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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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A B S T R A C T

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-HCl 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