# Synbiotic Ice Cream Containing Germinated KDML105 Rice Flour and Lactobacillus acidophilus LA-5: Physicochemical, Probiotic Viability and Sensory Evaluation 

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#### Abstract

Nutraceutical and functional food like probiotics and prebiotics are increasingly incorporated in food industries and pharmaceutical application to promote and maintain health conditions. The feasibility of synbiotic ice creams, manufactured to include germinated brown rice (KDML105) flour as prebiotic sources, and Lactobacillus acidophilus LA-5 as probiotic culture was determined. Physicochemical and sensory attributes of synbiotic ice creams, as well as probiotic survivability over a 30 -day of storage were evaluated. 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). The physicochemical and microbiological analyses showed suitability with standards required by legislation, and all formulations had acceptable sensory attributes. Synb-1 and Synb-2 ice creams had higher counts of viable probiotic microorganisms compared to the other ice cream formulations. During a 30 -day storage period, the product matrix and pH maintained the viability of the probiotic microorganisms above $10^{6} \mathrm{CFU} / \mathrm{g}$, thus revealing the potential of the manufactured synbiotic ice creams.


Keywords: prebiotics, probiotics, functional food, germinated brown rice

## 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 daily products, such as milk and cream, and often combined with fruits or other ingredients and flavour. It is typically sweetened with sugar or sugar substitutes. Typically, flavourings and colourings are added in addition to stabilizers. The mixture is stirred to incorporate air spaces and cooled below the freezing point of water to prevent

[^0]detectable ice crystals from forming. The result is smooth, semi-solid foam that is solid at very low temperatures $\left(<2{ }^{\circ} \mathrm{C}\right)$. 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 goal'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). 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 et al., 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 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 improving its functional properties (Balthazar et al., 2015; Öztürk et al., 2018).

Probiotics are defined as live microorganisms that when consumed in adequate amounts ( $\geq 10^{6} \mathrm{CFU} / \mathrm{mL}$ ) 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 et al., 2016; Sangsila et al., 2016). Probiotic bacteria are most frequently included in the composition of dairy sector that represents the largest food market (Itsaranuwat et al., 2003; Matejčeková et al., 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 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 et al., 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 et al., 2018). The most common prebiotics come from cereals such as bean, rice, barley and wheat (Espinosa- Martos and Ruperez, 2006; Judprasong et al., 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 et al., 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 et al., 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 et al., 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 aimed 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 (colour, 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 preparation

The freeze-dried probiotic 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, at $37^{\circ} \mathrm{C}$ for 18 h (Miranda et al., 2014). The strain was activated by two subcultures in 100 mL MRS broth prior to experimental use (Ravulaand and Shah, 1998). After incubation, cell biomass was harvested by centrifugation at $8000 \times g$ for 15 min at $4^{\circ} \mathrm{C}$ and washed twice with Ringer solution (Merck). After the last centrifugation, cell biomass was suspended in 50 mL of UHT milk and held at $4^{\circ} \mathrm{C}$ until used ( 6 h ). The bacterial cell suspension at 10 or $11 \log \mathrm{CFU} / \mathrm{g}$ was used for ice cream manufacture.

## Preparation of germinated brown rice

Seeds of the rice cultivar KDML105 were obtained from local farms in Kalasin province, Northeast Thailand. Seed germination was carried out according to Moongngarm and Saetung (2010). Briefly, seeds were put into plastic bags 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 $30^{\circ} \mathrm{C}$ in the darkroom 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 $50^{\circ} \mathrm{C}$ to get $5 \%$ moisture content and the obtained seeds were 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, liquid ingredients, germinated brown rice flour (prebiotics), and L. acidophilus as shown in Table 1.

The synbiotic ice cream mix were performed according to Cody 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 ${ }^{\circ} \mathrm{C}$ for 30 min , followed by the addition of raw yolk when the temperature was
reduced to $40^{\circ} \mathrm{C}$. 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 . The final product was packaged in paper cups, and stored at $-20^{\circ} \mathrm{C}$ for up to 30 days. The product was evaluated for probiotic viability during storage for $1,7,15$ and 30 days. The experiment was conducted in triplicate.

Table 1. Ingredients used for synbiotic ice cream production

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

## Physicochemical analysis

The pH value was measured using a digital pH meter (Mettler Toledo, SevenMulti ${ }^{\text {TM }}$, Switzerland). Ice cream overrun was determined from a comparison of the weight of mix and ice cream in a fixed volume container (Özdemir et al., 2003) by using a 250 mL beaker, and the overrun percentage was calculated according to the equation: Overrun (\%) $=\left(W_{\mathrm{m}} / W_{\mathrm{ic}}-1\right) \times 100$, where $W_{\mathrm{m}}$ represents the weight $(\mathrm{g})$ of a given volume of mix and $W_{\text {ic }}$ denotes the weight ( g ) of the same volume of ice cream. The melting behavior, expressed as melting rate (\%), was evaluated as described by Santana et al. (2011). Briefly, $25-30 \mathrm{~g}$ of ice cream ( $-20^{\circ} \mathrm{C}$ ) were placed on a 2-mm wire mesh screen and left to melt into a 250 mL beaker at ambient temperature ( $25 \pm$ $2^{\circ} \mathrm{C}$ ) 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 $(\mathrm{g} / \mathrm{min})$.

## L. acidophilus viability

Ice cream samples were thawed and serially diluted in sterile $0.1 \%$ (w/v) peptone water, and viable cells were enumerated on MRS agar using pour plate technique as described by Vinderola et al. (2002). The counts of $L$. acidophilus were determined on MRS agar incubated aerobically at $37^{\circ} \mathrm{C}$ for 23 days and recorded as the $\log \mathrm{CFU} / \mathrm{g}$ of sample.

## Sensory evaluation

Before sensory evaluation, the synbiotic ice cream samples previously stored for one day at $-20^{\circ} \mathrm{C}$ were left to attain a temperature of -10 to $-8{ }^{\circ} \mathrm{C}$. For the 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 et al., 2011).

## Statistical analysis

Statistically significant was analysed 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, $\rho$-value $<0.05$ was considered.

## Results

## Overrun and melting rates

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). 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 $1.63 \mathrm{~g} / \mathrm{min}$, followed by Synb-2 (1.60 $\mathrm{g} / \mathrm{min}$ ), Synb-3 ( $1.58 \mathrm{~g} / \mathrm{min}$ ) and Synb-4 ( $1.56 \mathrm{~g} / \mathrm{min}$ ) ice creams ( $p<0.05$ ), as presented in Table 2.

Table 2. Overrun and melting rates of four synbiotic ice cream formulations at $25^{\circ} \mathrm{C}$.

| Parameters | Value $^{\mathbf{x}}$ |  |  |  |
| :--- | :---: | :---: | :---: | :---: |
|  | ${\text { Ice } \text { cream }^{\mathbf{y}}}$ |  |  |  |
|  | Synb-1 | Synb-2 | Synb-3 | Synb-4 |
| Overrun (\%) | $89.85 \pm 0.14^{\mathrm{d}}$ | $92.62 \pm 0.27^{\mathrm{c}}$ | $93.72 \pm 0.14^{\mathrm{b}}$ | $96.52 \pm 0.68^{\mathrm{a}}$ |
| Melting rate at 30/min | $1.63 \pm 0.03^{\mathrm{a}}$ | $1.60 \pm 0.01^{\mathrm{b}}$ | $1.58 \pm 0.01^{\mathrm{b}}$ | $1.56 \pm 0.01^{\mathrm{c}}$ |
| Different capital letters in the same row denote significant differences $(p<$ | $0.05)$ between |  |  |  |
| different ice cream formulation for the same parameter. |  |  |  |  |
| ${ }^{\mathrm{x}}$ means of seven replicates. |  |  |  |  |
| ${ }^{\mathrm{y}}$ see Table 1 for the description of the ice cream formulations. |  |  |  |  |

## Bacterial viability in synbiotic ice creams

The viable counts (expressed as $\log \mathrm{CFU} / \mathrm{g}$ ) of L. acidophilus LA-5 in the ice cream mix and during freezing of the synbiotic ice creams at $-20 \pm 1^{\circ} \mathrm{C}$ 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 $7.80 \log \mathrm{CFU} / \mathrm{g}$ 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 $3.16 \log \mathrm{CFU} / \mathrm{g}$ 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.

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 $1.09 \pm$ $0.02 \mathrm{ml} / \mathrm{minute}$ 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 $-20 \pm 1^{\circ} \mathrm{C}$ 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.


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

## 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 et al. (2011).

Table 3. Mean pH values (mean $\pm \mathrm{SD}$ ) of four synbiotic ice cream formulations during frozen storage $\left(-20 \pm 1^{\circ} \mathrm{C}\right)$ for 30 days.

| Storage period (days) | $\mathbf{p H}^{\mathbf{x}}$ |  |  |  |
| :---: | :---: | :---: | :---: | :---: |
|  | Ice cream $^{\mathbf{y}}$ |  |  |  |
|  | 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.
${ }^{x}$ Means of seven replicates.
${ }^{y}$ 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 Synb2 appearing to be of most preference despite significant differences observed when compared to Synb-1 and Synb-3.

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

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

Different letters in the same row indicate significant differences ( $p<0.05$ ) between different ice cream formulations for the same parameter.
${ }^{x}$ Means of 27 replicates.
${ }^{\mathrm{y}}$ See Table 1 for the description of the ice cream formulations.
Associations between ice cream formulations and viable counts frozen
storage
Associations between the ice cream formulations and viable counts during the frozen storage of the ice creams were predicted with the determination efficiency regression analysis ( $R^{2}$ ) model. Regression models were constructed to predict the overall acceptability of ice cream recipes and the number of viable frozen ice cream stores. In addition to counting microorganisms, pH and physical properties and properties of ice cream were
associated. Ice creams preserve probiotic bacteria and count the viable lactic acid bacteria that experiment after the lowest freeze storage to obtain probiotics.

The dependent variables 'Overall acceptability' mentioned above can be predicted 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 ) $\mathrm{R}^{2}=0.241$ with adjusted $\mathrm{R}^{2}=0.226$. Hence, the equation model for the overall acceptance of ice cream formulations was established as: Overall acceptability $=4.55-0.39$ Synb- $4+0.26$ 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 byKirmann \& Rasic (1991) at 6, (b) Store time $=0\left(\mathrm{~T}_{0}\right)$ with unstandardized coefficient $=2.375$ and standardized beta $=0.354$, (c) Store time $=30\left(\mathrm{~T}_{30}\right)$ with unstandardized coefficient $=-4.149$ and standardized beta $=-0.618$, (d) Store time $=15\left(\mathrm{~T}_{15}\right)$ with unstandardized coefficient $=-3.169$ and standardized beta $=-0.472$, $(\mathrm{e})$ 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 $=7\left(\mathrm{~T}_{7}\right)$ with unstandardized coefficient $=-0.773$ and standardized beta $=-0.115$, (h) $\mathrm{R}^{2}=0.241$ with adjusted $\mathrm{R}^{2}=0.226$. Hence, the equation model for the viable count was established as: Viable counts $=8.8+$ $0.35 \mathrm{~T}_{0}-0.62 \mathrm{~T}_{30}-0.47 \mathrm{~T}_{15}+0.34$ Synb-1 +0.24 Synb- $2-0.12 \mathrm{~T}_{7}$; Adjusted $\mathrm{R}^{2}=$ 0.87 .

## Discussion

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 et al., 2010). The incorporation of too much air produces fluffy ice creams and too little produces soggy, heavy products. 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 $4 \mathrm{~g} / 100 \mathrm{~mL}$ 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 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 et al., 2013).

The melting rate of ice creams 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 et al., 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.

For the viable counts of probiotic bacteria, as with the recommended minimum level of the viable bacterial count of $10^{6} \mathrm{CFU} / \mathrm{g}$ for synbiotic products (Nousia et al., 2011), Synb-1 and Synb-2 ice creams could be stored at $-20 \pm 1^{\circ} \mathrm{C}$ 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 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 et al. (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 $5.8 \mu \mathrm{~m}$. 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 $\left(\Delta H_{\mathrm{gel}}\right)$ of 13.81 and $12.32 \mathrm{~J} / \mathrm{g}$, 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 et al., 2015).

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.

The sensorial properties of probiotic ice cream are important for consumer acceptability. Ozcan et al. (2010) reported that rice pudding was considered suitable food for the delivery of probiotic micro-organisms, with sufficient viability and acceptable sensory characteristics. Moreover, it demonstrated similar acceptability to the control up to 14 days and declined thereafter. 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 ( $\mathrm{P}<0.05$ ). The $30 \%$ inulin ice cream
was significantly less sweet than the control and $10 \%$ and $20 \%$ inulin ice cream, less smooth and less vanilla flavour than the control, and less acceptable than the control and $10 \%$ inulin ice cream ( $\mathrm{P}<0.05$ ) (Wood, 2011).

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 show that Synb-1 and Synb-2 have more probiotic bacteria compared to other ice cream formulations. Based on the results of the product matrix and the pH , the ability of probiotic bacteria to be higher than $10^{6} \mathrm{CFU} / \mathrm{g}$ over a 30 day storage period illustrates the potential of synbiotic ice creams. Probiotics are living microorganisms which are used as a nutritional supplement for health by improving the balance of intestinal microbial in the human body. Lactic Acid and Bacteria (LAB) are the most important probiotics. Today they are included in a variety of foods. The quality of the product depends on the viability of the probiotic bacteria. We discuss about their therapeutic roles, their level of life guidance, and the choice of synbiotics and prebiotics modes. For probiotics to pass through the food, the number of cells prior to processing must be increased to determine cell loss during the production and/or storage stages.

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