany off flavours [14]. The presence of either La-05 or Bb-12 had no significant influence on sensory properties of ice creams.

<table>
<thead>
<tr>
<th>TABLE VII</th>
<th>ORGANOLEPTIC PROPERTY SCORES OF ICE CREAMS WITH DIFFERENT MILKS*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ice creams#</td>
<td>Colour and Appearance</td>
</tr>
<tr>
<td></td>
<td>(1–5)</td>
</tr>
<tr>
<td>SL</td>
<td>3.12 ± 0.05a</td>
</tr>
<tr>
<td>SB</td>
<td>3.17 ± 0.03a</td>
</tr>
<tr>
<td>CL</td>
<td>3.25 ± 0.03b</td>
</tr>
<tr>
<td>CB</td>
<td>3.20 ± 0.02b</td>
</tr>
</tbody>
</table>

* ice creams inoculated with Lactobacillus acidophilus (La-05) made using soy milk (SL) or coconut milk (CL); ice creams inoculated with Bifidobacterium bifidum (Bb-12) made using soy milk (SB) or coconut milk (CB).

# Values with different letters in the same column are significantly different (P<0.05) (Tukey test).

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