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A Study on Physiochemical, Proximate, Functional and Microbial Profile of Fermented Karuppukavuni Rice and Karunkuruvai Rice

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Abstract

Worldwide the consumption of fermented foods makes a significant contribution to human health. The cereal based probiotic foods are most common in Asia - pacific region as it's free of dietary allergens, gluten, and low in fat as well as vegan friendly when compared to dairy-based fermented products. The objective of the present investigation was to ferment two variations of black rice such as Karuppu kavuni rice and Karunkuruvai rice and to analyze its various quality attributes such as physiochemical, proximate, functional and microbial count. Both the black rice was pressure cooked at 1:3 ratio (Rice: Water) until well cooked. Both the cooked black rice were separately soaked in water and allowed to ferment overnight for 12 hours at room temperature (27℃). The findings revealed that the fermented KKR1 and KKR2 were found to be good source of Protein (6.9 ± 0.1g and 6.43 ± 0.25g) and vitamin B 12 (3.68 ± 0.03 µg and 4.58 ± 0.03 µg). The fermented KKR1 and KKR2 exhibited excellent antioxidant activity (74.49% and 62.63%). Both the fermented black rice variations possessed excellent probiotic counts ($49×10^3$CFU/ml and $38×10^3$CFU/ml). Hence the fermented KKR1 and KKR2 can be considered as a healthy and affordable non-dairy probiotic food for people suffering from lactose intolerance, cow's milk proteins allergy and people following vegan diet.

***Key words*:** Black rice, Karuppu kavuni rice, Karunkuruvai rice, Probiotics, Fermented rice

More than 50% of the world's population consumes rice (Oryza sativa), which accounts for more than 20% of all calories consumed globally, particularly in East and South Asia, the Middle East, the West Indies, and Latin America.Half of the world's population consume rice as a staple diet, making it the most important cereal crop (1). Worldwide the consumption of fermented foods makes a significant contribution to human health. Fermentation also improves the overall quality, digestibility and enhances its nutritional value (2). Cereals can be considered as potential ingredients for the development of probiotics, which have been linked to lower the risk of chronic diseases such as obesity, cardiovascular disease, and type II diabetes (3). Fermented cereal-based beverages might potentially provide useful substances such as antioxidants, dietary fibre, minerals, probiotics, and vitamins in addition to satisfying customer desire for non-dairy probiotic beverages (4).

Consumption of polished white rice which is deficient in dietary fibre, polyphenols, minerals and B vitamins may lead to nutritional deficiency as most of the essential nutrients are removed by processing (5). Additionally, white rice contains high glycaemic load (GL) and glycaemic index (GI), which leads to elevated postprandial blood glucose levels (6). Until 1970, India possessed more than 110,000 different rice varieties, but the Green Revolution, which placed a focus on monoculture and hybrid crops, resulted in the loss of many varieties of crops (7).

Many different types of white rice are grown in India, including *Joha*, *Ponni*, *Jyothi*, *Pusa*, *Basmati*,*SonaMasuri*, *Navara*,*Jaya*, *Kalajiri* (aromatic), *Boli*, and *Palakkad Matta* etc.The coloured variety includes Himalayan red rice; *Matta rice*, *Kattamodon*, *Kairali*, *Jyothy*, *Bhadra*, *Asha*, *Rakthashali* of Kerala*; Red Kavuni*, *Karuppu kavuni*, *Kaivara Samba*, *Mappillai Samba*, *Karun kuruvi*and *Poongar* of Tamil Nadu (7). Among these there are different types of black rice cultivated in India, divided into various groups based on its various sizes, forms, nutrient content, and colours. There are numerous types, including black glutinous rice, black emperor's rice, karuppu kavuni rice, karunkuruvai rice, black jasmine rice, and black forbidden rice (8). In particular the karuppu kavuni and karunkuruvai is the traditional rice varieties of Tamil Nadu and it has higher protein content, a lower fat content, a lower level of total carbohydrates, and higher quantities of phenolic acids, carotenoids, and flavonoids. It was well-known for its anti-diabetic qualities in addition to its nutritional properties (9).

Black rice is considered the most nutrient-dense variety of rice among all other varieties. These grains contain significant amounts of protein, dietary fibre, phytochemicals and antioxidants than polished white rice (10). Cyaniding-3-

glucoside and Peonidin-3-glucoside are the two main antioxidants found in predominant traditional black rice and in general polished white rice essentially lacks many vital nutrients (11).

Most of the probiotic products are made from cow's milk which won't be suitable for the people suffering from lactose intolerance, those allergic to cow's milk proteins, and those who prefer to follow vegan diet. Thus, the best alternate source for the people with these conditions and preferences are the lactose free, plant based probiotic products (12). In the past, fermented rice was made by fermenting leftover cooked rice with excess water overnight and consumed the next day. "Pazhaya sadham" or "palaya soru" refers to leftover cooked rice that has been allowed to ferment, while "pazhaya sadham kanchi" or "neeragaram" refers to fermented rice water. Pazhaya sadham is the staple and traditional food of south Indians (13).

Fermented traditional black rice provides the body high-energy, hydration boost, restores intestinal health, aids in the prevention of gastrointestinal illnesses, and strengthens the immune system, which are the best ways to reduce the prevalence of illnesses. It serves as a best alternative for non-dairy probiotic food (13). Hence the current study aimed to ferment traditional varieties of black rice such as karuppu kavuni rice and karunkuruvai rice and to evaluate its physiochemical, proximate, functional properties and microbial profile.

MATERIALS AND METHODS

The raw materials such as karuppu kavuni rice and karunkuruvai rice were obtained from a local supermarket in Velachery. The project has been approved by the Independent Human Ethical Committee (IHEC) conducted by the Department of Home Science, SDNB Vaishnav College for Women, Chromepet, Chennai – 44, on 01/10/2022. The Protocol No - SDNBVC/HSE/IHEC/2021/23.

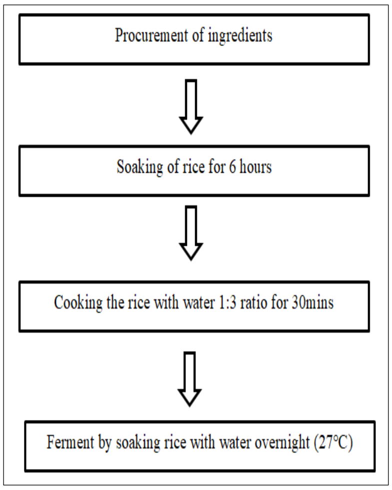

Fig 1 Preparation of Fermented Black Rice

Preparation of Fermented Black Rice

The figure 1 elucidates the flowchart of preparation of the fermented KKR1 and KKR2. Both the black rice were soaked for 6 hours, Soaking in the preliminary process of softening a solid food material by immersing it in a liquid, most preferably in the water. Soaking is a convenient way to remove antinutrients from foods while also reducing down on cooking time. Soaking provides crucial wet conditions in nuts, grains and other edible seeds, which are necessary for fermentation and related decreases in enzyme inhibitors and other anti-nutrients, hence improving digestibility and nutritional content (14). Both variations of black rice were pressure cooked in a pressure cooker at 1:3 ratio (Rice: Water). Both the cooked black rice were separately soaked in water and allowed to ferment overnight for 12 hours at room temperature. The fermentation of black rice was carried out as per the method suggested by (15) with slight modifications.

Quality Analysis of Fermented Black Rice

Physiochemical Analysis of Fermented Black Rice

The physiochemical properties of the fermented KKR1 and KKR2 such as total titratable acidity, pH, total soluble solids and viscosity were analyzed. The techniques used for analyses are provided in (Table 1).

Table 1 Physiochemical Analysis Technique and its References

Parameters	References
Titratable acidity	Sadiah and Handayani (2017)
pH	Sadiah and Handayani (2017)
Total Soluble Solids	Bhatt and Biswas (2001)
Viscosity	(Espirito-Santo *et al.* 2014)

Proximate Analysis of Fermented Black Rice

The proximate analysis of the fermented KKR1 and KKR2 includes energy, protein, fat, moisture, ash, carbohydrates and vitamin B-12. The techniques and references used for analyses are represented in (Table 2).

Table 2 Nutrient Analysis Technique and its References

Parameters	References
Energy	Amankwah *et al.* (2009)
Protein	Supriyati *et al.* (2015)
Total Fat	Supriyati *et al.* (2015)
Total Ash	Purwar *et al.* (2016)
Moisture	Ogodo *et al.* (2017)
Carbohydrates	Purwar *et al.* (2016)
Vitamin B-12	Nicolau *et al.* (2011)

Functional Analysis of Fermented Black Rice

The functional properties of the fermented KKR1 and KKR2 such as antioxidant activity, total phenol content and flavonoid content was evaluated and the techniques used for analyses are represented in (Table 3).

Table 3 Functional Properties Method References

Parameters	References
Total phenol content	Selvakumar *et al.* (2019)
Flavonoids content	Selvakumar *et al.* (2019)
Antioxidant activity	Ara and Nur (2009)

Microbial Analysis of Fermented Black

The microbial evaluation of the fermented black rice such as total bacterial count, yeast and mold count, and probiotic count were analyzed employing standard procedure. The total bacterial count was determined using the Plate count method whereas, the yeast and mold growth was determined using the spread plate method. The probiotic colonies were counted and expressed in CFU/g.

RESULTS AND DISCUSSION

Physiochemical Analysis of Fermented Black Rice

The physiochemical properties of fermented KKR1 and KKR2 such as total titratable acidity, pH, total soluble solids and viscosity were analyzed and statistically compared and explained with relevant literature. The obtained results are provided in (Table 4).

The total titratable acidity of fermented KKR1 and KKR2 was found to be 1.21 ± 0.08% and 2.21 ± 0.09%, respectively. It was clearly evident from the table that there was a statistically significant difference *($p < 0.01$)* identified between the both the samples. The results obtained in this current study were similar to the findings of (27) who reported the titratable acidity value of fermented green gram sprout as (1.05 ± 0.09%). The Total soluble solid of the fermented KKR1 and KKR2 was found to be 78.2 ± 0.92% and 88.6 ± 0.36%, respectively. It can be concluded from the research findings that both the samples exhibited statistically significant differences ($p < 0.01$). The total soluble solids content of fermented KKR1 and KKR2 was higher than the total solid content (53.7 ± 0.98) of fermented glutinous rice as reported by (28). The disparity between the both the research findings could be due to variations in ingredients, ratio of water used for cooking and duration of cooking.

Table 4 Physiochemical Properties of the Fermented KKR1 and KKR2

S. No.	Parameters	Fermented Karuppu Kavuni Rice	Fermented Karunkuruvai Rice	p – Value	Level of significance
1	Total Titratable Acidity	1.21 ± 0.08	2.21 ± 0.09	0.0001	S**
2	Total Solid Count	78.2 ± 0.92	88.6 ± 0.36	0.00	S**
3	Viscosity	5.73 ± 0.04	6.73 ± 0.04	0.00	S**
4	pH	6.96 ± 0.04	6.75 ± 0.03	0.002	S**

Values are represented as mean and standard deviation
All values are the mean of triplicate determination ± standard deviation
NS – Not-significant, S* - Significance at 5% level, and S** - Significance at 1% level

The pH of the fermented KKR1 and KKR2 was found to be 6.96 ± 0.04 and 6.75 ± 0.03 respectively. It was apparent that both the samples exhibited statistically significant differences ($p < 0.01$). The results obtained in this current study were similar to the findings of (29) who reported the pH of fermented brown rice flour as 6.38. The viscosity of the fermented KKR1 and KKR2 was found to be 5.73 ± 0.04% and 6.73 ± 0.04% respectively. It was clearly evident that both the samples exhibited statistically significant differences ($p < 0.01$). Traditional Turkish beverages made from fermented cereals called "Boza" possessed viscosity value (4.21%) as reported by (30), and it was clearly evident that both the fermented black rice has higher viscosity value than that of fermented cereal (Boza).

Proximate Analysis of Fermented Black Rice

The proximate parameters of the fermented KKR1 and KKR2 such as energy, protein, fat, ash, moisture, carbohydrates and vitamin B12 were analyzed for both the fermented black rice KKR1 and KKR2 which is statistically compared and explained with relevant literature. The results are provided in (Table 5).

Table 5 Proximate Composition of Fermented KKR1 and KKR2

S. No.	Parameters	Fermented Karuppu Kavuni Rice	Fermented Karunkuruvai Rice	p – Value	Level of Significance
1	Energy	198.1± 0.75	185.4 ± 0.56	0.00	S**
2	Protein	6.9 ± 0.1	6.43 ± 0.25	0.04	S*
3	Total Fat	2.06 ± 0.56	2.96 ± 0.40	0.08	0.089^{NS}
4	Carbohydrates	77.2 ± 0.40	84.3 ± 0.45	0.00	S**
5	Total Ash	2.2 ± 0.45	1.58 ± 0.04	0.08	0.081^{NS}
6	Moisture	12.75 ± 0.06	10.24 ± 0.08	0.00	S**
8	Vitamin B12	3.68 ± 0.03	4.58 ± 0.03	0.00	S**

Values are the means of triplicate determination ± standard deviation
NS – non-significant, S* - Significance at 5% level, and S** - Significance at 1% level

The calorific value of the fermented KKR1 and KKR2 was found to be 198.1± 0.75 kcal and 185.4 ± 0.56 kcal respectively. When statistically compared there was significant difference ($p < 0.01$) found between both the samples. The results provided by (31), who stated that the amount of energy present in fermented rice batter was 134kcal/g which was lesser that the fermented black rice. It can be concluded that the fermented KKR1 and KKR2 has higher calorific value compared to fermented rice batter. On the daily basis consumption of the fermented black rice gives more energy content. The protein content of the fermented KKR1 and KKR2 were determined to be 6.9 ± 0.10g and 6.43 ± 0.25g, respectively. There was a substantial difference between the samples when statistical comparisons were made ($p < 0.05$). The result of this study was supported by (32), who stated that the amount of protein present in fermented white rice flour was (7.60g). On the other hand, the fermented black rice possessed high protein content when compared to the protein content of glutinous raw white rice (2.02g) reported by (33).

The total fat of the fermented KKR1 and KKR2 was found to be 2.06 ± 0.56g and 2.96 ± 0.40g respectively. There was no statistically significant difference between the fat content ($p > 0.05$) of both the samples**.** The results obtained from the current study were also in agreement with the results produced by (34) who noted very less fat content (1.0 g) in Ambeli (a rice based fermented food) was (1g). Consuming cereal based fermented rice as a source of probiotics has the added benefit of having a very low-fat content which is ideal for the people suffering from obesity, hypercholesterolemia, and other heart diseases, whereas, consumption of dairy based probiotic food may increases the risk of obesity and cardiovascular disease as it is excellent source of saturated fatty acids and cholesterol.

The carbohydrate content of fermented KKR1 and KKR2 was found to be 77.2 ± 0.40g and 84.3 ± 0.45g respectively. When the values of the two samples were statistically analyzed, a significant difference ($p < 0.01$) was observed. The results obtained in this study are congruent with the finding of (33) who reported the similar carbohydrate content in brown rice (77.24vg). Rice has known to have high glycemic index, but International Rice Research Institute (2013) showed that glycemic index of rice varied widely, depending on the type of rice. The variation in glycemic index of rice could be due to the differences in the proportion of starch, particularly the ratio of amylose-amylopectin. In general, highly polished white rice (both raw and parboiled) possess high glycemic index when compared to black rice varieties (33). The ash value of the fermented KKR1 and KKR2 was found to be 2.2 ± 0.45% and 1.58 ± 0.04% respectively. There was no statistically significant difference in ash content *($p > 0.05$)* in both the samples**.** The ash content of fermented KKR1 and KKR2 was similar when compared to the findings of (22) where the ash content of fermented maize (*Zea mays*) was 1.88± 0.11%. The moisture content of the fermented KKR1 and KRR2 was found to be 12.75 ± 0.06% and 10.24 ± 0.08%. The values of the two samples differed significantly ($p < 0.01$) when statistical comparisons were made. The results conjugate with the moisture content of fermented maize (*Zea mays*) 10.82 ± 0.03% reported by (22).

The vitamin B12 of the fermented KKR1 and KKR2 was found to be 3.68 ± 0.03µg and 4.58 ± 0.03 µg. When statistically compared there was a significant difference ($p < 0.01$) found between the both the samples. The results obtained in this current research work was found to be higher than the results reported by (31) who reported the amount of vitamin B12 present in fermented brown rice (0.03 µg). Hence, both the fermented black rice variations are good sources of vitamin B12. The high Cobalamin content in fermented black rice could be useful in treating vitamin B12 deficiencies. One of the strategies for alleviation of micronutrient malnutrition including vitamins in rice is biofortification through fermentation (33).

Functional Analysis of Fermented Black Rice

The Functional properties of the fermented KKR1 and KKR2 such as antioxidant properties, total phenol, and total flavonoid content were analyzed and the results are represented in (Table 6). The antioxidant activity of the fermented KKR1 and KKR2 was found to be 74.49% and 62.63% of inhibition at a concentration of 400µg/ml respectively. The results obtained by (35), states that the antioxidant activity of the fermented red brown rice was 43.00 ± 10.79 mg/mL. Hence both the fermented KKR1 and KKR2 contain much higher antioxidant and free radical scavenging ability when compared to the fermented red brown rice. The total phenol content of both the fermented KKR1 and KKR2 was found to be 0.856 GAE/g and 0.514 GAE/g at a concentration of 400µg/ml. The Total phenolic concentration ranged from 1.73 to 89.2 mg/g for the whole black rice which was reported by (36). Thus, the high phenol content in the fermented black rice could be a reason for its good amounts of antioxidant property. The flavonoid content of both the fermented KKR1 and KKR2 was found to be 0.566 QE/g and 0.841 QE/g at a concentration of 400µg/ml respectively. According to the results provided by (37), the flavonoids value of the whole black rice was 1.97 ± 0.02 mg/g, which was lower when compared to the fermented KKR1 and KKR2 respectively. Thus because of its high phenol and flavonoids content could be beneficial in the prevention of certain non-communicable diseases such as arteriosclerosis, cancer, diabetes, neurogenerative diseases, and arthritis (37).

Table 6 Functional Properties of Fermented KKR1 and KKR2

S. No.	Concentration (µg/ml)	% of DPPH activity		Total Phenol Content GAE/g		Total Flavonoids Content QE/g	
		KKR1	KKR2	KKR1	KKR2	KKR1	KKR2
1	100	33.33	27.40	0.482	0.135	0.321	0.462
2	200	48.75	51.24	0.572	0.303	0.424	0.622
3	400	74.49	62.63	0.856	0.514	0.566	0.841

Microbial Analysis of Fermented Black Rice

The fermented KKR1 and KKR2 were examined for microbial profile and the results are tabulated in (Table 7).

Table 7 Microbial Examination of Fermented KKR1 and KKR2

Microbial Analysis	KKR1 (CFU/ml)	KKR2 (CFU/ml)
Total Bacterial Count	115×10^4	77×10^4
Yeast And Mould Count	<10	<10
Probiotic Count	49×10^3	38×10^3

For many commercial and research purposes, counting microorganisms is a crucial microbiological task. There are many tests used today to identify and count microorganisms, and one of the most popular methods is the viable bacterial plate count, also known as the Standard Plate Count (SPC), Total Viable Count (TVC), Heterotrophic Plate Count (HPC), and Aerobic Plate Count (APC). This method is based on counting the colony forming units (CFU) that are being developed on agar (38). The total bacterial count of the fermented KKR1 and KKR2 was found to be 115×10^4 CFU/ml and 77×10^4 CFU/ml respectively. According to (42), “Total Bacterial Count is the count of the number of bacterial colonies forming units present in the food sample per gram; Total bacterial count is an indicator of hygiene followed during the handling of food samples”. The obtained results for both the fermented black rice were found to be within the safe limit, and therefore it can be concluded that both the fermented black rice was microbially safe to consume. The microbial analysis of the Yeast and mold count showed that the yeast and mold count of the fermented karuppu kavuni rice and karunkuruvai rice was found to be Nil. According to (39), “the quality and possible safety of the product may not be affected since spoilage organisms were not observed or detected.” Therefore, it can be concluded that the fermented KKR1 and KKR2 were microbially safe for human consumption.

The probiotic count of the fermented KKR1 and KKR2 was found to be $49\times10^{3.}$ CFU/ml and 38×10^3 CFU/ml respectively. When compared to the viability count of both the samples, fermented karuppu kavuni rice has better growth of probiotic bacteria than the fermented karunkuruvai rice. The (40) recommends a probiotic count of about ($>10^7$ CFU/mL) to have beneficial effects as a probiotic. The findings show that both the fermented black rice can be considered as good source of probiotics, and regular consumption of it will improve intestinal health by the regulation of microbiota, and stimulation and development of the immune system, synthesizing and enhancing the bioavailability of nutrients, reducing symptoms of lactose intolerance, and reducing the risk of certain other diseases (41).

CONCLUSION

Nowadays consumers are health conscious and are becoming more aware of probiotic foods, in particular non-dairy probiotic foods are currently on trend. The majority of people in Tamil Nadu consume fermented rice as part of their traditional lifestyle. In the past, fermented rice was made by letting leftover cooked rice ferment overnight, adding a tiny bit of curd, and then eating the fermented rice the next day. This present study was intended to ferment two variations of indigenous black rice such as karuppu kavuni rice and karunkuruvai rice and to evaluate its various quality attributes. On overall investigation, the fermented rice was found to be rich in protein, low in fat, low in calories and possess a good amount of vitamin B-12 that can alleviate micro and macro nutrient deficiency in developing countries. The fermented rice also possessed excellent probiotics activity. It can be further concluded that both the variations of fermented black rice are comparatively more nutritious than the highly processed and polished white rice varieties. Both the fermented black rice exhibited desirable colour profiles and had significant antioxidant properties and phytochemical composition. Hence it can be concluded from the research findings that the fermented KKR1 and KKR2 can be considered as a healthy and affordable non-dairy probiotic food for people suffering from lactose intolerance, cow's milk proteins allergy and people following vegan diet.

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