



Formulation and Sensory Profiling of Dairy Free Probiotic Yogurt Mimics using Rice Milk

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(Received: 09 August 2024; Revised: 07 September 2024; Accepted: 07 October 2024; Published: 14 November 2024)

(Published by Research Trend)

ABSTRACT: The increased urge of human beings in search for novel food products with enhanced functional benefits and slighter environmental impact is on rise in the current era. Plant-based milk substitutes are in high demand since they are devoid of cholesterol and allergens and can be used as a vegan or lactose intolerant substitute. Hence, the present study focuses on developing a probiotic yoghurt mimic from rice milk and its sensory profiling to determine the consumer acceptability of the products. *Lactobacillus acidophilus* and *Streptococcus thermophilus* starter cultures were used for the fermentation. The study indicated that the pH values of the treatments decreased and titratable acidity of the treatments increased during storage period of 5 days. The sensory profiling results showed that the sensory qualities of the products samples were satisfactory and maintained a shelf life of 5 days when stored at 4°C in glass bottles without any growth of any pathogens. The microbiological quality analyses of the yoghurt mimics were evaluated by analyzing Total Bacterial Count, Total Fungal Count and Total Coliform Count for a period of 5 days. Coliform and Fungal microorganisms were absent in the product samples during storage period. More research is necessary to fully comprehend how the ingredients interact and react during fermentation and how this affects the product's mouthfeel, flavor, and texture.

Keywords: Yoghurt mimics, Rice milk, Probiotic, Dairy alternative, Sensory profile.

INTRODUCTION

Novel plant-based substitutes for animal origin products have been developed as a result of the world's population growth and the necessity for sustainable food supplies to meet human demands. Since milk is one of the most often used foods, there is a growing desire for vegan substitutes because of the negative effects of milk intake, such as lactose intolerance, cholesterolemia, and milk allergies (Kundu *et al.*, 2018). Nowadays, a variety of plant sources, including grains, pulses, nuts, and seeds, can be used as milk alternatives. Nondairy products are less harmful to the environment than dairy products, and as more people look for plant-based products, there will probably be a greater demand for plant-based nondairy substitutes in the upcoming years. Thus, plant-based milk extracts, such as cereal milk, could be a good substitute for food products made with milk.

Cereal grains are the promising grains with plenitude of bioactive phytochemical compounds along with major and minor nutrients. Rice (*Oriza sativa*) is one among the most commonly consumed staple cereal in the India and particularly in Kerala. A diet devoid of rice is considered a very unsatisfactory meal especially in Kerala. The bold red kernelled, non-stick parboiled rice that has undergone little processing is frequently preferred by Keralites (Suma *et al.*, 2018). A healthy

quantity of fibre, protein, and carbs may be found in rice. The rice proteins glutelin, prolamine, albumin, and globulin are good stabilisers, heat stable, and have surface-active qualities, and can also maintain mouthfeel and flavor (Dhakal *et al.*, 2023).

Rice is consumed as brown rice, raw milled rice, parboiled rice, and also as flour and other fermented forms. Milling and Parboiling are different processing techniques to improve the nutritional quality of rice. Parboiling is a common processing technique involving soaking, steaming, drying and milling of rice. This technique helps to improve the bioavailability of nutrients from rice. The development of brown rice involves dehusking the outer husk of the paddy and raw milled rice is made by removing the bran along with husk by milling process (Muchlisiyah *et al.*, 2023). The brown rice has all its parts bran, germ and endosperm except the hull, and it is converted to raw milled rice by taking of the bran and polishing it by milling technique (Kalita *et al.*, 2021). In the years ahead, consumers may find that rice-based yoghurt substitutes can serve as a hypoallergic vegan alternative to dairy-based yoghurts (Maheswari, 2018).

Microbes have been used in the development of fermented and probiotic beverages from ages. Probiotics are beneficial microorganisms that are crucial for supporting gut health and, consequently, general wellness. Probiotics are defined by the FAO

and WHO as live microorganisms that, when taken in sufficient quantities, have certain health advantages. The probiotics should be non-pathogenic, able to bind to human cell walls, tolerant of acid and bile, and able to flourish in the human gut. The gut microbiome is greatly influenced by an individual's dietary intake; foods high in prebiotics promote the proliferation of probiotics and aid in the body's transition to a eubiotic condition (Anbalagan *et al.*, 2024). Probiotics are therefore used in the formulation to develop a functional food that will enhance gut microbiota and have beneficial implications for health (Gupta *et al.*, 2016).

Yoghurts can be considered as one among the common foods of the consumer with light toned, smooth texture and pleasant sensory qualities (Gorlov *et al.*, 2019). The production of alternatives to milk using cereals aids in the development of a product that is high in dietary fibre, vitamins, and minerals and serves as a prebiotic to support the growth of probiotic bacteria (Smanalieva *et al.*, 2021). The fermentation process by probiotics acts as a natural preservation method there by extending the shelf life of the product while enriching the nutritional and sensory properties of the products. Therefore, the objective of the current study is to formulate probiotic yoghurt mimics utilizing rice milk and conduct sensory profiling of the product to determine its acceptance.

MATERIALS AND METHODS

Selection of raw materials. The rice varieties Jyothi, Kanchana, Revathi and Bhadra were collected from RARS Pattambi and RRS Moncompu. The starter cultures *Lactobacillus acidophilus* strain NCDC 13 and *Streptococcus thermophilus* strain NCDC 76 were procured from the culture collection of NCDC NDRI Karnal.

Processing of rice varieties. The collected paddy varieties Jyothi, Kanchana, Revathi and Bhadra were processed into brown rice, raw milled rice and parboiled milled rice. The first step in processing different types of rice is to clean the harvested paddy to get rid of any contaminants. To obtain brown rice, the paddy types are dehusked; to obtain raw milled rice, the husk and bran layer are removed; and to obtain parboiled milled rice, the rice is parboiled and then milled. The processed rice varieties are labeled and packed in air tight containers.

Preparation of starter culture. The freeze dried cultures of probiotics strains *Lactobacillus acidophilus* strain NCDC 13 and *Streptococcus thermophilus* strain NCDC 75 were rehydrated in sterile distilled water. The selective growth media MRS *Lactobacillus acidophilus* media and *Streptococcus thermophilus* media was selected for *Lactobacillus acidophilus* and *Streptococcus thermophilus* bacterium and the rehydrated culture aliquot was streak plated into both specific media plates and incubated at an optimal temperature of 37°C for 24 hours. The isolated colonies were picked and again streaked on fresh plates to confirm purity and a pure colony was transferred into *Lactobacillus acidophilus* broth and *Streptococcus*

thermophilus broth to grow the culture in bulk. The probiotic cultures were then centrifuged under sterile conditions and stored at 4°C for further use.

Formulation of yoghurt mimics. The yoghurt mimic samples were produced by several unit operations which are outlined in the flow chart. The plant based rice milk samples from brown rice, raw milled rice and parboiled milled rice involves soaking of the different rice treatments, wet milling (1:2 rice: water), straining the milk and heating the rice milk mixture to 95°C for 15 minutes and a known amount of sugar was added. The rice milk mixtures were cooled down to 40°C and inoculated with 6% probiotic culture and incubated at 37°C for 24 hours. The samples were stored in glass bottles at 4°C for the microbial and sensory analysis.

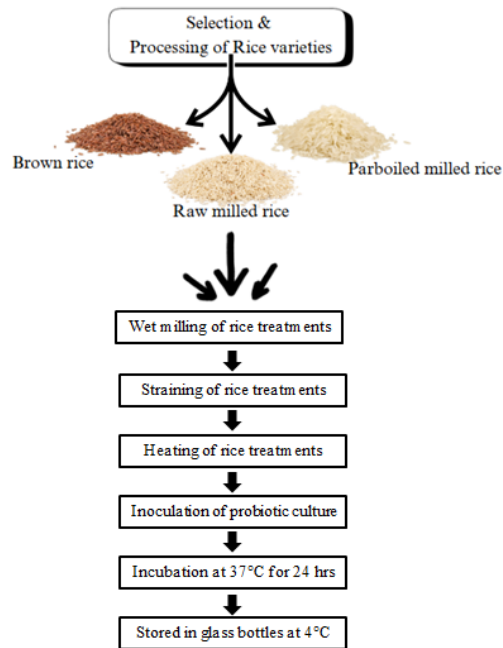


Fig. 1. Formulation of yoghurt mimics.

Table 1: Different treatments of Yoghurt Mimics.

Milling treatments	Rice treatments	Yoghurt mimics
Brown Rice (R1)	T ₁ -Kanchana	R1T1
	T ₂ -Jyothi	R1T2
	T ₃ -Revathi	R1T3
	T ₄ -Bhadra	R1T4
Raw Milled Rice (R2)	T ₁ -Kanchana	R2T1
	T ₂ -Jyothi	R2T2
	T ₃ -Revathi	R2T3
	T ₄ -Bhadra	R2T4
Parboiled Milled Rice (R3)	T ₁ -Kanchana	R3T1
	T ₂ -Jyothi	R3T2
	T ₃ -Revathi	R3T3
	T ₄ -Bhadra	R3T4

Quality analysis of developed yoghurt mimics. The pH and titratable acidity of the rice milk based yoghurt mimic samples were analyzed using a digital pH meter on the 1st, 3rd and 5th days of storage. The pH of the samples was measured using a digital pH meter (Hatami *et al.*, 2023). A titration method employing

0.1N NaOH and phenolphthalein indicator was used to determine the product samples' titratable acidity, which was measured as lactic acid concentration (g/L) (AOAC 2019).

Microbiological evaluation of developed yoghurt mimics. The total viable count of probiotic bacteria *Lactobacillus acidophilus* and *Streptococcus thermophilus* in the yoghurt mimics were determined using spread plate method in specific MRS *Lactobacillus acidophilus* media and *Streptococcus thermophilus* media. 1ml of yoghurt mimics samples were serially diluted and vortexed and from that 1µl of sample was plated to specific media plates and incubated by keeping upside down in an incubator for 24 hours at 37°C. Colonies present in each plate were counted and calculated using the formula to find the Colony Forming Unit. The percentage of viable cells were calculated using the formula as follows (Smanalieva *et al.*, 2021).

$$\text{Viable (\%)} = (\text{CFU at } n \text{ storage day} / \text{CFU at initial storage day}) \times 100$$

The microbial evaluation on the basis of Total bacterial, fungal and coliform count of various yoghurt mimics samples were carried out in Nutrient agar media, Rose Bengal Media and EMB agar media respectively using spread plate method. Serial dilutions of the samples were prepared and plated on the selective agar media plates and evenly distributed using a sterile rod and incubated at 28-37°C for a time period of 24-72 hours. All experiments were carried out in three replications. The colonies in the plates were counted and the Colony Forming Unit/ml of the samples were calculated (El-Sayed & Ramadan, 2020).

$$\text{Colony Forming Units (CFU/ml)} = (\text{Number of Colonies} \times \text{Dilution factor}) / \text{Volume of sample plated (mL)}$$

Sensory profiling of yoghurt mimics. The sensory profiling of the samples was carried out using 9 point hedonic scale method. To assess the sensory acceptance and suitability for consumption the degree of liking was measured across various attributes including appearance, aroma, consistency, taste, mouthfeel and overall acceptability. The sensory evaluation was carried out by 30 semi trained panel members, and they were handed over with evaluation forms and instructed with the related criteria. Samples were stored at cold storage under 4°C temperature prior to evaluation. Samples were placed on glass cups and coded randomly and served immediately after taking out from refrigerated temperature. The evaluation was conducted in the individual well lit cabin. Sensory panelists rinsed their mouth with drinking water between samples and scored the samples on a 9 point hedonic scale (1 – dislike extremely; 9 – like extremely). Acceptance of probiotic beverages as to appearance, aroma, consistency, taste, mouth feel and overall acceptability was evaluated. To guarantee similar outcomes, the beverages were taken from a single batch of production (Malyala *et al.*, 2018).

Statistical analysis. The experiments were carried out in three replications and statistically analyzed using

KAUGRAPES Version 1.1.0 Software. All data's were analyzed using CRD method to find the significant differences and the sensory profile data was analyzed using Kruskal Wallis test.

RESULT AND DISCUSSION

Quality analysis of developed yoghurt mimics. The probiotic starter culture ferments each rice milk treatments to create yoghurt-like samples, and the designed foods obtained a distinct organoleptic character when the pH dropped to 4.5. Table 2 summarizes the pH and titratable acidity values of the developed rice treatments on the 1st, 3rd and 5th day of refrigerated storage at 4°C. On the first and third days of storage, there was no discernible difference in the pH values of the yoghurt mimics samples developed from brown rice treatments; however, a small fluctuation was seen on the fifth day. During the storage time, it was discovered that the pH values of the yoghurt mimics samples R1T1, R1T2, R1T3, and R1T4 fell between 4.46-3.96. The pH values of the yoghurt mimics prepared from various raw milled rice treatments varied significantly between the first and fifth days of storage; however, no change was seen among samples on the third day. The pH range of the yoghurt mimics made from raw milled rice treatments R2T1, R2T2, R2T3, and R2T4 was in between 4.53–4.06. The pH values of the parboiled milled rice treatments R3T1, R3T2, R3T3, and R3T4 varied considerably from one another over the course of the storage period and ranged from 4.56 – 3.9. All of the yoghurt mimic samples from each milling procedure had progressively lower pH values during the course of storage. During the storage span, the pH value has decreased due to the fermentation by probiotic bacteria. The obtained results were similar to the results found in the study done by Khan *et al.* (2021); El-Sayed & Ramadan (2020). The buildup of lactic and organic acids from the fermentation brought on by the probiotic bacteria *Lactobacillus acidophilus* and *Streptococcus thermophilus* may be the reason of the pH drop in all of the samples.

The titratable acidity study revealed that the probiotic culture and fermentation had an impact on both the pH and acidity levels. It was discovered that practically all of the samples had considerably varying acidity readings. The titratable acidity of every sample rose over the course of storage and has been determined to be within 0.460 and 1.133. A similar trend of rise in titratable acidity of fermented plant based beverages was recorded by several researchers (El-Sayed & Ramadan 2020; Hatami *et al.*, 2023; Khan *et al.*, 2021). The yoghurt mimics samples' titratable acidity is seen to be raised by the probiotics *Lactobacillus acidophilus* and *Streptococcus thermophilus*'s activity on the drink's accessible prebiotic components. The lowest pH value was showed by sample R1T4 and R3T4 and a titratable acidity value of 1.010 and 1.133 respectively. Hence the present study showed that after 5 days of storage in glass bottles at 4°C pH values of samples descended and titratable acidity value ascended during storage.

Table 2: pH and Titratable acidity values of yoghurt mimics during storage period.

Treatments	pH			Titratable acidity		
	Day 1	Day 3	Day 5	Day 1	Day 3	Day 5
R1T1	4.46	4.26	4.16 ^a	0.480 ^e	0.643 ^d	0.890 ^d
R1T2	4.43	4.23	4.06 ^{ab}	0.460 ^e	0.727 ^c	0.960 ^c
R1T3	4.46	4.16	4.06 ^{ab}	0.520 ^b	0.790 ^b	0.987 ^b
R1T4	4.43	4.1	3.96 ^b	0.590 ^a	0.840 ^a	1.010 ^a
±SE(m)	0.033	0.033	0.033	0.008	0.007	0.005
CV%	1.297	1.372	1.42	2.581	1.721	0.949
CD	0	0	0.109	0.025	0.024	0.017
R2T1	4.36 ^b	4.16	4.06 ^b	0.520 ^d	0.690 ^d	0.930 ^d
R2T2	4.36 ^b	4.23	4.16 ^a	0.540 ^e	0.740 ^c	0.980 ^c
R2T3	4.53 ^a	4.26	4.06 ^b	0.580 ^b	0.827 ^b	1.030 ^b
R2T4	4.5 ^a	4.16	4.20 ^a	0.630 ^a	0.893 ^a	1.080 ^a
±SE(m)	0.041	0.033	0.029	0.006	0.006	0.006
CV%	1.592	1.372	1.212	1.762	1.372	0.995
CD	0.133	0	0.0094	0.019	0.020	0.019
R3T1	4.56 ^a	4.26 ^{ab}	4.16 ^a	0.510 ^e	0.683 ^d	1.107 ^c
R3T2	4.46 ^{ab}	4.16 ^b	4.06 ^a	0.560 ^b	0.720 ^b	1.040 ^{bc}
R3T3	4.46 ^{ab}	4.33 ^a	4.16 ^a	0.540 ^b	0.703 ^c	1.087 ^{ab}
R3T4	4.36 ^b	4.36 ^a	3.9 ^b	0.517 ^c	0.750 ^a	1.133 ^a
±SE(m)	0.033	0.033	0.041	0.005	0.005	0.017
CV%	1.293	1.348	1.735	1.717	1.143	2.767
CD	0.109	0.109	0.133	0.017	0.015	0.056

(Treatments with same letters are not significantly different)

Microbiological quality evaluation of developed yoghurt mimics. The amount of live microorganisms in the finished product is a crucial factor in determining whether a product qualifies as a probiotic. The survival of selected probiotic strains during production and storage of samples is influenced by intrinsic factors such as pH, titratable acidity, and oxygen (Mishra *et al.*, 2023). To qualify as a probiotic functional food, yoghurt or any fermented product must contain at least 10^8 cfu/g/ml. Several study results suggest that the typical Lactic acid bacteria levels in yoghurt range from 10^4 - 10^9 cfu/ml (Smanalieva *et al.*, 2021). This study examined the effects of rice milk on probiotic viability throughout a 5-day storage period in glass bottles, as it serves as the main substrate for the starter cultures of *Streptococcus thermophilus* and *Lactobacillus acidophilus* to develop yoghurt mimics. The findings in Table 3 demonstrate how storage affects the total viable count of the probiotic bacteria *Streptococcus thermophilus* and *Lactobacillus acidophilus* after a five-

day refrigerated (4°C) storage period. The current study's reported counts were either greater or comparable to those of prior reliable count studies conducted by (Malyala & Aparna 2019; Padma *et al.*, 2021; Palou, 2014). During the first day of storage, the viable count of the probiotic bacteria *Lactobacillus acidophilus* was between 9.056 and 9.121 cfu ml⁻¹; after that, it dropped to between 8.812 and 8.931 cfu ml⁻¹. The viable count of probiotic bacterium *Streptococcus thermophilus* fell in the range of 9.017-9.991 cfu ml⁻¹ on the 1st day and declined to a range of 8.553-8.770 cfu ml⁻¹ on the 5th day of storage. The causes of viability losses may be due to post acidification, low pH values, or oxygen permeability (Malyala & Aparna 2019). These results are in harmony with the results of (El-Sayed & Ramadan, 2020) who reported a reduction in the viable count of probiotic bacteria during the storage period and indicates that all the samples have the required number of probiotic cultures.

Table 3: Total Viable Count of the formulated yoghurt mimics during storage.

Treatments	Total Viable Count logcfuml ⁻¹				Viability %	
	<i>L. acidophilus</i>		<i>S. thermophilus</i>		<i>L. acidophilus</i>	<i>S. thermophilus</i>
	Day 1	Day 5	Day 1	Day 5		
R1T1	9.089 ^c	8.875	9.017 ^a	8.716 ^c	97.56 ^{cd}	96.56 ^a
R1T2	9.056 ^d	8.812	9.037 ^c	8.748 ^b	97.26 ^c	96.7 ^a
R1T3	9.081 ^c	8.869	9.029 ^f	8.770 ^a	97.63 ^c	96.86 ^a
R1T4	9.121 ^a	8.895	9.046 ^c	8.672 ^d	97.46 ^d	96.13 ^a
R2T1	9.066 ^d	8.819	9.045 ^{cd}	8.707 ^c	97.16 ^c	96.13 ^a
R2T2	9.089 ^c	8.845	9.991 ^a	8.616 ^f	97.26 ^c	89.53 ^{bcd}
R2T3	9.064 ^d	8.819	9.959 ^b	8.672 ^d	97.16 ^c	86.83 ^d
R2T4	9.082 ^c	8.861	9.021 ^g	8.643 ^e	97.46 ^d	92.96 ^{abc}
R3T1	9.117 ^{ab}	8.250	9.034 ^{ef}	8.587 ^g	90.46 ^f	95.23 ^a
R3T2	9.059 ^{ad}	8.851	9.038 ^{de}	8.553 ^h	97.6 ^c	94.8 ^{ab}
R3T3	9.108 ^b	8.931	9.991 ^a	8.612 ^f	98 ^b	89 ^{cd}
R3T4	9.065 ^d	8.908	9.044 ^d	8.724 ^c	98.23 ^a	93 ^{abc}
±SE(m)	0.004	0.095	0.002	0.006	0.036	1.816
CV%	0.069	1.853	0.046	0.125	0.064	3.358
CD	0.011	0	0.007	0.018	0.105	5.30

(Treatments with same letters are not significantly different)

The microbiological quality evaluation for Total Bacterial Count (TBC), Total Fungal Count (TFC) and Total Coliform Count (TCC) was carried out on different agar media including Nutrient agar (TBC), Rose Bengal Agar (TFC) and EMB agar (TCC) using spread plate method on day 1 and day 5 of refrigerated storage in glass bottles. The TBC found in the initial day was too low to count and on the fifth day of incubation ranges from $15\text{-}34 \times 10^4$ CFU/ml. The yoghurt mimics samples did not reveal any count for TFC and TCC indicating the samples are free from pathogenic fungus or coliforms.

Sensory profiling of yoghurt mimics. The sensory characteristics of a rice based yoghurt mimic are influenced by many factors such as pH, fermentation, source of protein, sugar and fat (Dhakal *et al.*, 2023). Sensory profile is one of the most important characteristic that defines the overall quality of any product. The sensory profiling of rice based yoghurt

mimics considers various attributes including appearance, aroma, consistency, taste, mouthfeel, and overall acceptability using a 9 point hedonic scale (Malashree *et al.*, 2023). The sensory evaluation results of yoghurt mimics made from different rice varieties from three milling treatments are depicted under the Table 5. The yoghurt mimic developed from parboiled rice milk sample R3T4 was found to be the most acceptable product in terms of taste followed by the sample R1T4. The same treatments R3T4 and R1T4 scored higher for mouthfeel, aroma and appearance. R3T4 outperformed other treatment samples in case of consistency. The treatment R3T4 had highest overall acceptability compared to other treatment samples. The sensory profile data showed that the probiotic yoghurt mimics made from rice milk were within an acceptable range over a five-day storage period, and the yoghurt mimic samples' preferences fell between like very much and like moderately.

Table 4: Microbiological analysis of yoghurt mimics samples.

Treatments	Day 1			Day 5		
	TBC Logcfu/ml	TFC Logcfu/ml	TCC Logcfu/ml	TBC logcfu/ml	TFC logcfu/ml	TCC Logcfu/ml
R1T1	TLTC	ND	ND	31×10^4	ND	ND
R1T2	TLTC	ND	ND	27×10^4	ND	ND
R1T3	TLTC	ND	ND	34×10^4	ND	ND
R1T4	TLTC	ND	ND	29×10^4	ND	ND
R2T1	TLTC	ND	ND	25×10^4	ND	ND
R2T2	TLTC	ND	ND	32×10^4	ND	ND
R2T3	TLTC	ND	ND	25×10^4	ND	ND
R2T4	TLTC	ND	ND	22×10^4	ND	ND
R3T1	TLTC	ND	ND	20×10^4	ND	ND
R3T2	TLTC	ND	ND	23×10^4	ND	ND
R3T3	TLTC	ND	ND	15×10^4	ND	ND
R3T4	TLTC	ND	ND	19×10^4	ND	ND

(TLTC-Too Low To Count, ND-Not Detected)

Table 5: Sensory Evaluation of Yoghurt Mimics Samples.

Treatments	Appearance	Aroma	Consistency	Taste	Mouthfeel	Overall acceptability
R1T1	8.33	7.33 ^a	7.55	7.72 ^a	7.83	7.75
R1T2	8.38	7.50 ^{ab}	7.55	7.88 ^{ab}	8.0	7.86
R1T3	8.27	7.38 ^a	7.61	8.0 ^{ab}	7.88	7.83
R1T4	8.38	7.88 ^b	7.61	8.27 ^b	8.11	8.05
χ^2 value	0.236	8.441	0.152	8.039	1.317	6.876
p_value	0.972	0.038	0.985	0.045	0.725	0.076
R2T1	8.0	7.33	7.72	8.0	7.72	7.75
R2T2	8.0	7.38	7.72	8.05	7.83	7.80
R2T3	8.05	7.44	7.77	8.05	7.77	7.82
R2T4	8.16	7.66	7.83	8.44	7.94	8.01
χ^2 value	0.624	2.458	0.304	5.972	0.737	6.885
p_value	0.891	0.483	0.959	0.113	0.865	0.076
R3T1	8.22	7.61	7.88	8.11	7.88	7.94
R3T2	8.16	7.66	7.88	8.16	8.0	7.97
R3T3	8.16	7.66	7.94	8.16	7.9	7.97
R3T4	8.22	7.88	8.0	8.55	8.05	8.14
χ^2 value	0.087	1.505	0.406	6.296	0.303	3.095
p_value	0.993	0.681	0.939	0.098	0.959	0.377

(Treatments with same letters are not significantly different)

CONCLUSIONS

Rice is an inevitable staple cereal of the Indian population and is exploited in numerous ways to

develop novel sustenance. Since the Indian diet is diverse in a wide range of fermented foods made from cereals, utilizing rice to develop innovative functional foods has the potential to boost market expansion. The

present study help to facilitate the formulation of non-dairy yoghurt mimics from rice milk by inoculating a starter culture mix of *Lactobacillus acidophilus* and *Streptococcus thermophilus* with a shelf life upto 5 days.

FUTURE SCOPE

Further refinement in flavor and large-scale production studies could enhance the commercial viability and acceptance of this yoghurt mimics as it is a ready to drink solution for those looking for a quick, nutritious option that doesn't compromise on health benefits and perfectly into the busy lifestyle of modern consumers.

Acknowledgement. The authors are thankful to Kerala Agricultural University for providing the research grant and laboratory facilities to conduct the experiment.

Conflict of Interest. None.

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How to cite this article: Shruthi P. and Suma Divakar (2024). Formulation and Sensory Profiling of Dairy Free Probiotic Yogurt Mimics using Rice Milk. *Biological Forum – An International Journal*, 16(11): 26-31.