

## RESEARCH ARTICLE

### PRODUCTION PROCESSES OF SYNBIOTIC ICE CREAM CONTAINING GERMINATED KDML105 RICE FLOUR AND LACTOBACILLUS ACIDOPHILUS LA-5: PHYSICO-CHEMICAL, PROBIOTIC VIABILITY AND SENSORY EVALUATION

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#### ABSTRACT

An unprecedented rate of advanced medical technology, physical activity promotion, nutraceutical and functional food like probiotics, prebiotics are increasingly to promote and maintain good health, feasibility of synbiotic ice creams (SIC), manufactured to include germinated brown rice (KDML105) flour, as prebiotic sources, and Lactobacillus acidophilus LA-5 probiotic culture was experimented. Physicochemical and sensory attributes of SIC. Using probiotic survivability over 30-day storage was manufactured. Four SIC formulations that mixed of rice flour (RF) and corn flour (CF): Synb-1 (0%RF, 4.0%CF); Synb-2 (2.0%RF, 2.0%CF 2.0%); Synb-3 (3.0%RF, 1.0%CF); and Synb-4 (4.0%RF, 0%CF). The physicochemical and microbiological analyses showed suitability with standards required by legislation, and all formulations through acceptable sensory attributes. Synb-1 and Synb-2 SIC indicate that higher counts of viable probiotic microorganisms with formulations were compared. The product matrix and pH maintained the viability of the probiotic microorganisms as 10<sup>6</sup> CFU/g, the potential of the manufactured of the SIC are provided.

**Key words:** Producing Process, germinated brown rice, KDML105, Lactobacillus acidophilus LA-5, ice cream, and functional food.

#### INTRODUCTION

Historical background of ice cream (derived from earlier iced cream or cream ice Beeton, I. M. (1911)), is a sweetened frozen food typically eaten as a snack or desert. It is usually made from dairy products, such as milk and cream, and often combined with fruits or other ingredients and flavors. It is typically sweetened with sugar or sugar substitutes. Typically, flavorings and colorings are added in addition to stabilizers. The mixture is stirred to incorporate air spaces and cooled below the freezing point of water to prevent detectable ice crystals from forming. The result is smooth, semi-solid foam that is solid at very low temperatures (< 2 °C or 35 °F). It becomes more malleable as its temperature increases. The meaning of the phrase "ice cream" varies from one country to another. Phrases such as; "frozen custard", "frozen yogurt", "sorbet". "Gelato" and others are used to distinguish different varieties and styles. In some countries, such as the United States, the phrase "ice cream" applies only to a specific variety, and most governments regulate the commercial use of the

various terms according to the relative quantities of the main ingredients, notably the amount of cream (Barry, 2008). Products that do not meet the criteria to be called ice cream are labeled "frozen dairy dessert" instead (Barry, 2017). In other countries, such as Italy and Argentina, one word is used for all variants. Analogues made from dairy alternatives, such as goat's or sheep's milk, or milk substitutes (e.g., soy milk or tofu), are available for those who are lactose intolerant, allergic to dairy protein, or vegan. Ice cream may be served in dishes, for eating with a spoon, or in cones, which are licked. Ice cream may be served with other desserts, such as apple pie. Ice cream is used to prepare other desserts, including ice cream floats, sundaes, milkshakes, ice cream cakes, and even baked items, such as Bakes Alaska (BBC News, 2009). In this research study, focusing on ice cream is an excellent source of nutritive compounds providing high dietary energy to consumers and is regarded as the most preferred and consumed frozen dairy desserts among others (Matias, Padilha, Bedani, and Saad (2016). This dairy product is a food complex system, consisting of a frozen matrix containing air bubbles, fat globules, ice crystals, and an unfrozen serum phase (Soukoulis, and *et al.*, 2014). As ice cream is appreciated globally and has significant impacts on human health, the supplementation with probiotics and prebiotics can add value to this product by

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improving its functional properties (Balthazar, and *et. al*, 2015.; Öztürk, Demirci, and Akin, 2018). Probiotics are defined as live microorganisms that when consumed in adequate amounts ( $\geq 10^6$  CFU/mL) provide health benefits to the host by improving the intestinal microbial balance, lowering the risk of gastrointestinal diseases through stimulation of the growth of beneficial microorganisms along with pathogen reduction, and detoxification of mycotoxin (Bansal, and *et al* 2016; Sangsila, Marquis, Leszkowicz, and Itsaranuwat, 2016). Probiotic bacteria are most frequently included in the composition of dairy sector that represents the largest food market (Itsaranuwat, Al-Haddad, and Robinson, 2003; Matejčeková, Liptáková, and Valík, 2017). Ice cream representing one of the important dairy products could, therefore, be an ideal food matrix for delivering probiotics owing it is high consumer acceptability. However, it is technologically challenging to guarantee probiotic viability, texture and sensory acceptance of the final product, in which lower overrun and correct choice of cryoprotectants are required (Parussolo, and *et al* 2017). In probiotic dairy products, the most preferred probiotic bacteria are *Lactobacillus* and *Bifidobacterium* strains, with *Lactobacillus* strain being more preferred for ice cream manufacture due to its superior tolerance to oxygen generated during processing and freezing (Öztürk, Demirci, and Akin, 2018).

Prebiotics are defined as non-digestible food ingredients that beneficially affect the host health by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon (Zhang, and *et al* 2018). The most common prebiotics come from cereals such as bean, rice, barley and wheat (Espinosa- Martos and Ruperez, 2006; Judprasong, Tanjor, Puwastien and Sungpuag, 2011). Some oligosaccharides are non-digestible and currently considered to be as prebiotics. Several types of sugar and oligosaccharides, namely maltotriose, isomaltotriose, maltotetraose and maltoheptose, were detected during the germination of the rough rice seed (Saman, Vázquez, and Pandiella, 2008). In this sense, prebiotics can be added in ice cream formulations in order to promote the growth and viability of probiotics. Over the past decade, cereals and its ingredients have been accepted as functional food and nutraceuticals owing to their dietary fiber, proteins, energy, minerals, vitamins and antioxidants required for human health, which can be used as fermentable substances for the growth of probiotic bacteria. Brown rice, wheat, buckwheat, oat, barley, flaxseed, psyllium and soy products are notified as the most common cereal based functional food and nutraceuticals (Das, Raychaudhuri, and Chakraborty, 2012). Brown rice which contains bran and embryo rice has a higher nutritional value when compared to white rice because the bran and embryo contain numerous nutritional and bioactive components including dietary fiber, functional lipids, amino acids, vitamins, phytosterols, phenolic compounds, gamma-aminobutyric acid (GABA) and minerals (Cho, and Lim, 2016). More importantly, rice bran, the outer layer of the rice grain, is a natural and rich source of prebiotics that can be metabolized by the gut microbiome to modulate mucosal immune responses, reduce intestinal colonization of enteric pathogens, increase numbers of native probiotic lactobacilli (Nealon, Worcester, and Ryan, 2017). Due to its bioactive compounds germinated brown rice is of particular interest and has been used as a nutritive ingredient in many foods (Tan, and Norhaizan, 2017). Hence, the addition of germinated brown rice as a prebiotic in ice cream can add value to this product by fortifying its functional properties.

This study was carried out to develop synbiotic ice cream, containing germinated KDML105 rice flour as prebiotic and *Lactobacillus acidophilus* LA-5 as probiotic, and to evaluate its physicochemical (overrun, viscosity, melting rate and pH) and sensory (color, aroma, fatty taste, smoothness, stickiness and solubility in mouth) properties, as well as its probiotic viability over a 30-day storage period.

## MATERIALS AND METHODS

**Probiotic Culture and Enumeration:** The freeze-dried probiotic culture of *L. acidophilus* LA-5 was kindly provided by Christian Hansen (Hørsholm, Denmark). An aliquot (5.0 g) of the freeze-dried culture was inoculated in 100 mL of MRS broth (Merck, Darmstadt, Germany) and incubated anaerobically, without agitation, at  $37 \pm 1$  °C for 18 h (Miranda, Carvalho de, and Nero, 2014). The strain was activated by two subcultures in 100 mL MRS broth prior to experimental use (Ravulaand, and Shah, 1998). An aliquot (50 mL) of the second subculture was used to inoculate 1000 mL of MRS broth, which was incubated under the aforementioned conditions. After incubation, cell biomass was harvested by centrifugation at  $8000 \times g$  for 15 min at 4 °C and washed twice with Ringer solution (Merck). After the last centrifugation, cell biomass was suspended in 50 mL of chilled UHT milk and held at 4 °C until used (< 6 h). The bacterial cell suspension at 10 or 11 log CFU/g was used for ice cream manufacture.

**Preparation of Germinated Brown Rice, as Prebiotic Sources:** Seeds of the rice cultivar KDML105 were obtained from local farms in Kalasin province, Northeast Thailand. Seed germination was carried out according to Moongnarm and Saetung (2010). Briefly, seeds (1.0 kg) were put into plastic bags ( $16 \times 15$  in<sup>2</sup>) to obtain a seed-to-bag volume ratio of 1:5. Each bag was then filled up with tap water at a water-to-bag volume ratio of 2:5 for seed-soaking for 10 h at  $30 \pm 0.5$  °C in the darkroom ( $3 \times 3 \times 3$  m<sup>3</sup>) with relative humidity kept constant at 90-95% and drainage conducted at 5-h intervals. After transferring properly soaked seeds to plastic baskets for seed germination for 0, 12, 24 and 36 days, germinated seeds were oven-dried at  $50 \pm 2$  °C to approx. 5% moisture content and the obtained seeds were mechanically dehusked to produce germinated brown rice. To prepare germinated brown rice flour, the germinated brown rice was finely ground and sieved through a 335 mesh screen.

**Manufacture of Synbiotic Ice Cream:** The synbiotic ice cream was manufactured using the following ingredients: solid ingredients (milk powder, corn flour and sugar), liquid ingredients (fresh milk, raw yolk and whipping cream), prebiotic (germinated brown rice flour), and probiotics (*L. acidophilus*) as listed in Table 1. The synbiotic ice cream mix and batch pasteurization were performed according to Cody, and *et al.* (2007). Briefly, after solid and liquid ingredients were separately prepared, the resultant mixtures were mixed together to gain adequate mixture. To prepare the liquid mixture, fresh milk and whipping cream were mixed and heated at 50 °C for 30 min, followed by the addition of raw yolk when the temperature was reduced to 40 °C. The synbiotic ice cream was produced immediately after the addition of the prebiotic and probiotic to the mixture in a two-stage homogenizer at 300 psi for 10 min and at 500 psi for 15 min.

**Table 1. Ingredients used for the production of the synbiotic ice cream formulations utilizing germinated brown rice flour (KDML105) as the prebiotic and *L. acidophilus* LA-5 as the probiotic**

Ingredients (g/100 mL)	Ice cream			
	Synb-1	Synb-2	Synb-3	Synb-4
<i>Variable ingredients</i>				
Germinated brown rice flour	0.00	2.00	3.00	4.00
Corn flour	4.00	2.00	1.00	0.00
<i>Fixed ingredients</i>				
Fresh milk	50.50	50.50	50.50	50.50
Whipping cream	20.00	20.00	20.00	20.00
Milk powder	6.50	6.50	6.50	6.50
Sugar	15.00	15.00	15.00	15.00
Raw yolk	4.00	4.00	4.00	4.00

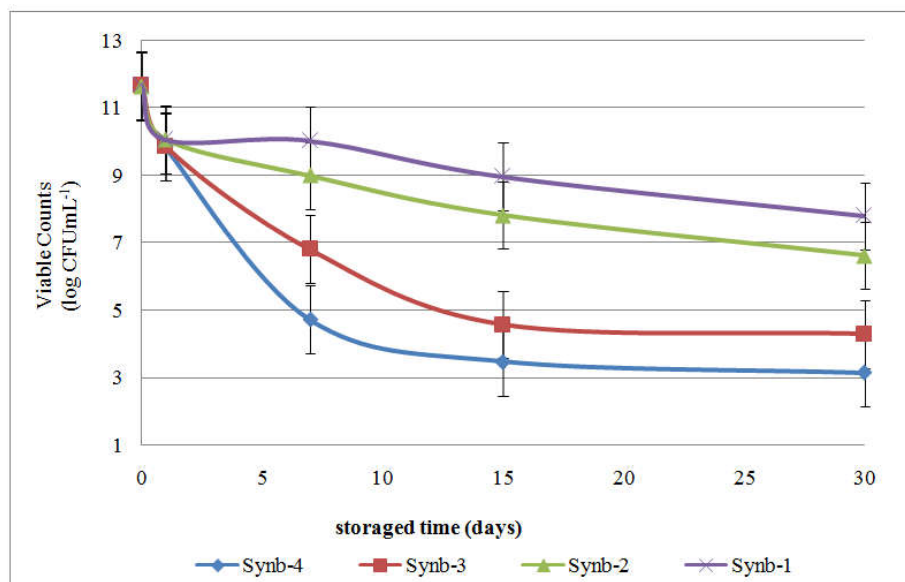
**Table 2. Overrun and melting rates of four synbiotic ice cream formulations at 25 °C**

Parameters	Value <sup>x</sup>			
	Ice cream <sup>y</sup>			
	Synb-1	Synb-2	Synb-3	Synb-4
Overrun (%)	89.85±0.14 <sup>d</sup>	92.62±0.27 <sup>c</sup>	93.72±0.14 <sup>b</sup>	96.52±0.68 <sup>a</sup>
Melting rate at 30/min	1.63±0.03 <sup>a</sup>	1.60±0.01 <sup>b</sup>	1.58±0.01 <sup>b</sup>	1.56±0.01 <sup>c</sup>

Different capital letters in the same row denote significant differences ( $p < 0.05$ ) between different ice cream formulation for the same parameter.

<sup>x</sup> means of seven replicates.

<sup>y</sup> see Table 1 for the description of the ice cream formulations.

**Figure 1. Survival of *L. acidophilus* LA-5 in four ice cream formulations during storage for 30 days**

The final product was packaged in paper cups, each containing approx. 500 g of the product, and stored at  $-20 \pm 1$  °C for up to 30 days. The experiment was conducted in triplicate. The final product was assessed for its crude prebiotic index to identify the best germination period of the prebiotic. The product was also evaluated for the viability of the probiotic during storage for 1, 7, 15 and 30 days to find the possible length of the storage period that ensured the acceptable count of the probiotic of 6 log cfu/g.

### Physicochemical Analysis

The pH of the ice cream was measured using a digital pH meter (Mettler Toledo, SevenMulti™, Switzerland). The ice cream overrun was determined from a comparison of the weight of mix and ice cream in a fixed volume container (Özdemir, Dagdemir, Celik, and Özdemir, 2003) by using a 250 mL beaker, and the overrun percentage was calculated according to the equation:  $\text{Overrun (\%)} = (W_m/W_{ic} - 1) \times 100$ ,

where  $W_m$  represents the weight (g) of a given volume of mix and  $W_{ic}$  denotes the weight (g) of the same volume of ice cream. The melting behavior, expressed as melting rate (%), was evaluated as described by Santana, Ribeiro, and Iguti (2011). Briefly, 25-30 g of ice cream ( $-20$  °C) were placed on a 2-mm wire mesh screen and left to melt into a 250 mL beaker at ambient temperature ( $25 \pm 2$  °C) until 50% of the sample was melted. The weight of the melted ice cream was recorded every 5 min to obtain a sigmoidal curve representing the kinetics of the melting process. From the linear part of the curve, the most probable straight line was calculated, with its slope representing the melting rate (g/min).

### *L. acidophilus* counts and viability

To determine the counts of viable *L. acidophilus*, the ice cream samples were thawed and serially diluted in sterile 0.1% (w/v) peptone water, and viable cells were enumerated using the pour plate technique as described by Vinderola, Mocchiutti, and Reinheimer (2002). The counts of *L. acidophilus* in the ice

cream samples were determined on MRS agar incubated aerobically at  $37 \pm 1$  °C for 3 days and recorded as the logarithm of cfu/g of sample (log cfu/g).

### Sensory Evaluation

Before sensory evaluation, the synbiotic ice cream samples previously stored for one day at -20 °C were left to attain a temperature of -10 to -8 °C. For the laboratory acceptance testing, the ice cream samples were provided to 27 untrained panelists, who evaluated the products using an acceptability test for colour, aroma, fatty taste, smoothness, stickiness, solubility in the mouth and overall acceptability. This test was based on a 7-point hedonic scale, with 1 as 'dislike very much' and 7 as 'like very much' (Nousia, Androulakis, and Fletouris, 2011).

### Statistical Analysis

Statistically significant was analyzed and carried out in triplicate from the same bulk of samples and the obtained data with one-way analysis of variance (ANOVA). The mean values were compared with Duncan's multiple range tests. In all cases,  $p$ -value < 0.05 was considered.

## RESULTS AND DISCUSSIONS

**Overrun and Melting Rates:** Overrun, which is the percent increase in ice cream volume relative to that of the ice cream mix on account of the incorporation of air into the ice cream mix during processing and freezing, is an important physical characteristic of ice creams since it affects the ice creams' quality, interfering with their texture, softness and stability (Cruz, and et., al, 2010). The incorporation of too much air produces fluffy ice creams and too little produces soggy, heavy products. In this study, significant differences in the overrun of the synbiotic ice creams were detected, with the highest value observed for Synb-4 (96.52%), followed by Synb-3 (93.72%), Synb-2 (92.62%) and Synb-1 (89.95%) creams (Table 2). In general, there is a relation between viscosity and overrun, and in this study, the concentrations of stabilizers had a profound effect on viscosity, and therefore, on overrun. For all the ice cream formulations, an increase in the levels of stabilizers was found to reduce viscosity, thus improving overrun. By contrast, an inordinate increase in viscosity as a result of stabilizers had a negative effect on overrun. Thus, Synb-1 containing 4 g/100 mL corn flour showed the lowest overrun, thereby exhibiting the highest recrystallization (Sofjan and Hartel, 2004). The overrun value for the synbiotic ice cream formulations in this study was much higher than those previously reported by Parussolo, and *et al* (2010) for probiotic products containing *L. acidophilus* NCFM (overrun of 29.49, 31.25 and 32.93%).

High overrun values may be related to ice cream manufacturing and freezing processes. The amount of fat in the ice creams also affect the overrun as coalescing fat droplets trap larger amounts of air in ice cream (Sun-Waterhouse, Edmonds, Wadhwa, and Wibisono, 2013). The amount of air incorporated also determines the melting rate of ice creams. As a result of its lowest overrun of 89.85%, Synb-1 had the significantly highest melting rate of 1.63 g/min, followed by Synb-2 (1.60 g/min), Synb-3 (1.58 g/min) and Synb-4 (1.56 g/min) ice creams ( $p < 0.05$ ), as presented in Table 2. The results obtained in this study revealed that greater melting

resistance of ice creams was correlated to higher overrun. The meltdown rate of ice creams is affected by many factors, including the amount of air incorporated, the nature of the ice crystals and the network of fat globules formed during frozen storage. In an earlier study (Sakurai, Kokubo, Hakamata, Tomita, 1996), it is found that ice creams with low overruns melted quickly while those with high overruns began to melt slowly and had a good melting resistance.

**Bacterial Viability in Synbiotic Ice Creams:** The viable counts (expressed as log CFU/g) of *L. acidophilus* LA-5 in the ice cream mix and during freezing of the synbiotic ice creams at  $-20 \pm 1$  °C for 30 days are depicted in Figure 1. It was obvious that the freezing process resulted in a significant decrease in the viability of *L. acidophilus* LA-5. Synb-1 displayed the highest viable counts of *L. acidophilus* LA-5 over the study period, as compared to other synbiotic ice cream formulations, with the viable bacterial counts of 10.03, 8.97, and 7.80 log CFU/g after freezing for 7, 15 and 30 days, respectively. Meanwhile, Synb-2 exhibited the second highest bacterial counts, followed by Synb-3 and Synb-4 ice creams. At the end of storage, the viable counts of *L. acidophilus* LA-5 dropped by 7.80, 6.63, 4.30 and 3.16 log CFU/g in Synb-1, Synb-2, Synb-3 and Synb-4, respectively, corresponding to a 22.00, 33.70, 57.00 and 68.40% log decrease with respect to its initial bacterial counts. As with the recommended minimum level of the viable bacterial count of  $10^6$  CFU/g for synbiotic products (Nousia, Androulakis, and Fletouris, 2011), Synb-1 and Synb-2 ice creams could be stored at  $-20 \pm 1$  °C for up to 30 days, while Synb-3 and Synb-4 could be kept for only 7 and 4 days, respectively.

The results obtained from this study clearly demonstrated that corn flour in Synb-1 ice creams was more effective than germinated brown rice (KDML105) flour in maintaining the viability of probiotic bacteria, which might be due to the differences in amylose/amylopectin contents. Amylose is an amorphous and soluble polysaccharide, whereas amylopectin is insoluble and exhibits a highly organized structure of densely packed double helices formed between neighbouring linear chains (Jiang, and *et al* 2010; Raguin and Ebenhöh, 2017). In general, during frozen storage, the viability of probiotic bacteria in ice creams is differentially affected by starch gels with different syneresis properties. Amylose is known to undergo retrogradation at a higher rate than amylopectin, and greater syneresis has been reported with higher amylose content for corn and potato starches (Sandhu and Singh, 2007), while no correlations between amylose content and syneresis are observed for rice starches. An earlier, Singh, Kaur, Sandhu, Kaur, and Nishinari (2006) reported that normal wheat and the starch separated from 19 different indica rice cultivars was evaluated for physicochemical, morphological, thermal and rheological properties. The relationships between the different properties of starches were determined using Pearson correlation analysis. The amylose content of starches from different rice cultivars differed significantly. PR-103 starch showed the lowest amylose content (4.1%), whereas PR-113 starch showed the highest (16.4%). The starch granular size ranged between 1.5 and 5.8  $\mu$ m. The starch granules were observed to be polyhedral and irregular in shape. Starch from PR-113 and RYT-2492 mainly consisted of large size polyhedral granules while that from PR-103 and IR-64 had small size irregular granules in a fairly large number. IR-64 and Sasyasree starches showed higher gelatinization enthalpy ( $\Delta H_{gel}$ ) of 13.81 and

12.32 J/g, respectively, Phospholipids in normal cereal starches could also influence syneresis which has a tendency to form helical complexes with amylose during starch gelatinization and therefore, the viability of probiotic bacteria is dependent on the high syneresis levels of two starch gels (Pon, Lee, and Chong, 2015).

The existence of probiotic bacteria had linearized the behavior of ice melting, especially at a high concentration of probiotic bacteria. This can be seen clearly in Figure 1, where the melting rates were more stable with probiotic bacteria and increments of probiotic bacteria concentration. As an average, melting rates were  $1.50 \pm 0.01$ ,  $1.32 \pm 0.01$ ,  $1.19 \pm 0.01$ , and  $1.09 \pm 0.02$  ml/minute for control, formulations of Synb 1, Synb 2, Synb 3, and Synb 4, respectively (Table 2). However, the growth and viability of probiotic culture are also affected by pH of the synbiotic products (Table 3). The products were monitored for the microbial population and pH during storage at  $-20 \pm 1$  °C for up to 30 days. Even though the viability of the probiotic bacteria was reduced over 30 days of storage, the viable cell concentrations were still sufficient to obtain the desired therapeutic impact in Synb-1 and Synb-2.

**Table 3. Mean pH values (mean  $\pm$  SD) of four synbiotic ice cream formulations during frozen storage ( $-20 \pm 1$  °C) for 30 days**

Storage period (days)	pH <sup>x</sup>			
	Ice cream <sup>y</sup>			
	Synb-1	Synb-2	Synb-3	Synb-4
0	5.75 $\pm$ 0.01	5.87 $\pm$ 0.01	5.91 $\pm$ 0.01	5.95 $\pm$ 0.01
1	5.75 $\pm$ 0.01	5.87 $\pm$ 0.01	5.91 $\pm$ 0.01	5.95 $\pm$ 0.01
7	5.75 $\pm$ 0.01	5.87 $\pm$ 0.01	5.91 $\pm$ 0.01	5.95 $\pm$ 0.01
15	5.75 $\pm$ 0.01	5.85 $\pm$ 0.01	5.88 $\pm$ 0.01	5.92 $\pm$ 0.01
30	5.75 $\pm$ 0.01	5.85 $\pm$ 0.01	5.88 $\pm$ 0.01	5.92 $\pm$ 0.01

Different capital letters in the same row denote significant differences ( $p < 0.05$ ) between different ice cream formulations for the same day of storage. Different lower-case letters in the same column denote significant differences ( $p < 0.05$ ) between different days of storage for the same ice cream formulation.

<sup>x</sup> Means of seven replicates.

<sup>y</sup> See Table 1 for the description of the ice cream formulations.

### Mean pH Values in Synbiotic Ice Creams

The mean pH values of the synbiotic ice creams range from 5.75 to 5.95 during frozen storage (Table 3), which was in a pH range optimal for the growth of *L. acidophilus* (5.5-6.0) as suggested by Mohammadi, Mortazavian, Khosrokhavar, and Cruz (2011), the incorporation of probiotic bacteria into ice cream is highly advantageous since, in addition to making a functional healthy food, ice cream in itself contains beneficial substances such as dairy raw materials, vitamins and minerals, and is consumed by the general population. Also, compared with fermented milks as a vehicle, ice cream supports considerably greater viability of probiotic strains during production and especially storage. However, losses in the viability of probiotic bacteria in ice cream unavoidably occur during product formulation, processing, storage and melting. During these stages, probiotic cells are subjected to different stresses related to pH, acidity, redox potential, freezing, oxygen (especially in overrun processing), sugar concentration and osmotic effects, hydrogen peroxide, antagonistic impact of co-cultures (in fermented ice creams), and mechanical shearing. In addition, significant pH variations were not detected for all the ice cream formulations during a 30-day frozen storage.

**Table 4. Sensory acceptability of four synbiotic ice cream formulations based on a 7-point hedonic scale, (n = 27)**

Sensory attributes	Score <sup>x</sup>			
	Ice cream <sup>y</sup>			
	Synb-1	Synb-2	Synb-3	Synb-4
Color	4.48 $\pm$ 1.05 <sup>b</sup>	5.03 $\pm$ 1.16 <sup>a</sup>	4.56 $\pm$ 1.22 <sup>ab</sup>	4.19 $\pm$ 1.17 <sup>b</sup>
Aroma	4.56 $\pm$ 1.60 <sup>b</sup>	5.03 $\pm$ 1.53 <sup>a</sup>	4.89 $\pm$ 1.08 <sup>a</sup>	4.81 $\pm$ 1.30 <sup>a</sup>
Fatty taste	4.63 $\pm$ 1.54 <sup>a</sup>	5.15 $\pm$ 1.02 <sup>a</sup>	4.89 $\pm$ 0.97 <sup>a</sup>	4.85 $\pm$ 1.25 <sup>a</sup>
Smoothness	4.89 $\pm$ 1.50 <sup>b</sup>	5.03 $\pm$ 1.15 <sup>b</sup>	5.74 $\pm$ 0.90 <sup>a</sup>	5.00 $\pm$ 1.30 <sup>b</sup>
Stickiness	4.85 $\pm$ 1.28 <sup>a</sup>	5.44 $\pm$ 0.93 <sup>a</sup>	5.44 $\pm$ 1.30 <sup>a</sup>	4.56 $\pm$ 1.47 <sup>b</sup>
Solubility in mouth	4.70 $\pm$ 1.30 <sup>a</sup>	5.37 $\pm$ 1.30 <sup>a</sup>	5.15 $\pm$ 1.26 <sup>a</sup>	5.04 $\pm$ 1.11 <sup>a</sup>
Overall acceptability	5.74 $\pm$ 1.22 <sup>a</sup>	5.81 $\pm$ 0.74 <sup>a</sup>	5.67 $\pm$ 1.03 <sup>a</sup>	4.65 $\pm$ 1.20 <sup>b</sup>

Different letters in the same row indicate significant differences ( $p < 0.05$ ) between different ice cream formulations for the same parameter.

<sup>x</sup> Means of 27 replicates.

<sup>y</sup> See Table 1 for the description of the ice cream formulations.

### Sensory Characteristics of Synbiotic Ice Creams

The sensory scores of the synbiotic ice cream formulations are given in Table 3. The points allocated for colour, aroma, fatty taste, smoothness, stickiness and solubility in mouth showed that the addition of prebiotic (germinated brown rice flour) had no effect on the aroma, fatty taste and solubility in a mouth of the ice creams. On the other hand, significant effects were observed for colour, smoothness and stickiness, resulting in significant differences in the overall acceptability of the ice creams. Total evaluation in terms of colour, aroma, fatty taste, smoothness, stickiness and solubility in mouth showed good acceptability for all the ice cream formulations, with Synb-2 appearing to be of most preference despite significant differences observed when compared to Synb-1 and Synb-3. The sensorial properties of probiotic rice pudding demonstrated similar acceptability to the control up to 14 days and declined thereafter. Ozcan, Yilmaz-Ersan, Akpinar-Bayizit, Sahin, and Aydinol (2010) reported that rice pudding was considered suitable food for the delivery of probiotic micro-organisms, with sufficient viability and acceptable sensory characteristics. Suggestions that, the prebiotics are non-digestible food ingredients that beneficially affect the host by selectively stimulating growth and/or activity of beneficial bacteria in the colon are designed. To determine consumer acceptability of ice cream with prebiotic ingredients substituted for part of the sugar and to determine sensory attributes of sweetness, smoothness, and vanilla flavor. A commercial ice cream mix was made substituting 0%, 10%, 20%, or 30% of the sugar for either Fructooligosaccharides (FOS) or inulin (Wood, 2011). Sensory analyses were conducted using 95 non-trained panelists. Overall consumer acceptability and sensory attributes were measured on a 175 mm anchored hedonic scale. When 10% and 20% inulin ice cream were compared to the control (0%), no significant differences in sweetness, smoothness, vanilla flavor or overall acceptability were found ( $P < 0.05$ ). The 30% inulin ice cream was significantly less sweet than the control and 10% and 20% inulin ice cream, less smooth and less vanilla flavor than the control, and less acceptable than the control and 10% inulin ice cream ( $P < 0.05$ ) (Wood, 2011).

### Regression

Regression models were established in order to predict the overall acceptability of the ice cream formulations and viable counts during the frozen storage of the ice creams. In addition to microbial counts, pH, and physical and functional properties of the ice creams were evaluated. The experimental ice creams preserved the probiotic bacteria and had counts of viable lactic

acid bacteria after frozen storage that met the minimum required to achieve probiotic effects. For the dependent variable 'Overall acceptability', the predictors were obtained as follows: (a) Constant = 4.55 with minimum = 1 and maximum = 7, (b) Synb-4 with unstandardized coefficient = -1.043 and standardized beta = -0.393, (c) Stickiness with unstandardized coefficient = 0.231 and standardized beta = 0.258, (d)  $R^2 = 0.241$  with adjusted  $R^2 = 0.226$ . Hence, the equation model for the overall acceptability of the ice cream formulations was established as: Overall acceptability =  $4.55 - 0.39\text{Synb-4} + 0.26\text{Stickiness}$ ; Adjusted  $R^2 = 0.23$ . For the dependent variable 'Viable counts', the predictors were obtained as follows: (a) Constant = 8.802 with satisfied line claimed by Kirmann and Rasic (1991) at 6, (b) Store time = 0 ( $T_0$ ) with unstandardized coefficient = 2.375 and standardized beta = 0.354, (c) Store time = 30 ( $T_{30}$ ) with unstandardized coefficient = -4.149 and standardized beta = -0.618, (d) Store time = 15 ( $T_{15}$ ) with unstandardized coefficient = -3.169 and standardized beta = -0.472, (e) Synb-1 with unstandardized coefficient = 2.126 and standardized beta = 0.342, (f) Synb-2 with unstandardized coefficient = 1.462 and standardized beta = -0.235, (g) Store time = 7 ( $T_7$ ) with unstandardized coefficient = -0.773 and standardized beta = -0.115, (h)  $R^2 = 0.241$  with adjusted  $R^2 = 0.226$ . Hence, the equation model for the viable count was established as: Viable counts =  $8.8 + 0.35T_0 - 0.62T_{30} - 0.47T_{15} + 0.34\text{Synb-1} + 0.24\text{Synb-2} - 0.12T_7$ ; Adjusted  $R^2 = 0.87$ .

## Conclusions

This study has elucidated the feasibility of synbiotic ice creams, manufactured to include germinated brown rice (KDML105) flour as prebiotic and *Lactobacillus acidophilus* LA-5 culture as probiotic, for elderly people. The results showed that Synb-1 and Synb-2 ice creams displayed greater counts of viable probiotic bacteria compared to the other ice cream formulations. As a result of the product matrix and pH, the viability of the probiotic bacteria was kept above  $10^6$  CFU/g during a 30-day storage period, thus revealing the potential of the manufactured synbiotic ice creams. Probiotics are live microorganisms used as food supplements to provide health benefits by improving intestinal microbial balance in the human body. Lactic, Acid, and Bacteria (LAB) are the most important probiotics. Today they are incorporated into wide variety of foods. The quality of the product is based on the viability of the probiotic bacteria. We will discuss about their therapeutic role, suggested levels, viability and selection, mode of action, synbiotics and prebiotics. For probiotics delivered through foods, additional amounts of cells are likely required prior to processing to account for the loss of cells during the processing and/or storage phases.

## DISCUSSIONS

Research on experimental analysis to analyze according to the synbiotic ice cream containing germinated KDML105 Rice Flour and *Lactobacillus Acidophilus* LA-5: physicochemical, probiotic viability and sensory evaluation was to grow at an unprecedented rate as a result of advanced medical technology, and therefore other than physical activity promotion, nutraceutical and functional food like probiotics and prebiotics are increasingly needed to promote and maintain good health. Administration to four synbiotic ice cream formulations were manufactured: Synb-1 (0% rice flour and 4.0% corn flour); Synb-2 (2.0% rice flour and 2.0% corn

flour); Synb-3 (3.0% rice flour and 1.0% corn flour) and Synb-4 (4.0% rice flour and 0% corn flour) to prepare germinated brown rice flour, the germinated brown rice was finely ground and sieved through a 335 mesh screen were provided. Focused on ice cream was an excellent source of nutritive compounds providing high dietary energy to consumers and is regarded as the most preferred and consumed frozen dairy desserts among others. This product was used a food complex system, consisting of a frozen matrix containing air bubbles, fat globules, ice crystals, and an unfrozen serum phase for improving the intestinal microbial balance, lowering the risk of gastrointestinal diseases through stimulation of the growth of beneficial microorganisms along with pathogen reduction, and detoxification of mycotoxin. The prebiotics can be added in ice cream formulations in order to promote the growth and viability of probiotics Das, Raychaudhuri, and Chakraborty, (2012). The final product was packaged in paper cups, each containing approx. 500 g of the product, and stored at  $-20 \pm 1$  °C for up to 30 days. The experiment was conducted in triplicate that this process is consistent with the results of Cody, Olabi, Pettingell, Tong, and Walker (2007). This ice cream overrun product was determined from a comparison of the weight of mix and ice cream in a fixed volume container that confirms the study of Özdemir, Dagdemir, Celik, and Özdemir (2003) and Vinderola, Mocchiutti, and Reinheimer (2002) who determined the counts of viable *L. acidophilus* on MRS agar incubated aerobically at  $37 \pm 1$  °C for 3 days for probiotic products containing *L. acidophilus* NCFM of air in ice cream that follows by Sun-Waterhouse, Edmonds, Wadhwa, and Wibisono (2013) reported. The viability of probiotic bacteria in ice creams is differentially affected by starch gels with different syneresis properties that confirms as the study by Raguin and Ebenhöf (2017). However, the growth and viability of probiotic culture are also affected by pH of the synbiotic products that consistent with the results of the study by Pon, Lee, and Chong (2015). Finally, the sensorial properties of probiotic rice pudding demonstrated similar acceptability to the control up to 14 days, that rice pudding was considered suitable food for the delivery of probiotic microorganisms, with sufficient viability and acceptable sensory characteristics, which this result is reported by Ozcan, Yilmaz-Ersan, Akpinar-Bayizit, Sahin, and Aydinol (2010).

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## REFERENCES

- Balthazar, C. F., Silva, H. L. A., Celeguini, R. M. S., Santos, R., Pastore, G. M., Junior, C. A. C., Freitas, M. Q., Nogueira, L. C., Silva, M. C. and AG Cruz, A. G. 2015.



- Effect of galactooligosaccharide addition on the physical, optical, and sensory acceptance of vanilla ice cream. *J. Dairy Sci.* 2015, 98; pp. 4266-4272.
- Bansal, S., Mangal, M., Sharma, S. K., Yadav, D. N. and Gupta, R. K. 2016. Optimization of process conditions for developing yoghurt like probiotic product from peanut. *LWT - Food Sci. Technol.*, 73; pp. 6-12.
- Barry, D. 2017. Ice cream's identity crisis. *New York Times*. Retrieved on 15 April 2017 from <https://www.nytimes.com/2013/04/17/dining/remembering-when-breyers-ice-cream-was-you-know-ice-cream.html>
- BBC News. 2009. The origin of ice-cream. Retrieved on 26 October 2009 from [http://news.bbc.co.uk/1/hi/find\\_out/guides/tech/ice-cream/newsid\\_3634000/3634978.stm](http://news.bbc.co.uk/1/hi/find_out/guides/tech/ice-cream/newsid_3634000/3634978.stm)
- Beeton, I. M. 1911. *Mrs Beeton's Cookery Book*. pp. 258–60.
- Cho, D. H. and Lim, S. T. 2016. Germinated brown rice and its bio-functional compounds. *Food Chem.* 196; pp. 259-271.
- Cody, T. L., Olabi, A., Pettingell, A. G., Tong, P. S. and Walker, J. H. 2007. Evaluation of rice flour for use in vanilla ice cream. *J. Dairy Sci.* 90; pp. 4575-4585.
- consuming 'health-promoting' fermented dairy products: a brief update. *International Journal of Dairy Technology*, 56 (4); pp. 203-210.
- Cruz, A. G., Faria, J. A. F., Saad, S. M. I., Bolini, H. M. A., Sant'Ana, A. S. and Cristianini, M. 2010. High pressure processing and pulsed electric fields: potential use in probiotic dairy foods processing. *Trends Food Sci. Technol.* 21; pp. 483-493.
- Das, A., Raychaudhuri, U. and Chakraborty, R. 2012. Cereal based functional food of Indian subcontinent: a review. *J. Food Sci. Technol.* 49; pp. 665-672.
- Espinosa-Martos I., Ruperez P. 2006. Soybean oligosaccharides. Potential as new ingredients in functional food. *NutrHosp*, 21; pp. 92-96.
- Itsaranuwat, P., Al-Haddad, K. S. H. and Robinson, R. K. 2003. The potential therapeutic benefits of
- Jiang, H., Lio, J., Blanco, M., Campbell, M. and Jane, J. 2010. Resistant-starch formation in high-amylose maize starch during kernel development. *J. Agric. Food Chem.* 58; pp. 8043-8047.
- Judprasong, K., Tanjor, S., Puwastien, P. and Sungpuag, P. 2011. Investigation of Thai plants for
- Kirmann, J. A., and Rasic, J. L. 1991. *The health potential of products containing bifidobacteria*. In: RK Robinson (eds.). *Therapeutic Properties of Fermented Milks*. Elsevier Applied Science Publishers, London; pp. 117-157.
- Matejčeková, Z., Liptáková, D., and Valík, L. 2017. Functional probiotic products based on fermented buckwheat with *Lactobacillus rhamnosus*. *LWT - Food Sci. Technol.* 81; pp. 35-41.
- Miranda, R. O., Carvalho de, A. F. and Nero, L. A. 2014. Development of a selective culture medium for bifidobacteria, Raffinose-Propionate Lithium Mupirocin (RP-MUP) and assessment of its usage with Petrifilm™ Aerobic Count plates. *Food Microbiol.* 39; pp. 96-102.
- Mohammadi, R., Mortazavian, A. M., Khosrokhavar, R. and Cruz, A. 2011. Probiotic ice cream: Viability of probiotic bacteria and sensory properties. *Ann. Microbiol.* 2011; 61; pp. 411-424.
- Moongngarm, A. and Saetung, N. 2010. Comparison of chemical compositions and bioactive compounds of germinated rough rice and brown rice. *Food Chem.* 122; pp. 782-788.
- Nealon, N. J., Worcester, C. R. and Ryan, E. P. 2017. *Lactobacillus paracasei* metabolism of rice bran reveals metabolome associated with *Salmonella Typhimurium* growth reduction. *J. Appl. Microbiol.* 122; pp. 1639-1656.
- Nousia, F. G., Androulakis, P. I., and Fletouris, D. J. 2011. Survival of *Lactobacillus acidophilus* LMGP-21381 in probiotic ice cream and its influence on sensory acceptability. *Int. J. Dairy Technol.* 64; pp. 130-136.
- Ozcan, T., Yilmaz-Ersan, L., Akpınar-Bayazit, A., Sahin, O. I., and Aydinol, P. 2010. Viability of *Lactobacillus acidophilus* LA-5 and *Bifidobacterium bifidum* BB-12 in rice pudding. *Mljekarstvo.* 2010; 60; pp. 135-144.
- Özdemir, C., Dagdemir, E., Celik, S., and Özdemir, S. 2003. An alternative ice cream production for diabetic patients. *Milchwissenschaft*, 58; pp. 167-166.
- Öztürk, H. I., Demirci, T. and Akın, N. 2018. Production of functional probiotic ice creams with white and dark blue fruits of *Myrtus communis*: The comparison of the prebiotic potentials on *Lactobacillus casei* 431 and functional characteristics. *LWT - Food Sci. Technol.* 2018; 90; pp. 339-345.
- Parussolo, G., Busatto, R. T., Schmitt, J., Pauletto, R., Schons, P. F., and Ries, E. F. 2017. Synbiotic ice cream containing yacon flour and *Lactobacillus acidophilus* NCFM. *LWT - Food Sci. Technol.* 82; pp. 192-198
- Pon, S. Y., Lee, W. J. and Chong, G. H. 2015. Textural and rheological properties of stevia ice cream. *Int. Food Res. J.* 2015, 22; pp. 1544-1549.
- potential sources of inulin-type fructans. *J. Food Comp. Anal.*, 24; pp. 642-649.
- Pupillo, M. 2008. Ice cream labeling - what does it all mean? *International Foodservice Distributors Association*. Retrieved on 9 August 2008 from <https://web.archive.org/web/20080514083818/http://www.idfa.org/facts/icmonth/page5.cfm>
- Raguin, A., and Ebenhöf, O. 2017. Design starch: stochastic modeling of starch granule biogenesis. *Biochem. Soc. Trans.* 45; pp. 885-893.
- Ravulaand, R. R. and Shah, N. P. 1998. Selective enumeration of *Lactobacillus casei* from yogurt and fermented milk drinks. *Biotechnol Tech.* 12; pp. 819-822.
- Sakurai, K., Kokubo, S., Hakamata, K., Tomita, M., S., Y. 1996. Effect of production conditions on ice cream melting resistance and hardness. *Milchwissenschaft*, 51; pp. 451-454.
- Saman, P., Vázquez, J.A., and Pandiella, S.S. 2008. Controlled germination to enhance the functional properties of rice. *Process Biochemistry.* 43(12); pp.1377-1382.
- Sandhu, K. S. and Singh, N. 2007. Some properties of corn starches II: physicochemical, gelatinization, retrogradation, pasting and gel textural properties. *Food Chemistry* 101, 1499-1507.
- Sangsila, A., Marquis, V.F., Leszkowicz, A., and Itsaranuwat, P. 2016. Detoxification of zearalenone by *Lactobacillus pentosus* strains. *Food Control.* 62 : 187-192.
- Santana, I. A., Ribeiro, E. P. and Iguti, A. M. 2011. Evaluation of green coconut (*Cocos nucifera* L.) pulp for use as milk, fat and emulsifier replacer in ice cream. *Procedia Food Sci.* 1; pp. 1447 – 1453.
- Singh, N., Kaur, I., Sandhu, K. S., Kaur. and Nishinari, K. 2006. Relationships between physicochemical, morphological, thermal, rheological properties of rice starches. *Food Hydrocoll* 20; pp.532-542.

- Sofjan, R. P. and Hartel, R. W. 2004. Effects of overrun on structural and physical characteristics of ice cream. *International Dairy Journal*, 14; pp. 255-262.
- Soukoulis, C., Yonekura, L., Gan, H. H., Behboudi-Jobbehdar, S., C Parmenter, C. and Fisk, I. 2014. Probiotic edible films as a new strategy for developing functional bakery products: The case of pan bread. *Food Hydrocoll.* 2014; 39; pp. 231–242.
- Sun-Waterhouse, D., Edmonds, L., Wadhwa, S. S. and Wibisono, R 2013. Producing ice cream using a substantial amount of juice from kiwifruit with green, gold or red flesh. *Food Res. Int.*,50; pp. 647–656.
- Tan, B. L. and Norhaizan, M. E. 2017. Scientific evidence of rice by-products for cancer prevention: chemopreventive properties of waste products from rice milling on carcinogenesis *In Vitro* and *In Vivo*. *Bio. Med. Res. Int.* 2017; pp. 901-912.
- Toussaint-Samat, M. 2006. The history of ice cream and the ice cream cone, translated by Anthea Bell, Barnes and Noble Books: New York, 1992 (pp. 749-50).
- Vinderola, C. G., Mocchiutti, P. and Reinheimer, J. A. 2002. Interactions among lactic acid starter and probiotic bacteria used for fermented dairy products. *J. Dairy Sci*, 85; pp. 721-729.
- Wood, J. M. 2011. *Sensory evaluation of ice cream made with prebiotic ingredients substituted for sugar*. Ph.D. Thesis, Nutrition and Health Sciences Dissertations.18. University of Nebraska-Lincoln.
- Zhang, S., Hu, H., Wang, L., Liu, F. and Pan, S. 2018. Preparation and prebiotic potential of pectin oligosaccharides obtained from citrus peel pectin. *Food Chemistry*, 244, pp. 232-237.

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