Research Article

Estimation of Nutrient Content of Unfermented and Fermented Rice Drinks

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ABSTRACT

Background: Fermentation of cooked rice has shown to enhance its nutrient content, palatability, organoleptic characteristics and bioavailability of nutrients. Although fermented rice is known to provide immense health benefits, there is not enough literature validating this claim and no scientific data with respect to changes in the nutrient content. **Materials and Methods:** This study comprised of an experimental design to estimate the nutrient content in fermented and unfermented cooked rice. Standardized procedures using methods from Association of Official Agricultural Chemists, High-performance Liquid Chromatography, 3, 5-Dinitrosalicyclic Acid, Thin Layer Chromatography and data from FSSAI Manual were used to estimate the nutrient content. **Results:** In 200 mL of fermented rice drink, macronutrient such as carbohydrate and protein content were found to be 2.4 g, dietary fibre content was 19 g and resistant starch content was 19.73 g. Whereas, in 200 mL of unfermented rice drink, protein content was found to be 1.94 g, dietary fibre content was 2.6 g and resistant starch content was 5.33 g. Total calories were found to be increased after fermentation. Micronutrients such as zinc, selenium, iron, vitamins B2, B3 and B5 also showed an increase after fermentation. **Conclusion:** Based on this evidence, fermented rice drink can be promoted as a traditional, cost-effective health drink.

Keywords: Fermentation, Nutrients, Rice Drink, Health, Nutritional Status, Rice.

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INTRODUCTION

One of the oldest food preservation techniques used for various food products is fermentation. A few of the globally consumed products which are fermented includes cereals, legumes, vegetables, tubers, fruits, milk, meat and fish products.[1,2] The main microorganism used in this fermentation process is the Lactic Acid Bacteria (LAB). They are mostly non-spore forming, non-motile, gram-positive cocci and rods. Few of them can survive in environments with very low levels of oxygen, but a majority of them are anaerobic in nature.[3,4] Their ability to produce an acidic environment is the key preservative activity. The main reasons to use LAB in fermentation process includes its abilities to enrich nutrients, increase its bioavailability and health benefits, enhance food safety and improve organoleptic properties.[5-7]

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Although fermented dairy products are the most popularly consumed food group, plant substitutes have been gaining popularity.[8] The possible reasons for this demand could be due to increased environmental awareness, aversion to cruelty of animals and most importantly a desire to live a life free of illness.[9] Cereals are the most preferred alternative source of non-dairy fermented products due to their supportive physiological effects which helps in the growth of *Lactobacillus* and *Bifidobacterium*.[10,11] Rice-based beverages are the most commonly consumed plant-based beverages due to its enhanced nutritional properties.[12] A few fermented beverages that were found to possess health benefits after consumption includes fermented milk, in which its consumption was studied on 34 hypercholesterolemic women aged 18 to 65 years for a period of four weeks. It was supplemented three times each day and the researchers found that LDL cholesterol was reduced among them.[13] Another study was carried out among 94 borderline hypertensive patients for 8 weeks. It was observed that their sympathetic activity was reduced indicating a change in ACE-inhibitor activity.[14] In a group of 82 patients with dyspepsia symptoms and *H. pylori* infection, kefir was supplemented two times each day for 2 weeks. It was found that their abdominal pain, diarrhoea and headache reduced considerably.[15] Therefore, fermented rice drink could be further studied to identify its nutrient content and potential health benefits.

Although there are many claims regarding the nutritional content of this drink, more experiment-based studies are needed to validate the effectiveness of supplementation with this drink. A few studies on fermented rice drink (commonly known as *Pazhayadhu* in Tamil Nadu) have shown health benefits pertaining to management of stomach and Gastrointestinal (GI) issues such as diarrhoea, bloating and constipation. Fermented rice has also been used as a preventive measure for GI conditions such as ulcerative colitis, irritable bowel syndrome, duodenal ulcers, Crohn's disease, candida infection and celiac disease.[16,17] One study by Varnakulendran[18] has shown fermented rice to regulate body temperature and can be suggested as a protective measure in the treatment of acne and blisters.

Due to improvement in the bioavailability of nutrients after fermentation, fermented rice drink can provide alternative solutions to improving the nutritional status of a population, where signs of malnutrition may be evident. In India where malnutrition is still prevalent among various populations, nutrient-dense fermented rice can be advocated as part of the dietary intervention in improving the nutritional status at the community level. Studies have also suggested the use of traditionally available, low-cost staple foods to be an effective strategy to improve the nutritional status among people of all age groups.[19] Thus, this study on the estimation of nutrients in fermented rice drinks will contribute to evidence-based data for the presence of various macro and micronutrients needed to improve the nutritional status of communities at large.

MATERIALS AND METHODS

The objective of this study was to estimate the nutrient content of unfermented and fermented rice drink. The design of the study was an experimental research design.

Preparation of the rice drinks

Ingredients with FSSAI Standards were purchased from the local market. White raw rice, plain Greek yogurt, iodized salt and RO purified drinking water were used to prepare the unfermented and fermented rice drinks. For 200 mL of fermented rice drink, 30 g of cooked rice was soaked in water for 10 to 12 hr to ferment, after which 15 g of yogurt and a pinch of salt was added. 200 mL of unfermented rice drink contained the same composition as for fermented rice drink, but the cooked rice was not fermented.

The rice drinks were prepared under hygienic conditions and were transported in ice packs to International Organization for Standardization/National Accreditation Board for Testing and Calibration Laboratories/ Food Safety and Standards Authority of India approved laboratories. The food analyses were carried out in reputed laboratories in Chennai.

The following tests were estimated for unfermented and fermented rice drinks

Estimation of macronutrients

Total calories were calculated by quantifying carbohydrates, proteins and fats and using the conversion factors from grams to kilocalories to estimate the total calories of the drinks. Total carbohydrates were estimated using Thin Layer Chromatography (TLC).[20] Protein content was estimated according to the Indian Standards 7219: 1973 through the determination of total nitrogen protein by Kjeldhal method.[21] Saturated and unsaturated fat content of unfermented and fermented rice drinks was estimated using capillary gas chromatography.[22]

Amino acid profiling

Essential amino acids such as histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine and non-essential amino acids which included alanine, arginine, aspartic acid, cysteine, glutamic acid, glycine, proline, serine and tyrosine were estimated using High-Performance Liquid Chromatography (HPLC) technique using solvents phenol and collidine.[23]

Estimation of vitamins

Fat-soluble and water-soluble vitamins were determined by HPLC method.[24] The solvent used for estimation of Vitamin A was Pentane: 2-propanol (2:8) and methanol for D2, D3, E, K1, K2. 0.1 N Hydrochloric acid was used to estimate vitamins B1, B3 and B6. 0.1 N Sodium hydroxide for B2 and B9, water for B5, B12 and C and 0.10 mL of Ammonia and water for estimating vitamin B7.

Estimation of minerals

Sodium was estimated through AOAC 21st edition 969.23: 2019.[25] Potassium was estimated through AOAC 21st edition 920.87: 2019. Zinc was estimated through extractive spectrophotometry using 1-phenyl-1-hydrazonyl-2-oximino propane-1,2-dione reagent.[26] Selenium content was determined through fluorometry.[27] Iron was estimated through spectrophotometry,[28] Calcium was estimated through titrimetric method[29] and Magnesium was estimated through complexometric titration using EDTA disodium salt.[30]

Estimation of dietary fibre

Total Dietary Fibre residue was estimated through Enzymatic-Gravimetric-Liquid Chromatographic method.[31] 10g of the sample was accurately weighed in 10 mL of phosphate buffer solution (pH 6.6). The mixture was stirred with a magnetic stirrer until the sample was completely dispersed in the solution. 50 µL of α-amylase solution was heated in a water bath at 98-100ºC and was incubated for 30 min. It was then cooled to 60ºC. 100 µL of protease solution was added in a water bath at 60ºC for 30 min. After incubation, the pH was adjusted to 4.8. 100 µL of α-amyloglucosidase solution was added and heated at 60ºC in a water bath for 30 min. After incubation, 200 mL of 95% EtOH was added and kept incubated overnight. The solution was filtered and filtrate was washed twice with 10 mL of 95% EtOH and acetone. The portion was weighed for dietary fibre. Empty crucible was weighed and the filtrate was placed and kept overnight for complete ash at 550ºC. The total dietary fibre was calculated using the below formula:[32]

Total Dietary Fibre = weight (residue) – weight (ash+protein)

Estimation of resistant starch

The resistant starch was calculated using the following formula as mentioned by Parchure and Kulkarni:[33]

Resistant Starch=Glucose (mg) × 0.9

The glucose content was estimated using the 3,5-Dinitrosalicylic acid (DNSA) method.[34] In this, 5 dry test tubes were taken. At the range of 0 to 3 mL, standard sugar solution was pippetted out in different test tubes and the volumes of all test tubes were made up to 3 mL with 0 to 250 mg concentrations of distilled water. 1 mL of DNSA reagent was added in each test tube and the tubes were plugged with cotton and boiled in a water bath for 5 min. They were then cooled to room temperature. The extract was read against the blank at 540 mm

RESULTS

Estimated macronutrients

The macronutrient estimation in 200 mL of unfermented and fermented rice drinks has been presented below.

Table 1: Macronutrient content in 200 mL of Unfermented and Fermented Rice Drinks

Nutrients	Estimated Nutrient Content	
	Unfermented Rice Drink (200 mL)	Fermented Rice Drink (200 mL)
Energy (kcal)	145	162.6
Protein (g)	1.94	2.4
Carbohydrates (g)	35.82	34.88
Dietary Fiber (g)	2.60	19.00
Resistant Starch (g)	5.33	19.73
Fat (saturated and unsaturated) (g)	Below quantifying level (<0.1)	Below quantifying level (<0.1)

Estimated amino acid profile

The amino acid profiling has been tabulated below in 200 mL of both unfermented and fermented rice drinks.

Table 2: Amino Acid Profiling of Unfermented and Fermented Rice Drink (g / 200g).

Sl. No.	Amino Acids	Estimated Amino Acid Content	
		Unfermented Rice Drink (g/200 mL)	Fermented Rice Drink (g/200 mL)
1	Tryptophan	0.00	0.04
2	Aspartic Acid	0.16	0.24
3	Serine	0.12	0.16
4	Glutamic Acid	0.44	0.56
5	Glycine	0.20	0.24
6	Histidine	0.08	0.08
7	Arginine	0.60	0.80
8	Threonine	0.48	0.48
9	Alanine	0.08	0.12
10	Proline	0.32	0.36
11	Cystine	0.40	0.40
12	Tyrosine	0.00	0.00
13	Valine	0.00	0.00
14	Methionine	0.12	0.16
15	Lysine	0.08	0.08
16	Isoleucine	0.08	0.08
17	Leucine	0.16	0.16
18	Phenylalanine	0.16	0.16

The estimation of fat- and water-soluble vitamins in 200 mL of unfermented and fermented rice drinks has been presented below.

Table 3: Estimation of Fat-Soluble and Water-Soluble Vitamins in Unfermented and Fermented Rice Drinks (mg/200 mL).

Sl. No.	Vitamins	Estimated Vitamin Content	
		Unfermented Rice Drink (g/200 mL)	Fermented Rice Drink (g/200 mL)
A	**Fat Soluble Vitamins**		
1	Alpha Tocopherol (Vitamin E)	<0.025	<0.025
2	Cholecalciferol (Vitamin D3)	<0.025	<0.025
3	Ergocholecalciferol (Vitamin D2)	<0.025	<0.025
4	Menaquinone (Vitamin K2)	<0.025	<0.025
5	Phylloquinone (Vitamin K1)	<0.025	<0.025
6	Retinol (Vitamin A)	<0.025	<0.025
B	**Water Soluble Vitamins**		
1	Ascorbic Acid (Vitamin C)	<0.025	<0.025
2	Biotin (Vitamin B7)	<0.025	<0.025
3	Calcium Panthothenic Acid (Vitamin B5)	0.059	0.078
4	Cyanocobalamine (Vitamin B12)	<0.025	<0.025
5	Folic Acid (Vitamin B9)	<0.025	<0.025
6	Nicotinic Acid (Niacin) (Vitamin B3)	<0.025	0.035
7	Pyridoxine Hydrochloride (Vitamin B6)	<0.025	<0.025
8	Riboflavin (Vitamin B2)	0.030	0.036
9	Thiamine Hydrochloride (Vitamin B1)	<0.025	<0.025

Estimated minerals

The estimation of mineral content of 200 mL of unfermented and fermented rice drinks are tabulated below.

Table 4: Estimation of Mineral contents in 200 mL of Unfermented and Fermented rice drinks.

Sl. No.	Minerals	Unfermented Rice Drink (g/200 mL)	Fermented Rice Drink (g/200 mL)
1	Sodium (mg)	189	194
2	Potassium (mg)	337.33	342.67
3	Iron (mg)	1.2	2.53
4	Calcium (mg)	146.67	560
5	Magnesium (mg)	320	320
6	Zinc (mg)	67.85	119.85
7	Selenium (µg)	46.8	73.6

DISCUSSION

It can be inferred from Table 1 that, energy and protein content indicated a slight increase on fermentation, although carbohydrate content was found to be slightly lower in fermented rice drink compared to unfermented rice drink. The carbohydrates present in rice becomes available and easily digestible due to the lactic-acid bacteria, which produces glycoside hydrolase enzyme that helps in the conversion of polysaccharides or the complex indigestible carbohydrates into simpler forms.[35] The starch content in rice facilitates the growth of microbes which may utilize the sugars and thus show a decreased sugar content.[36,37] However, in this study, complex carbohydrates such as dietary fibre and resistant starch has shown a considerable increase after fermentation. A study by Adebo *et al.*[38] has shown an increase in the cellulolytic materials such as lignocellulose, which is a main component in the plant cell walls comprising of fermentable cellulose and hemicellulose. A similar increase in the cellulolytic materials may have contributed to the higher fibre content reflected in this study. A few studies have also indicated an increase in the fibre content of cereal flours, especially when fermented for 72 hr at 22°C.[39-42] As cooking increases the amylose content of the rice, the spatial three-dimension structure formed with amylose molecules increases the formation of resistant starch.[43,44] This could have improved the content of resistant starch in the fermented rice drink. Resistant starch and dietary fibre increase the Short-Chain Fatty Acid (SCFA) contents in the colon that proves to be beneficial to the gut.[45]

The increase in protein could have been due to an increase in the content of some amino acids (as indicated in Table 2) and also an increase in the fermentative microorganisms such as *Saccharomyces cerevisiae* which releases proteolytic enzymes for better digestion of proteins.[35] A similar study on fermented soybean indicated a slight increase in protein content compared to the unfermented counterpart.[46] Since rice is naturally found to be lower in fat content as indicated in various studies,[47] the present study also reflected saturated and unsaturated fats to be less than 0.1 g in both the samples.

On estimation of amino acids (Table 2), certain Amino Acids (AAs) were found to be higher in fermented rice drink when compared with unfermented rice drink. The reason for an increase in amino acid content can be attributed to the release of amino acids and peptides from food due to the microbial degradation of complex proteins after fermentation as suggested in some studies.[48] Among the essential amino acids, lysine, threonine and methionine are the most limiting AAs in rice. This limitation may further lead to reduced absorption and utilization of other AAs.[49-51] Although lysine and threonine remained unchanged after fermentation, methionine showed a slight increase from 0.12 g/100 mL to 0.16 g/200 mL. Methionine is a sulphur-containing, aliphatic AA and is a precursor of homocysteine, creatine, succinyl-CoA, carnitine and creatine. It has been found to regulate the innate immune system, metabolic processes, reduce fatty acid synthesis, counteract oxidative stress by glutathione biosynthesis, digestive functioning and endogenous antioxidant enzyme activation.[52]

The other amino acids that indicated an increase after fermentation includes tryptophan, which helps in synthesis of melatonin and serotonin.[53] Aspartic acid helps in transmitting chemical signals through the nervous system.[54] Serine helps in facilitating a range of cellular functions including protein synthesis, neurotransmission, synthesis of sphingolipids, phospholipids and sulphur-containing amino acids.[55] Glutamic acid helps in transmission of brain signals and aids in learning and memory.[56] Glycine acts as a neurotransmitter and is involved as an antioxidant, anti-inflammatory, cryoprotective and immunomodulatory in peripheral and nervous tissues.[57] Arginine improves cardiovascular, pulmonary, immune and digestive functions and protects against early stages of cancer.[58] Alanine is involved in tryptophan metabolism.[59] Proline contributes in stabilizing sub-cellular structures such as membranes and proteins.[60]

Fat-soluble vitamins were observed (Table 3) to be similar for both fermented and unfermented rice drinks. A considerable increase in the Vitamins B2 (riboflavin), B3 (Niacin) and B5 (Pantothenic Acid) content in the fermented rice drink was observed when compared with unfermented rice drink. The other water-soluble vitamins were found to be present in negligible quantities. Lactic Acid Bacteria and *Bifidobacterium* have been found to produce a range of metabolites such as low-calorie sugar alcohol (sorbitol and mannitol) and B vitamins (folate and riboflavin).[61] Riboflavin-producing-LAB were also reported in three other studies done in India.[62-64] Vitamin B3 has been found to be synthesized from tryptophan by intestinal bacteria such as Bifidobacterium sps. upon fermentation.[65-67] Vitamin B5 was found to be synthesized from simple pathways from β-alanine and 2-dihydropantoate.[67] These vitamins as coenzymes are necessary for the metabolism of macronutrients especially in energy producing pathways.[68]

From the tabulated data (Table 4), it is evident that all of the minerals except magnesium showed an increase after fermentation. An increase in the mineral content could be due to the reduction of phytic acid which otherwise binds to minerals and leads to their poor bioavailability.[69] Studies have indicated an overall reduction in phytic acid on fermentation. Fermentation also leads to an optimum pH which further aids in enzymatic phytate degradation.[70,71] A study by Liang[72] has also indicated a noticeable degradation of starch and phytate by α-amylase and phytase which loosens the mineral embedded complex matrix.

In this study, calcium content was found to be considerably higher in fermented rice drink. It is the most important mineral in the formation of healthy bones and teeth, blood clotting factors and in muscle contraction.[73] Iron, Selenium and Zinc also showed an increase after fermentation. Iron is needed for oxygen transport, DNA synthesis, electron transport and erythropoiesis.[74] Intake of selenium has also been associated with decreased risk of digestive system cancers, depression and improves sperm quality and PCOS symptoms.[75] Zinc is needed for the proper functioning of gastrointestinal, central nervous, immune, skeletal and reproductive systems.[76]

CONCLUSION AND SUMMARY

This study indicated a considerable increase in certain nutrients on fermentation of rice. An improvement in resistant starch and dietary fibre was found after fermentation and this is of significance because of the production of short-chain-fatty acids which have an effect on improving gut health. More fibre in the diet is important in managing blood glucose levels, reduction in serum lipid parameters and thus contributing to the prevention or management of non-communicable diseases. The fermented rice drink was also found to have a higher amount of certain essential amino acids contributing to an increase in the protein content. Micronutrients such as calcium, iron, zinc and B-complex vitamins were found to be higher in fermented rice when compared with unfermented rice. Thus, increase in certain nutrients of fermented rice drink can contribute towards addressing nutritional deficiencies among the vulnerable group Since this drink has been made from a staple cereal source which is readily available and cost-effective, fermented rice drink can be promoted as a natural health food for people across all age groups and belonging to various socio-economic strata.

Credit authorship contribution statement

All authors equally contributed to Conceptualization, Methodology, Formal Analysis,
Investigation, Writing and Visualization, under supervision of the corresponding author.

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ETHICS APPROVAL

This study protocol was presented and approved by the Independent Institutional Ethics Committee of Women's Christian College, Chennai. Protocol Number No. WCC/IEC/2022:02.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

AOAC: Association of Official Agricultural Chemists; **FSSAI:** Food Safety and Standards Authority of India; **HPLC:** High-performance Liquid Chromatography; **DNSA:** 3, 5-Dinitrosalicylic Acid; **LAB:** Lactic Acid Bacteria; **ISO:** International Organization for Standardization; **NABL:** National Accreditation Board for Testing and Calibration Laboratories; **RO:** Reverse Osmosis; **TLC:** Thin Layer Chromatography; **EtOH:** Ethyl Alcohol; **AA:** Amino Acid; **PCOS:** PolyCystic Ovarian Syndrome.

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