FUNCTIONAL FOOD DEVELOPMENT FROM INDIGENOUS PIGMENTED RICE: A STUDY ON KATTUYANAM–MAPILLAI SAMBA RICE COMPOSITE-BASED PROBIOTIC BEVERAGE AND ICE CREAM

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ABSTRACT

Objective: Functional probiotic foods derived from traditional pigmented rice enhance nutritional value and health benefits, while also improving consumer acceptance through innovative products like beverages and ice cream. This study highlights the development and characterisation of probiotic ice cream and beverages made using a Kattuyanam-Mappillai Samba rice composite (KRMR).

Methods: Physicochemical (pH, acidity, moisture), proximate, micronutrient (vitamins B6, B12, ICP-MS minerals), and probiotic properties (acid–bile tolerance, fermentation, adhesion) were evaluated using established methods. Antibiotic susceptibility and shelf-life stability were assessed via agar diffusion and microbial load (TBC, TYMC) during storage.

Results: The beverage had a lower pH, higher titratable acidity and moisture content compared to ice cream indicating improved fermentation. The beverage had higher levels of carbohydrate (475.26 ± 0.76 mg/g) and protein (1062.94 ± 1.79 mg/g), while ice cream had a higher fat content (47.10 ± 0.72%). The samples exhibited acid tolerance at pH 3.5 with survival rates of 52.94 ± 0.28% in ice cream and 53.48 ± 0.28% in beverage after 6 hours, and bile tolerance at 0.3% with viability of 56.57 ± 0.54% and 56.52 ± 0.07% respectively at 24 hours; adhesion efficiencies were recorded as 36.40 ± 0.36% for ice cream and 34.47 ± 0.25% for beverage, along with positive glucose fermentation. Antibiotic susceptibility profile revealed intrinsic resistance patterns without safety concerns and microbial growth was within FSSAI guidelines.

Conclusion: These findings offer credibility to the technological potential of producing rice-based fermented probiotic ice cream and beverages as stable functional food products.

Keywords: Kattuyanam; Mappillai Samba; Probiotic beverage; Probiotic icecream; Rice-based probiotics; Functional food

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INTRODUCTION

Functional foods have health benefits that extend beyond simple nutrition. Functional foods are believed to help lower the risk of diseases and promote optimal health. The Food and Drug Administration regulates functional food claims made by manufacturers concerning nutritional content, impact on disease, health, or body function. Probiotics are live organisms that, when taken in appropriate quantities, have a favourable influence on health. The International Scientific Association for Probiotics and Prebiotics defines probiotics as "live microbes that, when provided in adequate amounts, offer a health benefit on the host"[1].

"Probiotic bacteria varies amongst host systems. The genera Lactobacillus, Bifidobacterium, and Enterococcus are frequently used in human nutrition. Yeasts such as Saccharomyces cerevisiae are prevalent in ruminants, whereas Bacillus, Enterococcus, and Lactobacillus species are commonly used in pigs and poultry. A wider variety of microorganisms, such as Lactobacillus, Lactococcus, Leuconostoc, Enterococcus, Carnobacterium, Bacillus, Pseudomonas, and Saccharomyces, are used in aquaculture systems. Importantly, probiotic benefits are strain-specific and cannot be generalised to the species or genus level [2].

Cereals provide essential nutrients such as proteins, carbs, vitamins, minerals, and fibre to people globally. Cereals can be utilised to make fermented products with probiotics if they meet probiotic requirements and have acceptable physicochemical and organoleptic features [3]. Ice cream is a frozen food produced with milk solids, edible fats, and optional authorised additions. Its main purpose is to be frozen for storage, sale, and consumption. Ice cream, due to its global popularity and appealing sensory properties, is a great carrier for probiotics, prebiotics, and other nutrients. Its composition promotes the stability and long-term survival of integrated probiotic bacteria [4].

Rice-based fermented foods are quite popular in the Asia-Pacific region. While white rice is still extensively consumed, pigmented traditional varieties are gaining popularity due to their high polyphenol, antioxidant, and phytochemical levels. Research has demonstrated that the nutritional and bioactive characteristics of fermented and beverage items produced from pigmented rice are superior to those of their non-pigmented counterparts. There is an increasing desire to create traditional non-alcoholic beverages with useful health advantages because alcohol intake has been linked to numerous health problems [5].

MATERIALS AND METHODOLOGY

Selection and Procurement of Raw Materials

In our previous study [6,7], the Kattuyanam (KR), Mappillai Samba (MR) and Kattuyanam: Mappillai samba (1:1) (KRMR) were analyzed for its various physiochemical, probiotic and biological activities. Among the three samples, KRMR demonstrated significant bioactive properties. Therefore, the present study focuses on its supplementary products such as ice cream and beverage and its physiochemical, nutritional, probiotic, antibiotic susceptibility and shelf life analysis.

Formulation and Standardization

Ice cream

The standardisation of probiotic ice cream. Plain ice cream without the addition of probiotic rice powder was served as control (T0). The experiment was conducted in a completely randomised design (CRD) and was replicated thrice. The probiotic rice powder KRMR was added to ice creams at 0, 5, 10, 15, 20, 25 and 30 percent levels for the treatments. Raw milk, Milk powder, Sugar, Stabilizer underwent Heat treatment (85 °C, 1 min) Cooling (10 °C), and ripening for 24 h at 4 °C, and then Addition of KRMR and were Packed and stored (at -18 °C) respectively. The ice cream prepared with these formulations was evaluated for sensory qualities, and the level of addition was standardized on the basis of sensory qualities of ice cream

Table 1: Experimental Trails of Ice cream

S. no	Ingredient	T0	T1	T2	T3	T4	T5	T6	T7
1	Cows milk	45 ml	45 ml	45 ml	45 ml	45 ml	45 ml	45 ml	45 ml
2	Cream	15 ml	15 ml	15 ml	15 ml	15 ml	15 ml	15 ml	15 ml
3	Skimmed milk powder	7.5 gm	7.5 gm	7.5 gm	7.5 gm	7.5 gm	7.5 gm	7.5 gm	7.5 gm
4	sugar	15 gm	15 gm	15 gm	15 gm	15 gm	15 gm	15 gm	15 gm
5	Stabilizer (Xanthan)	0.3 gm	0.3 gm	0.3 gm	0.3 gm	0.3 gm	0.3 gm	0.3 gm	0.3 gm
6	Vanilla flavor agent	0.1 ml	0.1 ml	0.1 ml	0.1 ml	0.1 ml	0.1 ml	0.1 ml	0.1 ml
7	Water	10	10	10	10	10	10 ml	10	10

		ml	ml	ml	ml	ml		ml	ml
8	Fermented rice powder	-	5 gm	10 gm	15 gm	20 gm	25 gm	30 gm	35 gm

Beverage

The probiotic beverage formulations were prepared using varying proportions of KRMR, salt, and sugar, with potable water as the standard value. A total of 10 different combinations were developed based on a trial-and-error standardization method to optimize sensory and functional characteristics. All raw materials were accurately weighed according to the formulation design. The ingredients were blended and homogenized thoroughly to ensure uniform distribution and consistency of the mixture. The prepared formulations were then transferred into sterilized glass bottles under hygienic conditions. Subsequently, the bottles were sealed with airtight caps to prevent contamination and maintain product stability. Each bottle was labeled appropriately with essential details, including formulation number, date of manufacture, and batch identification, to facilitate traceability and quality control .

Table 2: Experimental Trails of Beverage

S. no	Ingredient	CONTROL	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10
1	Water (ml)	100	100	100	100	100	100	100	100	100	100	100
2	Salt (gm)	3	3	3	3	3	3	3	3	3	3	3
3	Sugar (gm)	5	5	5	5	5	5	5	5	5	5	5
5	Fermented rice powder (gm)	-	10	20	30	40	50	60	70	80	90	100

Sensory Analysis

The sensory evaluation of the optimized ice cream and beverage was done by 25 panelists from the Department of Food Science, Nutrition and Dietetics, Shrimathi Devkunvar Nanalal Bhatt Vaishnav College, Chennai. The sensory attributes like appearance, flavor, texture, color, taste and overall acceptability were given in the 9-point hedonic sensory scale

Physicochemical and Nutritional Properties

Physiochemical analysis

The pH of the beverage and ice cream was determined using a calibrated digital pH meter (LABMAN, LMPH9). The titrable acidity of the product was determined titrimetrically with phenolphthalein as indicator. Moisture content was determined by standard AOAC method [8].

Proximate Analysis

The total carbohydrates were determined spectrophotometrically using anthrone method [9]. The total protein using Lowry's method [10] and the crude fat was determined using soxhlet extraction method [11]. The crude fibre was determined by chemical digestion method [12].

Micronutrient Analysis

The total vitamin B6 content was determined spectrophotometrically by coupling with diazotized p-nitroaniline [13]. While the vitamin B12 was determined using using cyanocobalamin as standard [14]. The mineral composition of the samples was tested using Inductively Coupled Plasma-Mass Spectrometry (ICP-MS, Agilent 7900).

Probiotic Efficacy

Acid and Bile Salt Tolerance

The samples were added to MRS broth enriched with pH 3.5 and 0.3% bile salts. The culture was incubated and measured at 600 nm (OD600) to observe growth. The survival rate under acidic conditions and ability to withstand stress in bile was calculated by increase or decrease in OD values [15].

Glucose fermentation

Carbohydrate fermentation was analyzed using phenol red broth that included specific sugars (0.1% w/v glucose, lactose, sucrose, and maltose). After sterilizing at 121 °C for 15 minutes, each tube-containing a Durham tube for gas detection-was inoculated with the test isolate and incubated at 37 °C for 24-48 hours. A color change from red to yellow indicated the production of acid, while gas presence in the Durham tube confirmed fermentation activity [16]

Bacterial adhesion to stainless steel plates

The adherence assay was performed on stainless steel plates. Briefly, LAB were grown in sterile MRS broth. A sterile stainless steel plate was then set up, and the test tubes were then incubated for 24 hours at 37°C. The stainless steel plate was removed under aseptic circumstances, and the plate was washed and vortexed for 3 minutes in a sterile 1% peptone water to remove bacterial cells from the steel plate surface. Cell count was measured on MRS agar after 24 hours of incubation at 37°C [17].

Antibiotic susceptibility test

The determination of antibiotic susceptibility in isolates was conducted using the agar well diffusion method on MRS agar, following modified NCCLS guidelines. Standardized bacterial cultures were spread to establish a uniform lawn, and wells were filled with selected antibiotic solutions. After a 24-hour incubation at 37 °C, the diameters of the inhibition zones were measured, and isolates were categorized as resistant, moderately susceptible, or susceptible based on standard interpretive criteria [18].

Shelf life analysis

Microbial load was assessed by dissolving 0.1 g of sample in phosphate-buffered saline (pH 7.2) followed by serial tenfold dilutions (10^{-1}–10^{-6}). Aliquots (1 mL) from selected dilutions were aseptically transferred into sterile Petri plates. Plates were incubated at 37 °C for 48 h for total bacterial count (TBC). For total yeast and mold count (TYMC), selective media were used with incubation for 48–72 h. Colonies were counted and expressed as CFU/mL based on the dilution factor.

RESULT AND DISCUSSION

Sensory Analysis

Sensory analysis of beverage (T1-T7) (Table 3) and ice cream (T1-T10) (Table 4) formulations revealed that T3 consistently scored highest in all parameters, and was statistically comparable to the control ($p > 0.05$). T1, T2, T4, and T5 showed moderate acceptance (7.45–7.95) in beverages, but T6 and T7 had much lower scores (6.95–7.30; $p < 0.05$). Similarly, in ice cream, T2, T4, T5, and T6 exhibited moderate scores (7.50-7.80), while T8-T10 had the lowest ratings (6.15-6.60), substantially different from the top-performing samples ($p < 0.05$). Overall, T3 and the control were the most recommended of the two products. The findings correspond with earlier reports where moderate incorporation of natural or probiotic additives improved creaminess, taste, and consumer preference without affecting appearance [19]. Another research stated that fermented ice cream with rice bran and probiotics had better sensory properties than conventional ice creams in the early phases of manufacture [4].

Table 3: Sensory score of Beverage

Sensory Attribute	Control	T1	T2	T3	T4	T5	T6	T7
Colour	$7.90^{a}\pm.788$	$7.65^{ab}\pm.875$	$7.50^{ab}\pm.688$	$8.05^{a}\pm.759$	$7.65^{ab}\pm.671$	$7.45^{b}\pm.510$	$7.20^{b}\pm.410$	$6.95^{c}\pm.510$
Flavour	$7.85^{a}\pm.745$	$7.75^{ab}\pm.851$	$7.65^{ab}\pm.745$	$8.20^{a}\pm.834$	$7.95^{a}\pm.759$	$7.75^{ab}\pm.639$	$7.35^{b}\pm.489$	$7.00^{c}\pm.459$
Texture	$7.95^{a}\pm.605$	$7.65^{ab}\pm.745$	$7.40^{ab}\pm.503$	$8.15^{a}\pm.745$	$7.90^{a}\pm.553$	$7.80^{ab}\pm.410$	$7.45^{b}\pm.510$	$7.25^{b}\pm.639$
Appearance	$7.80^{a}\pm.696$	$7.70^{ab}\pm.801$	$7.55^{ab}\pm.605$	$8.00^{a}\pm.725$	$7.75^{a}\pm.639$	$7.60^{ab}\pm.503$	$7.35^{b}\pm.489$	$7.20^{b}\pm.523$
Taste	$7.85^{a}\pm.489$	$7.60^{ab}\pm.821$	$7.55^{ab}\pm.605$	$8.10^{a}\pm.718$	$7.90^{a}\pm.718$	$7.60^{ab}\pm.503$	$7.35^{b}\pm.489$	$7.15^{b}\pm.587$

Overall Acceptability	$8.25^{a}\pm.444$	$7.40^{b}\pm.598$	$7.35^{b}\pm.489$	$8.45^{a}\pm.510$	$7.95^{a}\pm.686$	$7.75^{ab}\pm.444$	$7.45^{b}\pm.510$	$7.30^{b}\pm.470$

Note: This table presents the mean sensory scores (± standard deviation) for different beverage treatments (T1–T7) compared to the Control group. Statistical groupings (a, b, c) indicate significant differences among treatments based on post-hoc analysis (Tukey's HSD).

Table 4: Sensory score of Ice cream

Sensory Attributes	**Control**	**T1**	**T2**	**T3**	**T4**	**T5**	**T6**	**T7**	**T8**	**T9**	**T10**
Overall Acceptability	$8.05^{a}\pm.945$	$7.85^{a}\pm.813$	$7.60^{ab}\pm.821$	$8.30^{a}\pm.657$	$7.80^{ab}\pm.768$	$7.75^{ab}\pm.639$	$7.50^{ab}\pm.513$	$7.25^{b}\pm.550$	$6.85^{bc}\pm.366$	$6.55^{c}\pm.510$	$6.20^{c}\pm.410$
Taste	$8.10^{a}\pm.968$	$7.75^{ab}\pm.716$	$7.80^{ab}\pm.768$	$8.25^{a}\pm.716$	$7.65^{ab}\pm.745$	$7.50^{ab}\pm.607$	$7.25^{b}\pm.444$	$7.20^{b}\pm.523$	$6.75^{bc}\pm.444$	$6.50^{c}\pm.513$	$6.15^{c}\pm.671$
Appearance	$8.15^{a}\pm.671$	$7.85^{a}\pm.813$	$7.75^{ab}\pm.786$	$8.25^{a}\pm.550$	$7.70^{ab}\pm.657$	$7.55^{ab}\pm.510$	$7.35^{b}\pm.489$	$7.15^{b}\pm.587$	$6.80^{bc}\pm.410$	$6.50^{c}\pm.513$	$6.30^{c}\pm.571$
Texture	$8.00^{a}\pm.973$	$7.95^{a}\pm.759$	$7.55^{ab}\pm.759$	$8.20^{a}\pm.768$	$8.00^{a}\pm.918$	$7.70^{ab}\pm.657$	$7.50^{ab}\pm.513$	$7.25^{b}\pm.716$	$6.70^{bc}\pm.470$	$6.35^{c}\pm.489$	$6.10^{c}\pm.641$
Flavour	$8.15^{a}\pm.745$	$7.80^{ab}\pm.696$	$7.75^{ab}\pm.910$	$8.15^{a}\pm.933$	$8.00^{a}\pm1.026$	$7.65^{ab}\pm.745$	$7.30^{b}\pm.470$	$7.05^{b}\pm.605$	$6.85^{bc}\pm.366$	$6.60^{c}\pm.503$	$6.40^{c}\pm.681$
Colour	$7.90^{a}\pm.788$	$7.80^{a}\pm.894$	$7.70^{ab}\pm.801$	$8.25^{a}\pm.716$	$7.80^{a}\pm.834$	$7.50^{ab}\pm.513$	$7.35^{b}\pm.489$	$7.15^{b}\pm.587$	$6.80^{bc}\pm.410$	$6.60^{c}\pm.503$	$6.30^{c}\pm.733$

Note: This table presents the mean sensory attribute scores (± standard deviation) for different treatments (T1–T10) compared to the control group. Statistical groupings (a, b, c) indicate which treatments are significantly different from each other based on post-hoc analysis (LSD).

Physicochemical and Nutritional Properties

Physiochemical Analysis

The physicochemical analysis revealed distinct differences between the ice cream and beverage samples (Table 5). The beverage exhibited a lower pH (4.86 ± 0.01) and higher titratable acidity (10.01 ± 1.51 g citric acid/g) compared to the ice cream, indicating a more acidic nature. Additionally, the beverage showed significantly higher moisture content (78.05 ± 0.38%) than the ice cream (50.32 ± 1.06%).Earlier studies also reported pH decreases (based on longer fermentation) from 6.3 to 3.4 in 48 hours (*L. plantarum*), from 5.75 to 4.63 in 60 hours (yeast tapai), and from 5.04 to 3.68 in 72 hours (mixed-culture yogurt) [20]. The slightly acidic pH and elevated titratable acidity of the beverage reflect active fermentation, consistent with earlier findings that rice fermentation lowers pH and raises acid content over time [3]. Similar to previous research, the pH of beverage was predominantly low. Previous studies reported moisture levels of 51.8% in probiotic ice cream and 61.8% in a stevia-based formulation, with the latter containing 38.2% total solids [21,22]. Liquid probiotic beverages often have a high moisture level, commonly around 80-90%, whereas dried or powder-form probiotic products have a significantly lower moisture content, usually in the range of 2.7-9.05%, to improve stability and shelf life [23,24] . Previous study results align with present research.

Table 5: Physiochemical parameters of Beverage and Icecream

S.No.	Parameter	Ice Cream	Beverage
1	pH	7.05 ± 0.01	4.86 ± 0.01
2	Titratable acidity (g citric acid/1g sample)	3.62 ± 0.83	10.01 ± 1.51
3	Moisture content (%)	50.32 ± 1.06	78.05 ± 0.38

Proximate analysis

Total carbohydrate content

The results revealed that the beverage contained a notably higher carbohydrate level (475.26 ± 0.76 mg/g) than the ice cream (75.53 ± 1.41 mg/g). The standard curve for estimation of carbohydrate is depicted in Fig. 1. Previous research revealed that the sheep-milk probiotic ice cream had carbohydrate content of 19.45–19.50 % in the final product [25], while soy based ice cream showed has a carbohydrate content of 12.17 g/100 g [26].

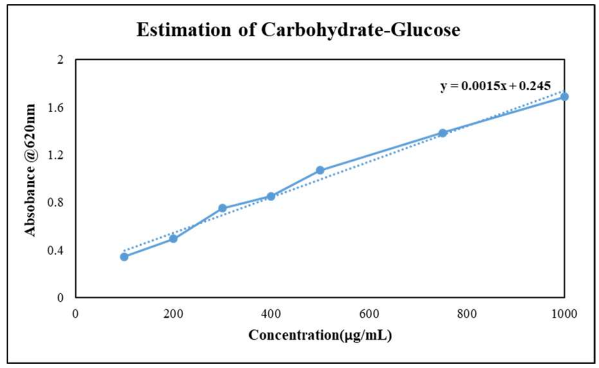

Fig. 1: Estimation of carbohydrates – Standard curve (Glucose)

Total protein content

The protein estimation executed by Lowry's method. The beverage recorded a slightly higher protein concentration (1062.94 ± 1.79 mg/g) when compared to the ice cream (1035.56 ± 0.71 mg/g) (Fig. 2). A recent study of vegan probiotic ice cream prepared from red kidney-bean milk found protein levels ranging from 2.05-2.20% [27]. Another study revealed the average protein content ranging from 3.00% to 3.03% in probiotic-enriched ice cream with fermented white kidney bean homogenate [28].

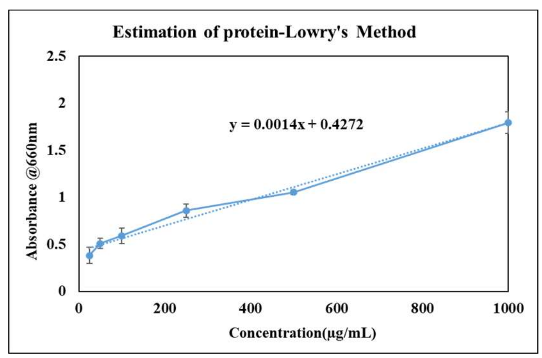

Fig. 2: Estimation of protein – Standard curve (Bovine Serum Albumin)

Crude fat

According to Soxhlet extraction, the crude fat content was 36.21 ± 0.66% in the beverage and 47.10 ± 0.72% in the ice cream. The higher lipid content in ice cream reflects the incorporation of milk fat and stabilizers that are vital for a creamy texture and flavor. The okara-based probiotic beverage contained approximately 1.87 % crude fat metabolism [29], while yogurt-based probiotic ice cream had 2 % - 10 % fat levels [30].

Crude fibre

The assessment of crude fibre content revealed that both formulations included considerable quantities of dietary fibre. The beverage had a somewhat higher fibre content (8.01 ± 0.38%) relative to the ice cream (7.53 ± 0.33%). The fermented tigernut probiotic beverages contained approximately 1.2–1.8 % crude fibre [31], while the probiotic beverage prepared from Kodo millet contained about 0.5 % crude fat [32].

Probiotic Efficacy

Acid and Bile salt tolerance

The results indicate that samples with a survival rate above 50% were considered tolerant to acidic pH (3.5) and bile salt (0.3%) conditions. Among the tested formulations, the ice cream sample recorded the highest survival rate, with 52.94 ± 0.28% at 6 hours under acid stress (Fig. 3a) and 56.57 ± 0.54% at 24 hours under bile salt exposure (Fig. 3b), surpassing the individual fermented rice samples. The specific survival percentages of the test samples are outlined in Table 6. Previous investigations have reported probiotic survival rates ranging from 74.6% to 87.1% at low pH and 45.35% to 97.11% at pH 2-3 for 3-6 hours. Bile tolerance tests reveal 91.37% to 97.22% survival at 0.3% bile, while long-term preservation data show 84.7% survival after 120 days with a 3.82-log drop under simulated gastrointestinal circumstances [33,34]. The length of exposure can affect survival rates. For example, after three hours, some strains had survival rates of 94.00% at pH 3 and 79.39% at pH 2.5, but after six hours, they fell to 38.40% and 71.50%, respectively [35].

Table 6: Acid and bile salt tolerance of supplementary products

S.No.	Sample	pH tolerance		Bile salt tolerance	
		3 h	6 h	3 h	24 h
1	Ice cream	76.316 ± 0.0636	52.941 ± 0.283	77.778 ± 0.0424	56.571 ± 0.537
2	Beverage	74.286 ± 0.0636	53.488 ± 0.283	76.923 ± 0.0424	56.522 ± 0.071

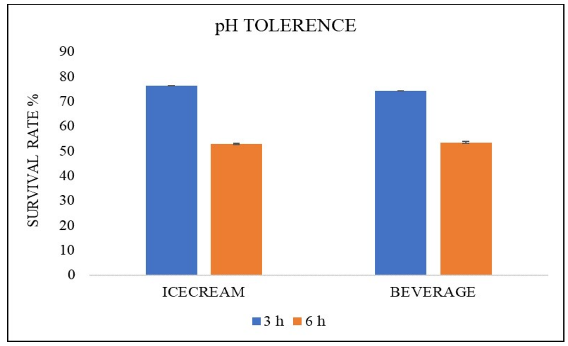

Fig. 3a: Acid tolerance of probiotics

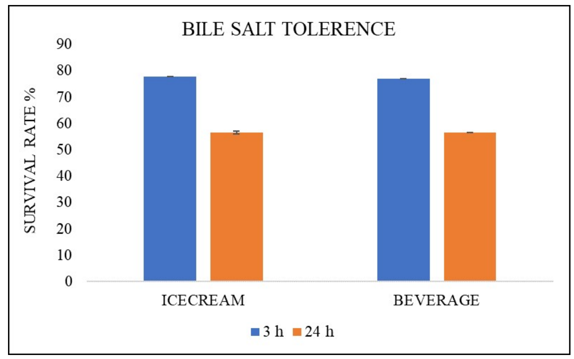

Fig. 3b: Bile salt tolerance of probiotics

Glucose fermentation

Phenol red glucose broth was utilized for glucose fermentation, incorporating inverted Durham tubes to detect gas production. The fermentation profiles for the control (A), ice cream (B), and beverage (C) are depicted in Fig. 4. Acid production was apparent in all three samples, as shown by a significant color change of the medium from red to yellow. The significant fermentative ability observed in both formulations indicates that the incorporated cultures remained metabolically active, ensuring ongoing acidification and preservation of product quality during storage, which is consistent with earlier findings by [36].

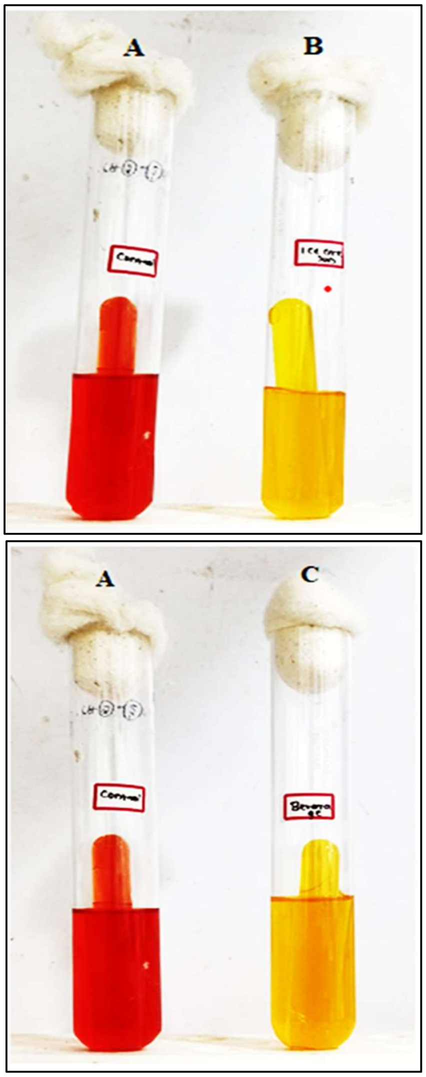

Fig. 4: Glucose fermentation assay

Bacterial adhesion to stainless steel plates

The adhesion of probiotic bacteria to the intestinal mucosa is a critical functional feature, as it determines their ability to survive, colonize, and deliver health benefits in the gastrointestinal tract. In this research, the probiotic culture included in the ice cream formulation showed an adhesion efficiency of 36.40 ± 0.36%, while the beverage formulation had an adhesion efficiency of 34.47 ± 0.25% (illustrated in Table 7 and Fig. 5 & 6).

Table 7: Percentage of probiotic adhesion

S.NO	SAMPLES	PROBIOTIC ADHESION %
1	Ice cream	36.40 ± 0.36
2	Beverage	34.47 ± 0.25

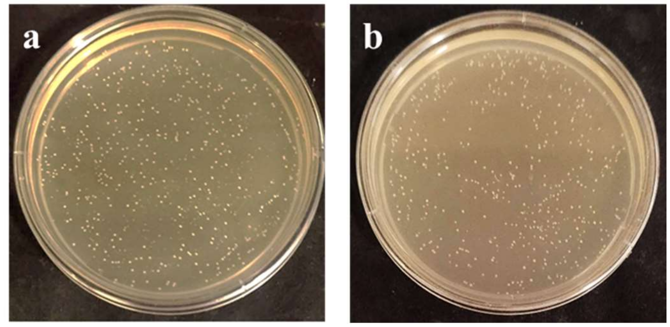

Fig. 5: Bacterial adhesion to stainless steel plate a) Ice cream and b) Beverage

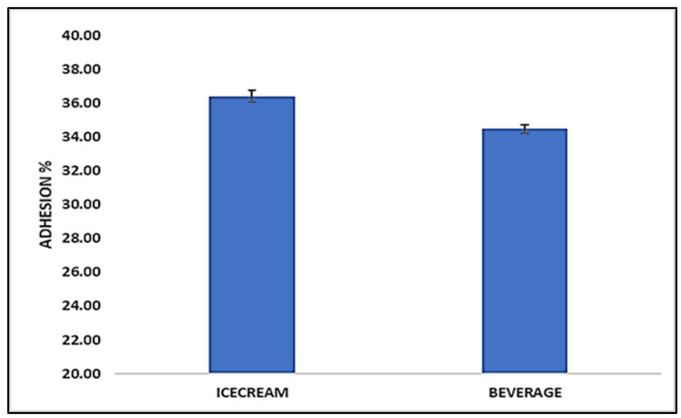

Fig. 6: Percentage of probiotic adhesion to stainless steel plate

Antibiotic susceptibility

The antibiotic susceptibility profiles of probiotic isolates derived from KRMR-based ice cream and beverage were evaluated through the agar well diffusion method, with results displayed in Table 8 and Fig. 7. Penicillin, and Streptomycin, which is consistent with earlier studies indicating that LAB naturally exhibit intrinsic resistance to these antibiotics due to the lack of specific penicillin-binding proteins or the low permeability of their cell walls [37,38]. This intrinsic resistance is regarded as non-transferable, thereby not raising safety concerns for probiotic use.

Table 8: Antibiotic susceptibility test

S. No.	Antibiotics	Ice cream (mm)	Beverage (mm)

1	Ampicillin	R	R
2	Erythromycin	29.61 ± 0.10	27.41 ± 0.36
3	Ciproflaxime	22.38 ± 0.45	23.17 ± 0.29
4	Cefotaxime	21.69 ± 0.18	20.69 ± 0.25
5	Rifampin	21.07 ± 0.12	22.50 ± 0.19
6	Tetracycline	36.31 ± 0.26	34.43 ± 0.28
7	Penicillin	R	R
8	Streptomycin	R	R

Fig. 7 a: Antibiotic susceptibility of ice cream

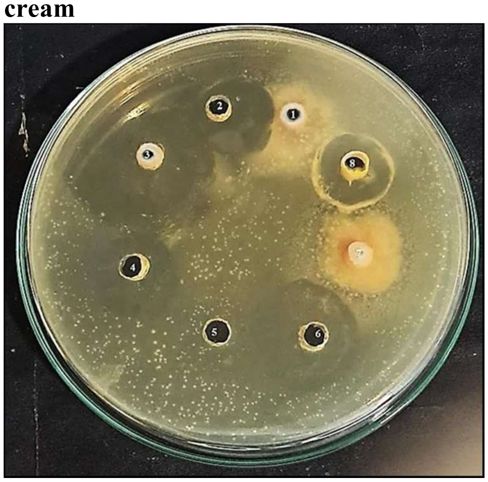

Fig. 7 b: Antibiotic susceptibility of beverage

Shelf life analysis

The total bacterial, yeast, and mold counts (Table 9 & 10) in both ice cream and beverage samples gradually increased during storage, indicating microbial growth. The microbial load was minimal on the first day and substantially rose by the 15th day, staying within permissible limits. However, by the 30th day, counts reached $\sim 10^5$ CFU/ml, indicating spoilage. The beverage's high moisture level, nutrient density, and acidic character all aided microbial multiplication. Furthermore, post-processing contamination and slight temperature changes during storage could have contributed to the elevated microbial burden. The total viable count ranged up to 10^4–10^5 CFU/g, which falls within the permissible limit specified by the Food Safety and Standards Authority of India (FSSAI), where the maximum acceptable limit is $\leq 2 \times 10^5$ CFU/g.

Table 9: Total bacterial count (TBC)

Ice cream		
S. No.	**Day**	**CFU/ml**
1	0^{th}	1.1×10^4
2	15^{th}	4.09×10^4
3	30^{th}	8.3×10^4
Beverage		
S. No.	**Day**	**CFU/ml**
1	0^{th}	1×10^4
2	15^{th}	4.8×10^4
3	30^{th}	9.9×10^4

Table 10: Total Yeast and Mold

Ice cream		
S. No	**Day**	**CFU/ml**
1	0^{th}	4.5×10^3
2	15^{th}	3.1×10^4

3	30th	8.7×10^4
Beverage		
S. No	**Day**	**CFU/ml**
1	0th	7.2×10^3
2	15th	3.9×10^4
3	30th	8.1×10^4

4. CONCLUSION

In conclusion, the current study successfully demonstrated the production of functional probiotic ice cream and beverages utilising a composite of Kattuyanam and Mappillai Samba rice (KRMR), showing their potential as nutritionally enriched functional foods. T3 had the highest sensory acceptability of the formulations, demonstrating that moderate integration of fermented rice powder improves product quality while maintaining customer preference. The beverage had increased acidity and moisture content, indicating active fermentation, while both products included high concentrations of carbohydrates, proteins, fibre, and fat, adding to their nutritional value. Probiotic efficacy tests revealed significant acid and bile tolerance, glucose fermentation ability, and adhesion capability, confirming the functional viability of the products. Furthermore, the products showed safe antibiotic resistance profiles, indicating that they are suitable for probiotic uses. Shelf-life testing found that both products remained under acceptable microbiological levels. Overall, KRMR-based probiotic ice cream and beverages are attractive functional foods with potential health benefits, as they combine traditional rice varieties.

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AUTHOR CONTRIBUTION

R. Subaratinam: Conceptualisation, data collection, design of the study, and draft. R. Vidya: Supervised the overall work, formal analysis and performed validation. V. Suganthi: Scientific accuracy verification, manuscript review and editing. All authors have read and agreed to the published version of the manuscript.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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