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Traditional fermented rice beverage enriched with zinc, calcium and iron

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Fermentation is a processing method passed on to us by our ancestors. Today, their benefit goes beyond preservation with the knowledge of probiotic organisms offering benefits to our gut health and various other therapeutic benefits. Cereal fermentation creates conditions that are optimal for degradation of anti-nutrients, otherwise complexed with micronutrients. The overall objective of the study was to develop a fermented rice beverage based on a traditional South Indian practice, enriched with micronutrients zinc, calcium and iron. The product incorporated ingredients such as sesame seeds rich in micronutrients iron, calcium and zinc and pumpkin seeds as a source of zinc and calcium along with red rice. The beverage was prepared by fermenting red rice with water and curd as a starter culture for 12 h as the control (T0). Roasted pumpkin seed and sesame seed powders were incorporated in formulations as variation 1 (5%), variation 2 (10%), variation 3 (15%). It was observed in sensory evaluation that V1 was the best accepted variation. Physiochemical analysis of V1 reveals pH 4.16±0.005 and acidity 0.1±0.01%. Macronutrient estimation showed 8±0.01 g/100 mL carbohydrate and 0.883±0.00 g/100 mL protein in V1. Micronutrient content of enriched rice biotic beverage was 0.235±0.001 mg/100 mL zinc, 0.375±0.001 mg/100 mL iron and 20±0.1 mg/100 mL calcium. Micronutrient content was significantly increased ($p<0.05$) in V1 when compared to the standard (T0). Isolation of bacteria showed presence of potential probiotics, 12×10^6 CFU/mL in T0 and 31×10^6 CFU/mL in V1. Bacteria were confirmed to be rod shaped and gram positive in character. The study concluded that an acceptable enriched rice biotic beverage was developed.

Keywords: Enriched, Fermentation, Micronutrients, Probiotic, Rice beverage

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Ancient people used these preservation methods primarily as a means to store the excess food, be it plant or animal origin. History of fermentation dates back to 7000 - 8000 B.C in the Indus valley civilization where it was presumed to have originated. There exists evidence of usage of clay pots in the fermentation of food and beverage during the Vedic period. Fermented foods and beverages still continue to be a key part of our food practices. It is commonly a method that can be performed with mostly simple techniques and equipment at the household level. Today fermentation is known for benefits that go beyond just preservation. One of the earliest mentions of health benefits was by a Roman historian named Pliny in 75AD. He mentioned fermented milk as use for treating gastrointestinal infections. Lavoisier in the late 1700s, was the first to publish the clear account of the chemical changes that took place during the process of fermentation. The role of microbes in particular to initiate and continue the process of fermentation was discovered by French chemist Louis Pasteur in the mid1800s. Further into the 1900s, it was proposed by a French paediatrician, Tisser that Bifidobacteria could be effective in prevention of infections in infants[1].

In India, various traditional fermented foods and beverages developed across the country in various preparations. Various parts of our country have different fermented preparations with various raw materials as their base. Fermented rice water traditionally known as 'Pazham Sooru Kanchi' or 'Nisineer' was used as healthy water in natural medicine in folklore practice of ancient times. It was used to provide energy, help with stomach issues like bloating, constipation, diarrhoea, prevent dehydration and act as an effective electrolyte solution[2].

The concept of these fermented foods being health beneficial has slowly matured over various scientific studies over past few decades, in particular. Hence there has been an increased awareness of traditional foods and their consumption in relation to health improvement and disease prevention in the recent

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years. These fermented foods are popular today due to the presence of beneficial microorganisms called probiotics. According to FAO and WHO[3] these probiotics are adequately administered live organisms that confer health benefits to the host. Lactobacillus, certain yeast strains and Bifidobacteria are some examples of commonly used organisms for this purpose. The various therapeutic benefits offered by these organisms such as maintaining of intestinal health by regulation of gut microbiota, antimicrobial action against pathogenic microbes, anti-carcinogenic effect, development and stimulation of immunity, short chain fatty acid production, anti-atherogenic effect, improved mucosal membrane barrier, reduction in lactose intolerance symptoms, production and enhancement of bioavailability of nutrients[4].

Cereal grains are used as a known source of carbohydrate, dietary fibre, protein minerals and vitamins. But their quality is generally seen to be poorer in terms of nutrition as compared to other matrices like dairy. This could be attributed to the content of protein being lower, being deficient in amino acids like lysine as well as anti-nutrients present. Fermentation on cereals could be a method of processing to help improve their nutritional properties. An optimum pH condition is created by fermentation for enzymes to degrade phytate of cereals, which usually exists as a complex with polyvalent cations like calcium, iron and zinc. Therefore reduction in the bound phytate may result in increased amount of nutrients like calcium, zinc and iron to be present in soluble forms and available to use[5]. Addition of inoculum like curds in fermentation of grains could also contribute to reducing antinutritional factors[6]. Fermentation also, loosens the complex matrix that embeds minerals[7].

Seeds are found to be good sources of micronutrients having the potential to be used to enhance nutrition value. Pumpkin seeds have been gaining attention in the recent years for their recognised nutrition value. It has been a part of traditional medicine practices throughout the world. They are usually thrown away during processing as agricultural wastes but are highly nutritious in nature. They are a good source of protein, fibre[8], vitamins and minerals like zinc and calcium[9]. Sesame seeds are well known to be a reservoir of nutrients. They are found to be rich in micronutrients like iron calcium as well as zinc[10]. Additionally they are also known for contributing pleasing aroma.

Cereals and legumes are considered as effective substrates for the production of probiotic-incorporated functional food, as they can be used as a source of non-digestible carbohydrates, which stimulate the growth of Lactobacilli and Bifidobacteria[11]. Cereals also have a capability of buffering effect for protecting the microorganisms in harsh conditions of intestines and also components of cereal have a capacity to buffer while protecting the organisms in harsh environments of intestine and naturally carries media for growth of micro organisms. Interest in cereal based fermented products has globally seen an increase today due to its various benefits and high potential for further development[1]. On this account, rice fermentation has been chosen for this research study with nutrition value addition.

The fermented food which is grain based are growing more popular today considering their benefits. Globally, lifestyle changes and incidence of various diseases has been on the increase. Also, the population today are growing to be nutritionally aware and conscious tending to seek out nutrition enriched and healthy food options. Hence this creates a need for innovation in the industry to develop attractive and desirable products that provide significant value addition in terms of nutrition, without compromising on taste. Sustainability is an important facet of food production and consumption today. Conversion of otherwise considered agricultural waste products like seeds which are actually rich nutrients, to value added products is a step towards sustainable nutrition. It is also seen in recent years that we have begun looking back to our traditional and cultural practices. Our ancestors integrated healthy lifestyle and food habits into their daily life. They used simple ingredients and processes to create healthy food options while being unaware of the entire benefits and sustainability of their practices. Some of these practices to date are not properly documented. Studying benefits of these naturally fermented foods have the potential to vindicate traditional food practices. Efforts are beginning to be made in the direction of documenting traditional food practices and understanding their benefits. This sustains the need for a well-designed scientific approach to study them. Hence, this study aims in documenting, studying as well as enriching this age old practice of rice fermentation.

The objectives of the study were to develop a fermented rice beverage based on a traditional south

Indian practice and enrich it with zinc, calcium and iron. And also to analyse the overall acceptability through a sensory analysis, estimate its physiochemical characteristics, mineral content and presence of potential probiotics in the beverage. The study stands for promoting traditional practices to be understood and brought back to use.

Materials and Methods

Selection of product

In the study, an enriched rice biotic beverage was developed with incorporation of sesame seeds and pumpkin seeds. Fermented rice beverages are traditional to various regions of our country and all over the world as well. Fermentation of rice overnight and using the water as a beverage is one of the traditional practices in South India. Today traditional beverages are coming back into practice due to their perceived benefits. But there are usually no standardised methods of preparing these beverages and not as many studies about their beneficial properties. This study aims to develop a standardised method of preparation of a traditional beverage practice while enriching it with micronutrients.

Ingredient procurement and preparation

The beverage was prepared using the ingredients red rice, sesame seeds and pumpkin seeds (Fig. 1). These ingredients were procured from a local market in Bangalore. The ingredients once procured were stored in dry, hygienic conditions till use.

Sensory evaluation

Sensory evaluation was conducted using a 9 point hedonic scale. The scale that was used consisted of nine categories ranging from 'extremely dislike' to 'extremely like'. The parameters taste, colour, consistency, aroma and overall acceptability of the beverages were evaluated by sensory test.

Physiochemical and mineral analyses

Moisture, pH, titratable acidity, protein, fat and carbohydrate of the control and best selected variation were analysed using standard methods of analysis of AOAC[12]. Mineral analysis for zinc, calcium and Iron content was conducted using Atomic Absorption Spectrometer following method of American Public Health Association[13].

Microbiological analyses

Isolation of Lactic Acid Bacteria (LAB)

1 g of the sample was dissolved in 9 mL of 0.15% buffered peptone water and diluted up to 10 folds. Diluted sample was inoculated on MRS agar plate ensuring pH of 6.5, incubated at 37°C for 48 h. A single colony was obtained by streaking.

Morphological characteristics

Subcultured MRS plates were examined for colony characteristics. Gram staining was done according to Forhad, *et al.*[14], and observed under light microscope.

Catalase test

The slide test was conducted on sample and variation. Glass slide was divided into two and a drop of saline was placed on each side with a cool sterilized loop. Small amount of culture was picked up from the Petri plate and one or two colonies emulsified on each drop, making a level suspension.

Table 1 — Formulation of standard and developed variations of enriched rice biotic beverage (100 mL)

Variation	Rice water	Pumpkin seed powder	Sesame seed powder
Control	100%	-	-
V1	90%	5%	5%
V2	80%	10%	10%
V3	70%	15%	15%

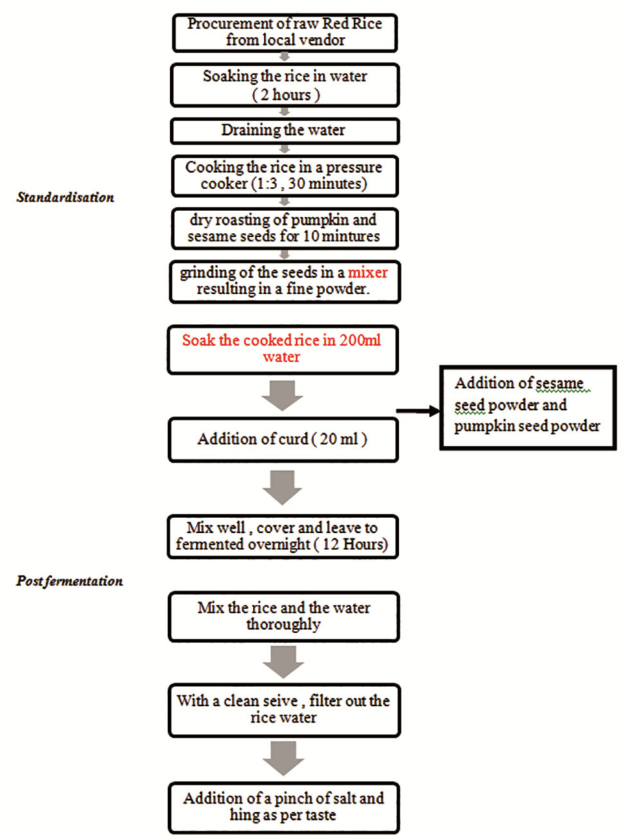

Fig. 1 — Ingredient procurement and preparation of beverage

A drop of hydrogen peroxide was added on each side and observed for gas bubbles.

Statistical Analyses

Statistical analysis was performed to determine the mean, standard deviation and f-test for the developed fermented rice water beverage. ANOVA test was conducted which helped in analysing the level of significance between control and variations. The ANOVA test also helped in determining most acceptable product. Karl Pearson's coefficient was calculated to determine coefficient between sensory parameters and addition of ingredients. Microsoft Excel and SPSS software were used in conducting statistical analysis.

Results and Discussion

Sensory evaluation

The mean sensory scores for the attributes like taste, colour, consistency, aroma and overall acceptability are depicted in Table 2. Highest score for overall acceptability (7.52±0.01) was observed for the sample V1 with 5% sesame and 5% pumpkin seeds. While V2 and V3 showed minimum overall acceptability (6.84±0.01).Variation V1 also showed highest scores in taste, consistency and aroma (7.28±0.01, 7.6±0.01, 7.32±0.01). Hence V1 was found to be the most accepted variation. Studies show that fermentation of cereals has a better sensory acceptance. According to studies fermentation results in improvement of sensory properties of cereals[15,16]. Fermentation improved taste increasingly from 0 to 12 h. Fermented traditional rice beverage Basi showed best acceptability on 12 h of fermentation when tested on a 9 point hedonic[17].

In this study, the correlation between the mean sensory scores and incorporation of sesame seed and pumpkin seed powders were calculated using Karl Pearson's coefficient. The relationship between the mean sensory scores of colour, taste, consistency, aroma and overall acceptability with the incorporation of seed powders showed a negative correlation (-0.26897, -0.82165, -0.81264, -0.87796, - 0.79406) respectively. This indicates that sensory scores decrease as the addition of seed powders increase. Addition of sesame seeds in increased proportion could cause bitter after taste resulted in lower sensory acceptability of V2 and V3. Reduced sensory properties with increased inclusion of sesame seed has also been reported by other studies[18,10].

Physiochemical analysis

The results of the physiochemical analyses after 12 h of fermentation are depicted in Table 3. High moisture content 95.26±0.56% and 95.92±0.82% was observed in both control and variation V1. Mean acidity 0.05±0.001% in the control (T0) and 0.1±0.01% in V1 was found on analysis. Statistical analysis showed significant difference (74.257*, $p<0.05$) in acidity of control (T0) compared to the variation (V1). The mean pH was 4.33±0.005 in the control (T0) and 4.16±0.005 in variation (V1). Statistical F-test on the results are significant at 5%

Table 2 — Mean Sensory Scores of enriched rice biotic beverage

Variation	Colour	Taste	Consistency	Aroma	Overall Acceptability
T0	7.35±0.01^{a}	7.15±0.01^{b}	7.44±0.01^{c}	7.32±0.01^{a}	7.32±0.01^{b}
V1	7.28±0.01^{a}	7.28±0.01^{c}	7.6±0.01^{d}	7.32±0.01^{a}	7.52±0.01^{c}
V2	6.96±0.01^{a}	6.72±0.01^{a}	7.28±0.01^{b}	6.72±0.01^{b}	6.84±0.01^{a}
V3	7.33±0.56^{a}	6.72±0.01^{a}	7.08±0.01^{a}	6.76±0.01^{a}	6.84±0.01^{a}
F-Value	1.241^{NS}	2534.75*	1484*	3372*	3564*
P Value	0.357	0.000	0.000	0.000	0.000

*significant at 5% level NS: Not significant

Data expressed as mean± standard deviation of triplicates. Means within the same column have no common superscripts are significantly different.

T0- Control, V1-5% sesame and pumpkin, V2-10% sesame and pumpkin, V3-15% sesame and pumpkin

Table 3 — Physiochemical analyses of enriched rice biotic beverage

Variations	Mean ± SD					
	Moisture (%)	Acidity (%)	Ph	Protein (g/100 mL)	Carbohydrate (g/100 mL)	Fat (g/100 mL)
Control (T0)	95.26±0.56	0.05±0.001	4.33±0.005	0.357±0.00	6±0.01	0.81±0.01
V1	95.92±0.82	0.1±0.01	4.16±0.005	0.883±0.00	8±0.01	0.64±0.01
F-value	1.272^{NS}	74.257*	1300.500*	2304*	297*	1.676^{NS}
P Value	0.322	0.001	0.00	0.01	0.00	0.26

*significant at 5% level NS: Not significant

Data expressed as mean ± standard deviation of triplicates.

level (1300.500*, $p<0.05$). A Kefir beverage produced from yam, enriched with sesame seed and bean extract showed to reach a pH of 4.3 on 24 h fermentation. Lesser time taken to reach a pH of about 4.4 to 4.6 during fermentation is desirable due to lesser modification of sensory properties and chances of development of pathogenic bacteria are lesser[19]. A probiotic beverage was developed from pomegranate, fermented with a prepared starter culture for 7 h. On chemical analysis, results showed titratable acidity as 0.540% expressed in terms of lactic acid which was produced as a result of metabolic activity of probiotic organisms. The pH was inversely proportional to the acidity of the medium .The pH was found to be 3.512 in the probiotic pomegranate beverage[20].

The mean protein value of the standard beverage was found to be 0.357±0.00 g/100 mL and that of V1 was 0.883±0.00 g/100 mL. Statistical analysis showed a significant difference (2304*, $p<0.05$) in mean protein value of the control (T0) in comparison to the variation (V1). The result showed that the control beverage containing mean carbohydrate 6±0.01g/100 mL and the variation V1 containing 8±0.01g/100 mL carbohydrate. Statistical analysis revealed that there was a significant difference (297*, $p<0.05$) between the control (T0) and the variation (V1) with respect to mean carbohydrate content. The mean fat content was found to be 0.81±0.01 in the control (T0) and 0.64±0.01 in the variation (V1). In a similar study a sprouted wheat based probiotic beverage was developed with *L. acidophilus* NCDC-14 strain. Analysis on the beverage (100 mL) provided 1.19% protein, 0.33% fat and 11.56% carbohydrates when prepared with the optimized formulation[21]. The proximate analysis done on Kunu, a local beverage of Nigeria on natural fermentation showed crude protein of 4.41% and fat 1.14%. Much of protein, fat content in cereal and other crops are often lost on processing which can be retained on enhanced fermentation with Lactic acid bacteria[22].

Mineral analysis

The result in the Table 4 shows the mineral content which was analysed for the standard beverage in comparison to the most accepted variation V1 (5% sesame seed and 5% pumpkin seed).

The results from the analysis of the micronutrients in the beverages are depicted in Table 4. It was revealed on analysis that mean zinc content of the enriched rice biotic beverage (V1) increased to 0.235±0.001 mg/100 mL from 0.170±0.001 mg/100 mL in the control. The mean iron content of the control beverage (T0) was found to be 0.317±0/.001 mg/100 mL which increased to 0.375±0.001 mg/100 mL in variation. With respect to the mean calcium content depicted in Table 4, an increase from 8±0.1 mg/100 mL in the standard (T0) to 20±0.1 mg/100 mL was observed in variation (V1) with added 5% of pumpkin and 5% sesame seed powders. The statistical analysis indicates that the micronutrients zinc, calcium and iron increased significantly ($p<0.05$) in the variation V1 as compared to the control (T0). Improvement of the micronutrients zinc, iron and calcium was reported that in fermented rice water beverage with added sesame seed and pumpkin milks[23]. Sesame seeds on fermentation lead to decrease in the anti-nutritional factors like phytic acid and oxalate which are otherwise complexed with minerals[24]. Fermentation is known to be the most effective method of processing in reduction of phytic acid. Results showed decrease of phytic acid from 31.59 mg/g in raw sesame to 24.17 mg/g after 24 h of fermentation. It was observed that oxalates reduced from 1.05 mg/g to 0.72 mg/g on 24 h of fermentation. A fermented cereal based beverage, Kanun-zaki drink native to Nigeria was studied and this drink was prepared using sorghum and supplemented with sesame seeds resulted in the improvement in the micronutrients iron and calcium. Based on the native beverage, another beverage was developed with addition of sesame seeds and results showed an increase of calcium from 11 mg/100 g to 12.67 mg/100 g , iron from 2.7 mg/100 g to 3 mg/100 g with 20% sesame formulation. Increase in % of sesame seeds showed an increase in micronutrient content[10]. Improvement in composition of minerals in cereals during fermentation was seen due to action of fermenting microbes which were more when there was addition of oil seed such as sesame. Similar results were observed in a flour that was made using

Table 4 — Mineral content of enriched rice biotic beverage

Variations	Mean ± SD		
	Zinc (mg/100 mL)	Iron (mg/100 mL)	Calcium (mg/100 mL)
Control T (0)	0.170±0.001	0.317±0.001	8±0.1
V1	0.235±0.001	0.375±0.001	20±0.1
F-value	3724.9*	5046.0*	2160000.0*
P Value	0.00	0.00	0.00

*significant at 5% level NS: Not significant
Data expressed as mean ± standard deviation of triplicates.

Table 5 — Morphological characteristics, plate count and catalase activity of colonies obtained on MRS agar

Sample	Agar	Size	Colour	Opacity	Margin	Elevation	Gram character	cfu/mL	Catalase activity
T0	MRS	1 mm	Cream	Opaque	Smooth	Slightly elevated	Gram positive rods	12×10^6	Negative
V1	MRS	1 mm	Cream	Opaque	Smooth	Slightly elevated	Gram positive rods	31×10^6	Negative

fermented sesame showed an improvement in values of calcium, iron and zinc in the flour. On evaluation of different processing methods on nutrition of sesame seed flour, mineral content of calcium, zinc and iron were seen to improve on fermentation of the flour. Improvement in mineral content could be as a result activity of the microorganisms and biosynthesis taking place during the processing[25].

Microbiological analysis

Results of the microbiology analyses (Table 5) revealed that there was presence of 12×10^6 cfu/mL organisms in the control beverage and 31×10^6 cfu/mL organisms in the variation on inoculation for 38-72 h using MRS media, which was used for the identification of *Lactobacillus* spp. It was observed in a study, minimum count of 10^6 CFU/g of probiotics is required to be present to extract health benefits. The isolated colonies were observed to be creamish in colour, opaque, round, smooth and slightly elevated. The isolated bacteria from both the control and variation samples on gram staining revealed to be gram positive rods in nature. Negative catalase activity was observed in both the samples on performance of the slide test[4]. Growth of small, shiny, round, whitish cream or brown coloured colonies which were morphological similar to *Lactobacillus* spp. on testing yogurt sample isolated on MRS agar were observed. Examination under the microscope showed the bacteria to be gram positive rods, short and medium, non-spore forming in nature, which is an indication to be a member of *Lactobacillus* spp. The isolates were also found to be catalase and oxidase negative which might confirm them as *Lactobacillus* spp[26]. Study on buffalo milk yogurt indicated round, off white shiny colonies similar to a reference *Lactobacillus* spp. grown on MRS media. On gram staining and observation, short to medium chain gram positive rod shaped bacteria were revealed which are typical of *Lactobacillus* spp. Biochemical characterization conducted showed negative catalase and oxidase activity[27].Another study reported resultant colonies which were diplococci, rod shaped gram positive and catalase negative indicating presence potential probiotic organisms like *Lactobacillus* spp. on isolation of bacteria from fermented red rice[28].

Conclusion

The overall objective of this study was to develop a micronutrient enriched beverage based on the traditional practice of rice fermentation in south India. Analyse it for sensory acceptability, physiochemical characteristics, mineral content and determination of presence of potential probiotic organisms. Sesame seeds and pumpkin seeds known to be rich in micronutrients zinc, calcium and iron, were used to enrich the traditional beverage. The results indicated that there was a significant improvement in the mineral content due to addition of the seeds after fermentation with the rice. It also showed the presence of potentially probiotic organisms in the beverage in adequate amounts. This study is a step towards looking back, documenting and studying our traditional practices which could help them to be slowly incorporated in our lifestyles again. Regular incorporation of such practices by our ancestors could have been one of the factors that contributed to maintenance of their health. The study concludes that simple ingredients like cooked red rice which is commonly prepared in households along with seeds like sesame and pumpkin which are inexpensive and easily available, can be used to create an acceptable beverage enriched in micronutrients zinc, calcium and iron. Seeds which are often overlooked and underutilized, could be incorporated as sources of micronutrients.

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Conflict of Interest

The authors declare no competing or conflict of interest.

Authors' Contributions

In this study SP helped with conceptualization, supervising of study, reviewing and editing manuscript. VV helped with conceptualizing, investigation of study, editing and manuscript preparation.

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