
Development and quality enhancement of fermented rice bran and probiotic incorporated ice cream

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Abstract *Bifidobacterium* spp., *Lactobacillus acidophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* (10^{12} CFU ml⁻¹) was used to ferment rice bran in order to produce probiotic incorporated ice cream. According to the testing of the ice cream produced from the fermented and non-fermented rice bran and probiotic, the highest consumer preferred the color and acceptability which recorded for fermented rice bran + probiotic (FRB+P) incorporated ice cream. The results were significantly differed in dry matter % (DM), ash %, and overrun % which observed in all rice bran incorporated ice cream. During storage, the pH of all treatments decreased while titratable acidity (TA) increased. The highest score for the taste was observed from the probiotic incorporated ice cream. A reduction of probiotic count (10^9 – 10^7 CFU ml⁻¹) was observed in (FRB+P). However, the value remained above the minimum therapeutic value (10^6 CFU ml⁻¹). The fermented rice bran (1%) and probiotic collectively enhanced the consumer preference and nutritional properties of ice cream without quality deterioration at -18 °C for 21 days.

Keywords: Colour, Fermentation, Nutritional properties, pH, Probiotics

Introduction

Rising consumer awareness of the connection between diet and health has led to an increased global demand for functional foods, including dairy products such as yogurt, cheese, and milk (Salem *et al.*, 2005; Barbosa *et al.*, 2015). Functional foods provide extra health benefits beyond basic nutrition, with one of the primary objectives in their development being the inclusion of beneficial

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microorganisms, especially probiotics, in these products (Di Criscio *et al.*, 2010). Food products with probiotics have therapeutic benefits such as promoting a healthy gut microbiota, enhancing the immune system, reduction of serum cholesterol, inhibition of bacterial pathogens by producing antimicrobial substances, prevention of lactose intolerance, help to prevent constipation, diarrhea and bowel cancer (Walstra *et al.*, 2005; Di Criscio *et al.*, 2010; Karlton-Senaye *et al.*, 2015). The increasing consumer preference for health-promoting foods, along with advances in food science, continues to fuel innovation of functional dairy foods. For probiotics to confer health benefits, they must be consumed in sufficient quantities, which requires careful formulation and adequate levels of viable probiotics, at least 10^6 – 10^8 CFU g⁻¹ are required to be present (Albadran *et al.*, 2015).

Ice cream is a frozen food made from milk solids, edible fats, and, if needed, additional approved ingredients. It is primarily designed to be stored, sold, and consumed while frozen (Alvarez, 2008). Due to its widespread popularity globally, ice cream is an ideal vehicle for delivering probiotics, prebiotics, and other nutrients. Its appealing texture, taste, and composition offer significant potential for using it as a medium for transferring beneficial probiotics (Akaln *et al.*, 2018). Ice cream serves as an effective medium for preserving the functional properties of health-promoting probiotics, over extended periods. The low-temperature environment, including frozen storage and protection from light (except during the homogenization and heat treatment process), helps achieving the viability of these probiotics (Soukoulis *et al.*, 2014).

The development of food products that incorporate both probiotics and prebiotics has gained increasing attention due to the potential benefits for both probiotic survival and gut health (Hasani *et al.*, 2016). Prebiotics are non-digestible food components that selectively promote the growth and activity of beneficial, naturally occurring probiotics (Jay *et al.*, 2008).

Rice bran, a byproduct of rice milling, is one of the most abundant and underutilized resources in rice processing, comprising approximately 10% of the raw rice weight. It is a complex mixture of endosperm, germ, seed coat, aleurone layer, subaleurone layer, and pericarp. Rice bran has a light color, a mildly oily texture, a sweet taste, and a slightly toasted nutty flavor. Its nutritional composition includes 10–15% moisture, 11–17% protein, 12–22% oil, 6–14% fiber, and 8–17% ash. It is also rich in natural antioxidants such as tocotrienols, oryzanols, and tocopherols (Zubaidah *et al.*, 2012; Sharif *et al.*, 2014).

Fermentation of rice bran enhances the bioavailability of its nutrients and improves the digestibility of its fiber fraction (Sharif *et al.*, 2014). The fermentation process also produces indigestible oligosaccharides, oligopeptides, and resistant starches, which act as prebiotics to promote the growth of probiotics

(Zubaidah *et al.*, 2012). However, despite its nutritional potential, its predominantly used in animal feed (Sharif *et al.*, 2014), and there is limited research exploring its use in human foods.

The study aimed to develop a probiotic ice cream that incorporates fermented rice bran as a prebiotic.

Materials and methods

Raw milk was obtained from the farm belongs to Faculty of Agriculture, Rajarata University of Sri Lanka in Anuradhapura, Sri Lanka. Probiotic culture (ABY-3 probiotic culture) that contain *Bifidobacterium* spp., *Lactobacillus acidophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* were purchased from Aletech International, Rajagiriya, Sri Lanka. The rice bran in BG-300 variety was ground into small particles, sieved and sterilized at 121 °C for 15 minutes before being used and it was cooled to 4 °C.

Fermentation of rice bran

Rice bran was inoculated with ABY-3 probiotic culture and incubated for 72 hours at 37 °C. A 2 mL of cell suspension containing 1.5×10^8 (CFU mL⁻¹) was added to 10 g of rice bran with 1 g of skimmed milk powder. The cell suspension was prepared according to the 0.5 McFarland standard by adjusting the absorbance at 600 nm to the 18 value 0.06 (Rajkowska and Kunicka-styczyn, 2012). After fermentation, rice bran was sterilized at 121 °C for 15 minutes, cooled to 4 °C and stored at -18 °C.

Preliminary trials for selection of rice bran level

Preliminary trials were conducted at 1%, 2% and 3% rice bran levels to select the best rice bran level for ice cream preparation (Yangilar, 2015a). Evaluation was practiced with five -point Hedonic scale with the participation of 30 untrained sensory panelists.

Physicochemical analyses of non-fermented and fermented rice bran

The dry matter, crude fat, crude fiber, crude protein, ash content, pH, and acidity of both non-fermented and fermented rice bran were determined using the methods described by AOAC (2010) and Yangilar (2015a). These parameters were analyzed to compare the nutritional and chemical properties of the rice bran before and after fermentation. The dry matter content was measured to determine the percentage of solids in the sample. Crude fat and crude protein were assessed

to evaluate the lipid and protein content, while crude fiber provided information on the fibrous components of the rice bran. The ash content was determined to quantify the mineral content, and the pH and acidity were measured to understand the potential changes in the acidity and overall chemical environment as a result of fermentation of rice bran.

Preparation of ice cream

Six types of ice cream were produced, including control sample (without adding rice bran or probiotic (C), probiotic (P), non-fermented rice bran (RB), non-fermented rice bran + probiotic (RB+P), fermented rice bran (FRB), fermented rice bran + probiotic (FRB+P). Fat content of cow milk was standardized up to 10% by adding cream and 11% solid nonfat, 17% sugar, 0.5% stabilizer and 0.3% vanilla flavor was added with standardized milk. Selected level of fermented rice bran was added to the mix separately and homogenized mixture was pasteurized at 85 °C for 15 minutes. After cooling to 15 °C and 0.1 g of ABY-3 probiotic culture was added. Then it was stored at -4 °C for 18 hours, aerated and frozen in an ice cream maker about 40 minutes and packed in plastic containers. The containers were kept at -18 °C until further analysis. Ultimately, the physicochemical characteristics, overrun %, color and microbiological analysis were done throughout the frozen storage at -18 °C.

Nutritional analyses of ice cream

Samples were analyzed for dry matter, crude fat, crude protein, ash and crude fiber using hot air oven (YCO-010, Taiwan), soxhelt extractor (MICROSIL, India), Kjeldahl unit (DK 20, Italy), and muffle furnace (DMF-05, Korea) and fiber analyzer (FIWE3, Italy) respectively according to the protocols described by AOAC (2010).

Measurement of pH and acidity

The pH of all ice cream samples was measured at 1, 7, 14, and 21 days of storage using a pH meter. Additionally, the acidity of the ice cream was determined in triplicate by titration with 0.1 N sodium hydroxide, using phenolphthalein as an indicator, in accordance with the AOAC (2010) method.

Evaluation of overrun%

The overrun values were determined following the method outlined by Akalin *et al.* (2018) and were expressed using the following formula:

$$\text{Overrun (\%)} = \frac{(\text{weight of ice cream mix} - \text{weight of ice cream})}{(\text{weight of ice cream})} \times 100$$

Evaluation of color

The color was determined by Chroma meter and defined using L* a* b* system, where L* = lightness, a* = red-green color, and b* = yellow-blue color.

Microbiological analyses

Microbiological analysis was conducted on ice cream samples stored at -18 °C for 1, 7, 14, and 21 days. A 1 g sample of each ice cream variant was transferred into a sterile test tube, then diluted with 9 mL of distilled water and mixed thoroughly. From the initial dilution, 1 mL was further serially diluted in 10-fold steps for microbial enumeration. Lactic acid bacteria (LAB) were cultured on MRS agar and incubated at 28°C for 48 hours. Yeasts and molds were enumerated on potato dextrose agar, (PDA) incubated at 28°C for 72 hours. Total coliforms were counted on MacConkey agar after incubation at 37°C for 24 to 48 hours (Cruz *et al.*, 2013).

Sensory analysis of final ice cream

The evaluation was conducted with the participation of 30 untrained sensory panelists. The panelists were asked to rate the color, texture, taste, aroma, and overall acceptability of the samples using a five-point hedonic scale.

Statistical analyses

All experiments were conducted in triplicates. The differences in microbiological and physiological properties among the ice cream samples were analyzed using a Completely Randomized Design (CRD). The results are presented as mean ± standard deviation (SD). One-way analysis of variance (ANOVA), performed using the Statistical Analysis System (SAS), version 9.0 (SAS, 2000), with a 95% confidence interval, was applied to assess differences between groups. Tukey's Standardized Range Test was used for mean separation. Sensory evaluation data were analyzed using SPSS software, with a 95% confidence interval, and the non-parametric Friedman test.

Results

Preliminary trials for selection of rice bran level

1% rice bran incorporated ice cream had the highest preference than other types (Table 1). Therefore, 1% rice bran level was selected for further studies.

Table 1. Result of preliminary sensory test

Type	Average Rank
1% rice bran incorporated ice cream	20.5
2% rice bran incorporated ice cream	42.5
3% rice bran incorporated ice cream	73.5

Physiochemical characteristics

The ash content was not shown a significant difference ($p > 0.05$) between the non-fermented and fermented rice bran samples (Table 2). However, the fiber content was significantly higher in non-fermented rice bran compared to the fermented variety. Following fermentation, the fiber content decreased significantly. Fermented rice bran exhibited higher levels of crude protein and crude fat than non-fermented rice bran. Additionally, the fermented rice bran had the highest TA% and the lowest pH (Table 2).

Table 2. Physiochemical properties of non-fermented and fermented rice bran

Nutrients	Non-fermented Rice Bran	Fermented Rice Bran
Crude protein (%)	10.99±0.06 ^a	12.00±0.04 ^b
Crude fat (%)	19.31±0.56 ^a	20.87±0.12 ^b
Crude fiber (%)	12.43±0.01 ^a	8.74±0.01 ^b
Ash (%)	7.81±0.06 ^a	8.58±0.48 ^a
pH	6.65±0.01 ^a	5.42±0.01 ^b
Acidity (%)	0.19±0.01 ^a	0.72±0.01 ^b

Note: the values in each row with the same overwritten letter are not significantly different ($p > 0.05$). Values are expressed as Means + SD. C=control, P=probiotic, RB=non-fermented rice bran, RB+P=non-fermented rice bran + probiotic, FRB=fermented rice bran, FRB+P=fermented rice bran + probiotic

Physiochemical characteristics of ice cream

The dry matter content in both the control and probiotic-incorporated ice creams was significantly lower ($p < 0.05$) than in other tested samples (Table 3). The addition of rice bran led to an increase in the dry matter content of the ice cream. In contrast, control and probiotic-incorporated ice cream samples had lower ash percentages compared to those containing rice bran. Ice creams made with fermented rice bran or fermented rice bran with probiotics had the highest levels of crude protein and crude fat, while control and probiotic only ice creams had the lowest values (Table 3).

Table 3. Physiochemical properties of final ice cream products

Treatment	Dry matter %	Crude Protein %	Crude fat %	Ash %	Overrun %
C	38.36±0.12 ^b	9.03±0.06 ^c	8.2±0.01 ^c	0.87±0.01 ^b	30.8±0.65 ^b
P	38.35±0.40 ^b	9.06±0.01 ^c	8.18±0.02 ^c	0.89±0.02 ^b	31.58±0.40 ^b
RB	39.25±0.26 ^a	9.36±0.04 ^b	8.50±0.02 ^b	0.93±0.01 ^a	34.37±0.52 ^a
FRB	39.17±0.35 ^a	9.72±0.04 ^a	8.82±0.03 ^a	0.92±0.02 ^a	34.33±0.59 ^a
RB + P	39.12±0.40 ^a	9.36±0.02 ^b	8.47±0.02 ^b	0.93±0.04 ^a	34.34±0.39 ^a
FRB + P	39.13±0.16 ^a	9.72±0.03 ^a	8.78±0.02 ^a	0.94±0.01 ^a	34.85±0.32 ^a

Note: the values in each column with the same overwritten letter are not significantly different ($p > 0.05$). Values are expressed as Means + SD. C=control, P=probiotic, RB=non-fermented rice bran, RB+P=non-fermented rice bran + probiotic, FRB=fermented rice bran, FRB+P=fermented rice bran + probiotic

pH and acidity evaluation

The changes in pH and TA% of the samples were monitored over 21 days of frozen storage (Figure 1 and Figure 2). The pH values of all treatments were decreased over time, with significant differences ($p < 0.05$) throughout the storage. Ice creams with fermented rice bran and those with both fermented rice bran and probiotics exhibited the lowest pH levels. By the 21st day of storage, the pH of the fermented rice bran + probiotic incorporated ice cream was significantly declined. The TA% values were increased during storage.

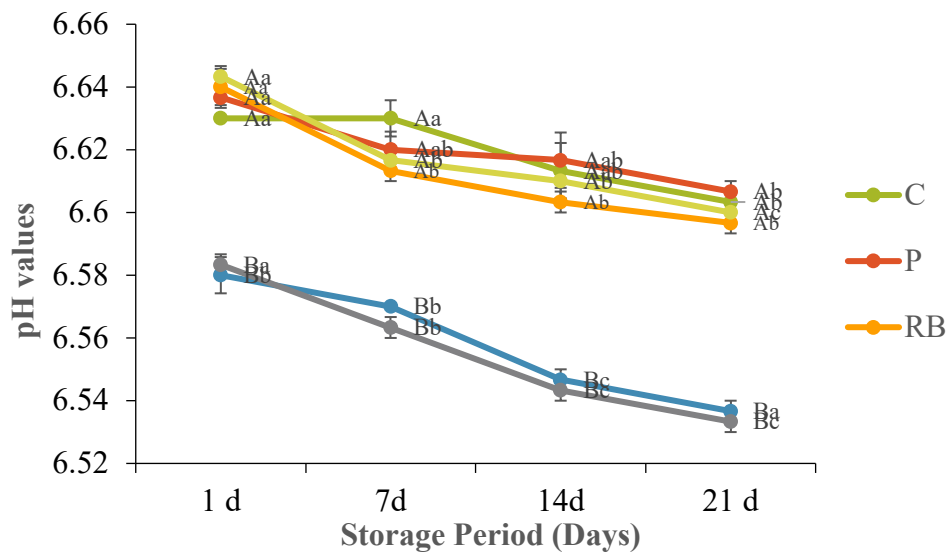


Figure 1. Pattern of pH variation during storage of ice cream A, B showed the significant difference among the treatments. a, b, c showed significant difference of the treatments during the storage period

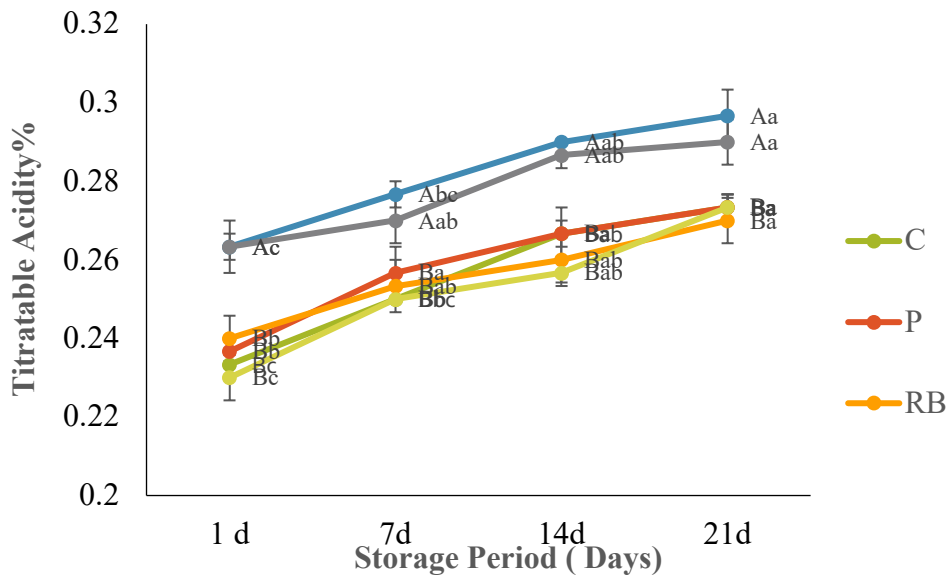


Figure 2. Pattern of titratable acidity variation during storage of ice cream A, B showed significant difference among the treatments. a, b, c showed significant difference of the treatments during the storage period

Effect of storage on color attributes (L, a, and b*) of rice bran and probiotic formulated ice cream

The factors of both ice cream formulation and storage time were significantly ($p < 0.05$) impacted the L* and a* values (Table 4). The color of the ice cream was notably influenced by the inclusion of rice bran, which imparted a brownish hue to the product. Ice cream containing rice bran exhibited significantly higher a* values and lower L* values compared to the control and only probiotic incorporated samples. During storage, the lightness (L*) values of all ice cream formulations decreased significantly ($p < 0.05$). However, the control and probiotic only ice creams were maintained the highest L* values throughout the period of storage.

Table 4. Color variation during storage of ice cream

Treatment	Storage Period (Days)			
	1 day	7 day	14 day	21 day
L* value				
C	81.43±0.02 ^{Aa}	81.40±0.01 ^{Aa}	81.25±0.01 ^{Ab}	80.56±0.01 ^{Ac}
P	81.42±0.02 ^{Aa}	81.41±0.01 ^{Aa}	81.22±0.03 ^{Ab}	80.56±0.01 ^{Ac}
RB	77.14±0.05 ^{Ba}	76.66±0.1 ^{Bb}	76.42±0.02 ^{Bc}	76.12±0.02 ^{Bd}
FRB	77.15±0.14 ^{Ba}	76.70±0.1 ^{Bb}	76.42±0.01 ^{Bc}	76.13±0.01 ^{Bd}
RB + P	77.18±0.10 ^{Ba}	76.71±0.02 ^{Bb}	76.40±0.01 ^{Bc}	76.13±0.01 ^{Bd}
FRB + P	77.10±0.10 ^{Ba}	76.68±0.02 ^{Bb}	76.39±0.01 ^{Bc}	76.13±0.01 ^{Bd}
a* value				
C	1.62±0.01 ^{Bc}	1.64±0.01 ^{Bc}	1.65±0.01 ^{Bb}	1.68±0.01 ^{Ba}
P	1.62±0.01 ^{Bb}	1.64±0.01 ^{Bb}	1.66±0 ^{Ba}	1.68±0.01 ^{Ba}
RB	2.53±0.01 ^{Ad}	2.58±0.01 ^{Ac}	2.57±0.01 ^{Ab}	2.59±0.01 ^{Aa}
FRB	2.53±0.01 ^{Ab}	2.56±0.01 ^{Aa}	2.57±0.01 ^{Aa}	2.58±0.01 ^{Aa}
RB + P	2.53±0.01 ^{Ac}	2.56±0.01 ^{Ab}	2.58±0.01 ^{Aa}	2.58±0.021 ^{Aa}
FRB + P	2.54±0.01 ^{Ac}	2.55±0.01 ^{Abc}	2.57±0.01 ^{Aab}	2.59±0.01 ^{Aa}
b* value				
C	9.18±0.02 ^{Aa}	9.18±0.01 ^{Aa}	9.20±0.01 ^{Aa}	9.21±0 ^{Aa}
P	9.18±0.02 ^{Aa}	9.19±0.01 ^{Aa}	9.20±0.01 ^{Aa}	9.21±0.01 ^{Aa}
RB	9.19±0.01 ^{Aa}	9.20±0.01 ^{Aa}	9.20±0.01 ^{Aa}	9.21±0.01 ^{Aa}
FRB	9.17±0.01 ^{Aa}	9.18±0.01 ^{Aa}	9.20±0.01 ^{Aa}	9.21±0.01 ^{Aa}
RB + P	9.18±0.01 ^{Ac}	9.19±0.01 ^{Abc}	9.21±0.01 ^{Aab}	9.22±0.01 ^{Aa}
FRB + P	9.18±0.01 ^{Ab}	9.19±0.01 ^{Aab}	9.19±0.01 ^{Aab}	9.22±0.02 ^{Aa}

Note: the values in each column with the same overwritten letter are not significantly different ($p > 0.05$). Values are expressed as Means + SD. C=control, P=probiotic, RB=non-fermented rice bran, RB+P=non-fermented rice bran + probiotic, FRB=fermented rice bran, FRB+P=fermented rice bran + probiotic A, B show significant difference among the treatments. a, b, c, d show significant difference of the treatments during the storage period.

The a^* values, which are associated with red intensity, increased significantly ($p < 0.05$), indicating a gradual shift toward a red hue. The highest a^* value, representing a more intense red color, was observed in the rice bran fortified ice cream. The addition of rice bran provided a slight increase in b^* values (which corresponds to the yellow-blue axis), although the effect was not statistically significant. Nonetheless, the b^* values were shown a slight upward trend.

Sensory evaluation

The overall acceptability, color, and texture values for the fermented rice bran and probiotic incorporated ice cream were significantly higher ($p < 0.05$) compared to the other formulations (Figure 3). The most acceptable taste score has been given to probiotic incorporated ice cream. Fermented rice bran had a notable effect on the sensory characteristics that fiber enriched ice cream formulations receiving lower scores for overall acceptability and sensory characteristics ($p < 0.05$). Specifically, the samples containing higher fiber content, including those with rice bran, tended to have less favorable sensory evaluations.

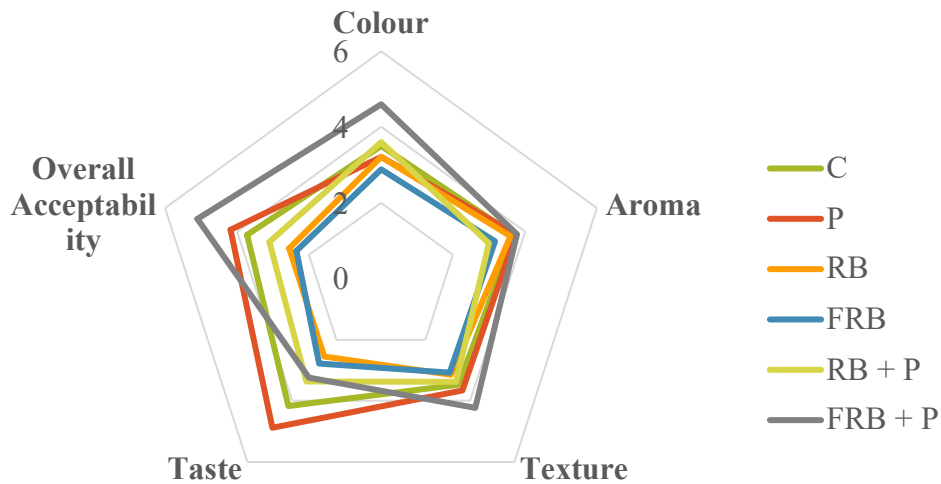


Figure 3. Pattern of variation in sensory properties during storage of ice cream

Viability of probiotic bacteria

A slight decline in the viable count of probiotics was noted up to the 7th day of storage, after which the count decreased more significantly (Figure 4).

The reduction in probiotic count was more pronounced in the probiotic-only ice cream. The survival rate of probiotics was notably higher in both rice bran-enriched ice creams (fermented and non-fermented), likely because rice bran served as a prebiotic that supported the growth and viability of the probiotics (Zubaidah *et al.*, 2012).

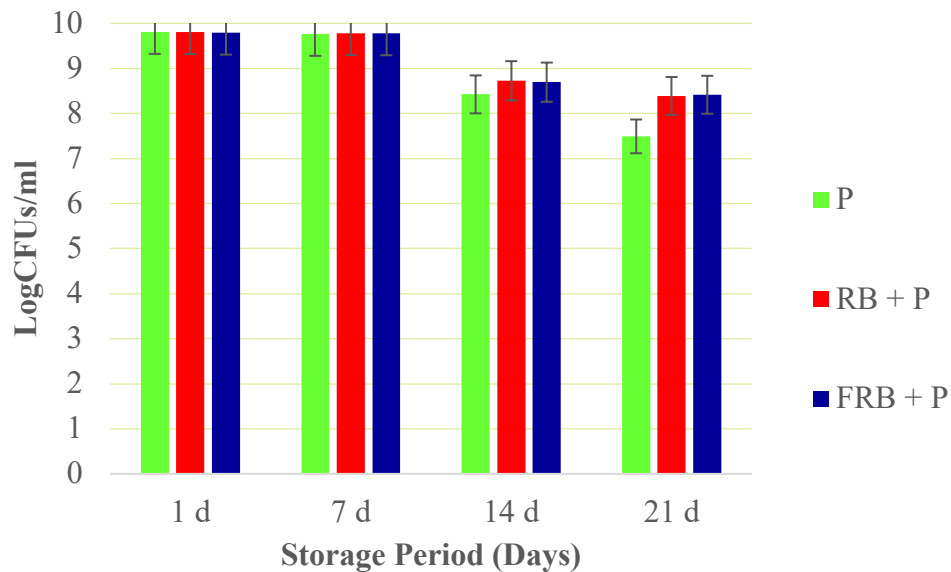


Figure 4. Pattern of population variation in probiotic bacteria during storage of ice cream

Microbial count during storage

There was no coliform and yeast and mold growth during 21 day storage period.

Discussion

Physiochemical properties of non-fermented and fermented rice bran

Ash is one of the energy sources of fermenting microorganisms (Bhosale and Vijayalakshmi, 2015), and because of that, the level of ash content may

decrease during the fermentation process of rice bran. In probiotic culture, there was anaerobic *Bifidobacterium*. Therefore, the oxygen exposure may contribute to the death of anaerobic bacteria. Therefore, the microbial population of the *Bifidobacterium* spp. might have an effect. During the fermentation of rice bran, several biochemical changes occur that enhance its nutritional profile. According to Muhakka *et al.* (2015), bacteria involved in the fermentation process hydrolyze cellulose and hemicellulose, breaking them down into simpler sugars. This breakdown not only reduces the crude fiber content but also releases nitrogen, which can contribute to an increase in protein levels. The fermentation process also increases the availability of proteins and fats, as highlighted by Bhosale and Vijayalakshmi (2015). The fermentation not only makes these nutrients more bioavailable but also leads to an overall increase in protein and fat, further enhancing the nutritional value of the fermented rice bran. Furthermore, certain probiotic microorganisms have the ability to convert saturated fats into healthier unsaturated fats, as noted by Yadav *et al.* (2011). This conversion enhances the fatty acid profile of the fermented rice bran, making it more beneficial for health.

During fermentation, lactic acid bacteria (LAB) ferment glucose into organic acids, which lowers the pH (Zubaidah *et al.*, 2012). LAB primarily produce lactic acid and acetic acid (De Vuyst and Leroy, 2020), both of which contribute to the decrease in pH (Sun *et al.*, 2021).

Physicochemical properties of ice cream

Direct inoculation of probiotics prior to the freezing step and produced unfermented probiotic ice cream. Because of that, the addition of probiotics does not change the composition, the viscosity, and physicochemical characteristics and the overrun values (Soukoulis *et al.*, 2014). Incorporation of rice bran might influence the significantly lower ash percentage. This is similar to the findings of Akalın *et al.* (2018), Yangilar (2015a), and Yangilar (2015b). The overrun ratios of the studied samples were low, and similar to the findings of Di Criscio *et al.* (2010), and Hashim and Shamsi (2016). The current study used the batch-type freezing machine. In the batch-type freezing system, the air is incorporated into the mix at atmospheric pressure. The low overrun values may have occurred due to the batch freezing (Akalın *et al.*, 2018) and the lack of homogenization. The introduction of dietary fibers into the medium increased the overrun of the tested ice cream samples (Akalın *et al.*, 2018).

Evaluation of pH and acidity

A decrease in pH during fermentation is likely due to increased lactic acid production, which results from the growth of LAB over time. The dietary fibers in rice bran may have supported the growth and activity of LAB, enhancing lactic acid production during the storage period. However, the activity of LAB in both the control and probiotic-incorporated ice cream remained low due to the absence of rice bran's dietary fibers.

These results were in agreement with previous studies by Inoue *et al.* (1998), Singh *et al.* (2014), and Salem *et al.* (2005). It is also noted that the pH of unfermented ice cream typically ranges around 6.3, depending on manufacturing practices (Soukoulis *et al.*, 2014). The increase in acidity observed in the ice cream during storage is likely due to the production of lactic acid by both LAB and psychrophilic bacteria (Murtaza *et al.*, 2004).

Although probiotics were directly added, they did not significantly affect the pH, which remained comparable to the control. These findings align with those of Murtaza *et al.* (2004), Abdullah *et al.* (2003), and Singh *et al.* (2014), who also have observed an increase in acidity during the storage of ice cream. According to the Sri Lanka Standards Institution (SLSI), the acceptable acidity level in ice cream should be around 0.25 g per 100 g. Since this product is considered a value-added item, it falls under the "complex ice cream" category, where acidity levels may vary due to its more complex composition. Therefore, all treatments and control samples in this study remained within the acceptable titratable acidity range.

Sensory evaluation of final ice cream

The present study, the highest taste score was observed in the probiotic-incorporated ice cream. In contrary Yangilar (2015b), reported that fortifying ice cream with date fiber significantly affected all sensory properties except sweetness.

Viability of probiotic bacteria

Bifidobacterium spp., *Lactobacillus acidophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, and *Streptococcus thermophilus*, have been observed to act synergistically, potentially offering a symbiotic relationship. Incorporating these symbiotic cultures, particularly *L. acidophilus* and *S. thermophilus* into ice cream has been shown to reduce the detrimental effects of frozen storage on probiotic viability. This is reflected in the maintenance of β -galactosidase enzyme activity, a marker of probiotic health and function (Soukoulis *et al.*, 2014). Such

interactions are believed to contribute to the high probiotic viability observed during storage.

If probiotics to be considered functional, it is crucial that they maintain a viable concentration of approximately 10^6 viable cells per mL of the product at the time of consumption (Di Criscio *et al.*, 2010). In the present study, all the probiotic-incorporated ice cream samples exceeded this therapeutic minimum, indicating that the viability of the probiotics was successfully preserved throughout storage of 21 days.

Microbial count during storage

During the 21 day storage period, no coliforms, yeasts, or molds were observed, indicating that proper hygienic conditions were maintained throughout the production process. Additionally, the rice bran used in the ice cream preparation was sterilized both before and after fermentation to ensure product safety and quality. Sterilized fermented and non-fermented rice bran were stored at frozen storage ($-18\text{ }^{\circ}\text{C}$) until used. A study conducted by Ahmed and El Zubeir (2015), also reported no growth of coliform of a ice cream made by camel milk, honey, vanilla, sugar, skim milk powder, coconut and gum arabic.

It can be concluded that ABY-3 probiotic culture consisted of *Bifidobacterium* spp., *Lactobacillus acidophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* was effectively used for the fermentation process of rice bran-based ice cream. Fermented rice bran and probiotic incorporated ice cream was exerted more favorable sensory attributes than the other ice cream during the initial stages after production. The ice cream formulations incorporating fermented rice bran, both with and without probiotics, demonstrated superior physicochemical and nutritional properties compared to the control. During the 21 days of frozen storage period at $-18\text{ }^{\circ}\text{C}$, these probiotic-enriched ice creams maintained acceptable levels of pH, titratable acidity (TA%), and microbial safety, including yeast and mold counts. Furthermore, the probiotic content of the rice bran based ice creams remained within the desired range, indicating the stability and viability of the probiotics throughout 21 days of storage. These findings suggested that the inclusion of fermented rice bran and probiotics not only enhances the nutritional value of the ice cream but also ensures its safety and quality over time with in the 21 days of frozen storage.

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