




# Preparation of rice fermented food using root of *Asparagus racemosus* as herbal starter and assessment of its nutrient profile

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## Abstract

The popularity of traditional fermented food products is based on their healthiness. The addition of a starter brings consistent, desirable, and predictable food changes with improved nutritive, functional, and sensory qualities. The addition of a mixture of plant residues as a starter or source of microbes is an age-old practice to prepare traditional fermented food and beverages, and most of the reported data on traditional foods were based on the analysis of the final product. The contribution of an individual starter component (plant residue) is not experimentally substantiated for any traditional fermented food, but this data are very essential for the formulation of an effective starter. In this study, *Asparagus racemosus*, which used as a common ingredient of starter for preparation of rice fermented food in the Indian sub-continent, was used as a starter for the preparation of rice fermented food under laboratory scale, and its microbial and nutrient profile was evaluated. The fermented product was a good source of lactic acid bacteria, *Bifidobacterium* sp., yeast, etc. The food product was acidic and enriched with lactic acid and acetic acid with titratable acidity of 0.65%. The content of protein, fat, minerals, and vitamins (water-soluble) was considerably improved. Most notably, oligosaccharide (G3-matotriose), unsaturated fatty acids ( $\omega$ 3,  $\omega$ 6,  $\omega$ 7, and  $\omega$ 9), and a pool of essential and non-essential amino acids were enriched in the newly formulated food. Thus, the herbal starter-based rice fermented food would provide important macro- and micronutrients. They could also deliver large numbers of active microorganisms for the sustainability of health. Therefore, the selected plant part conferred its suitability as an effective starter for the preparation of healthier rice-based food products.

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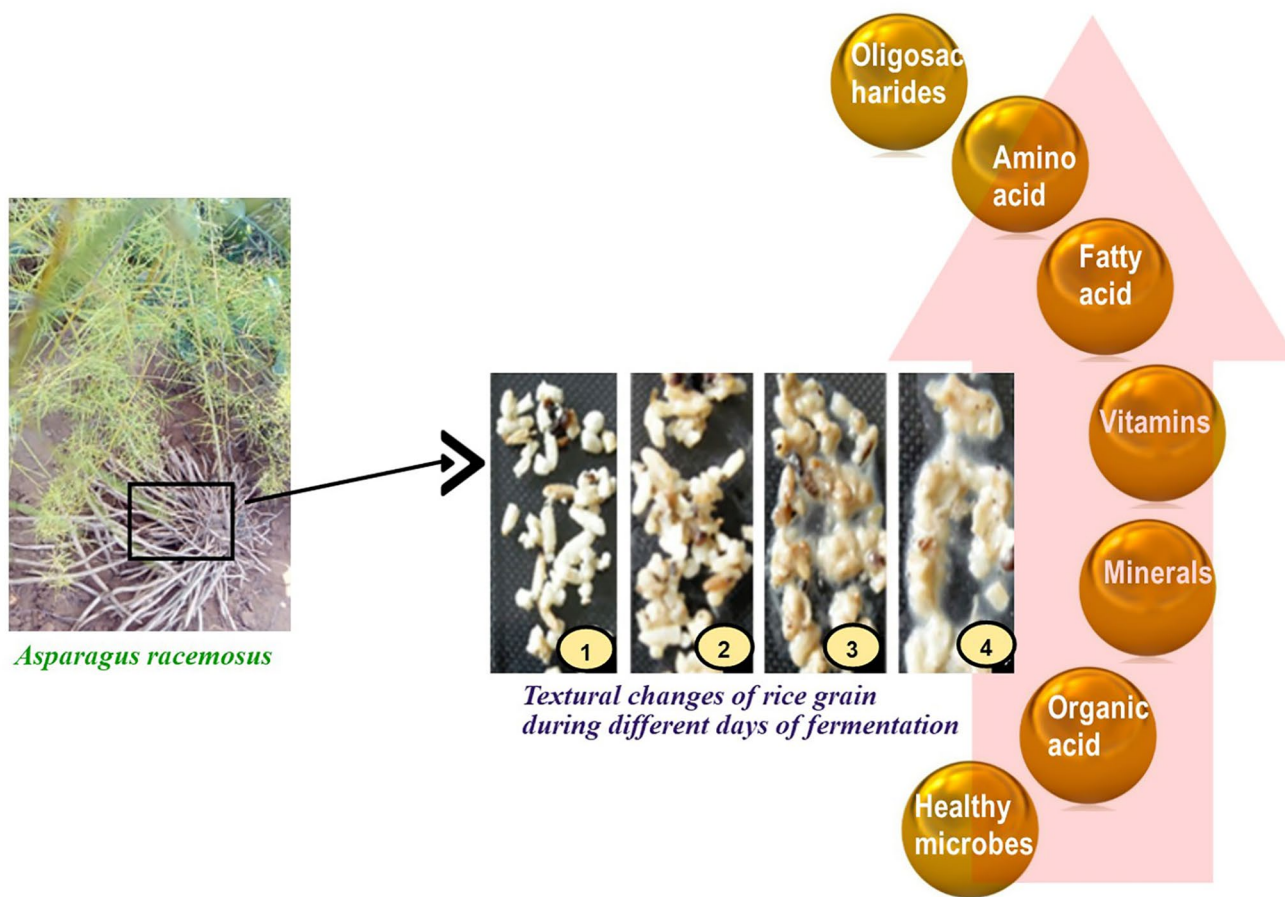
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## Graphic abstract



**Keywords** Food fermentation · Starter · *Asparagus racemosus* · LAB · Bifidobacterium spp. · Macro- and micronutrients

## Introduction

Fermented foods and beverages have long been manufactured with or without starter cultures. The addition of a starter culture brings consistent, desirable, and predictable changes of the food with improved nutritive value and sensory qualities [1]. Traditional methods of starter culture for fermented food preparation include backslopping, mixing a small amount of aged ferment, using the special container, and adding specific natural products containing active microorganisms [2]. These traditional methods facilitated the preparation of individual/varieties of fermented foods and beverages and are still practiced for small- to mid-scale production units, particularly in household-type product manufacturing [3]. In contrast, naturally fermented foods are prone to slow or failed fermentations, contamination, and inconsistent quality.

In some Asian, African, and East European countries, plant residues are used as a starter to prepare varieties of

fermented food and beverages [4–8]. Over the generations, this pioneering practice was followed by the native people to prepare many foods for sustained nutrition to meet nutritional needs. Recent scientific focus indicated that a group of wild microbes in plant parts (as endophytic organisms) could participate in the multi-stage and multi-species fermentation process. Ghosh et al. [9] mentioned the use of 3–7 plant parts (mostly tuber) to prepare *haria*, a very popular rice fermented mildly alcoholic beverage in Central, Eastern, and North-Eastern India. The initial stages (up to 2 days) of fermentation of *haria* is facilitated by the molds that saccharify the boiled rice and decomposed it; therefore, an anaerobic condition is created that favors the growth of lactic acid bacteria and yeasts at the latter stage of fermentation [10]. The beverage contains a mine of nutraceuticals, including phenolics, flavonoids, oligosaccharides, fatty acids, minerals, bioactive peptides, and many others [10–12]. They also believed that this beverage confers protection, particularly against several gastrointestinal disorders, skin, eye, hair,

and heart diseases. A similar type of microbial dynamics in different phases of fermentation was also noted during the preparation of *tarhana*, a popular herbal-based wheat fermented food in Turkey and other neighboring countries [4]. This unique multi-stage fermentation and microbial dynamics cannot be possible by adding old ferment or selective microbes. The tribal people, by their ancestral experience and mastery, selected few plant parts as a starter and added a desirable amount for the preparation of many fermented foods/beverages. To till day, no study has been undertaken to elucidate the role of individual herb/plant residues during food fermentation.

Considering this perspective, the present study dealt with the preparation of a rice fermented food using the root (rhizome) of *Asparagus racemosus* (local name *sata-muli*), a well-recognized ethnomedicinal plant [13] and used extensively for the preparation of many rice-based traditional foods/beverages [9]. This study aimed to explore the potentialities of single plant residues towards preparing nutrient-rich functional food and simultaneously scientifically support the age-old culture of using herbal residues as an adjunct for traditional food preparation. The microbial composition and whole spectra of the nutrient profile were reported to establish this fermented food as health beneficial.

## Materials and methods

### Plant collection and optimization of fermented food preparation

The rhizome of *Asparagus racemosus* was collected from the different locations in lateritic forest of the Jangalmahal area of West Bengal, India. It was repeatedly washed with sterile water, dried under sunlight, and then grounded using a mixture grinder. Whole grain rice (*Oryza sativa*) was procured from the local market and cooked under boiling water for about 45 min (rice grain becomes tender but without any residual water). The rice grain is sun-dried up to its moisture content of around 80%. After that, 100 g of semi-dried rice was kept in an Erlenmeyer flask and autoclaved. Fresh root dust (0.5%, w/w) was mixed with sterilized boiled rice (100 g) and fermented at room temperature for 4 days. Parametric optimization of the fermentation condition was studied following the OVAT (one variable at a time) approach for considerable scale-up of the process. The fermentation process was optimized by varying the amount of starter (0.1, 0.5, 1.0 and 2.0%, w/w), the incubation period (2nd to 5th day), and collected starter from three distinct locations (*Belpahari*, 22.6321° N, 86.7643° E; *Gopiballavpur*, 22.2059° N, 86.8948° E; and *Jhilimili*, 22.8140° N, 86.6384° E) during post-monsoon season. The smell with mild acid-alcoholic aroma was being selected

as the endpoint of fermentation [2]. The fermented rice has been designated as a test sample (Ts). Similarly, a control sample (Cs) was prepared by autoclaving after the addition of an herbal starter.

### Analysis of microbial community

The quantity of the prevalent group of microbes in the food samples (direct sample) was enumerated based on colony-forming units (cfu). The dominant microbes in the fermented food were enumerated using strain-specific selective media (HiMedia, India) [14, 15]. Briefly, 1.0 g of the sample was mixed with 10 ml of phosphate buffer saline (pH 7.2) and used as stock for the microbial count. Total aerobic bacteria were quantified using Plate Count Agar (PCA) media and incubated at 37 °C. The lactic acid bacteria (LAB), *Bifidobacterium* sp. and total anaerobic bacteria were cultured in Rogosa SL agar (supplemented with 0.132% acetic acid), Bifidobacterium agar supplemented with Bifidobacterium Selective Supplement and reduced Wilkins chalgren agar media, respectively, and plates were incubated in a CO<sub>2</sub> incubator (5% CO<sub>2</sub>), at 37 °C. Yeast and mold were enumerated using yeast and mold agar and Potato Dextrose Agar (PDA) media, respectively, and incubated at 30 °C. The mycelial and round convex colonies were recorded for the mold and yeast counts, respectively.

### pH and titratable acidity (TA)

The pH of the fermented product was determined by homogenizing it with sterile distilled water in a ratio of 1:10, followed by shaking for 5 min. The pH of the fermented substrate was then measured by a glass probe digital pH meter (ELCO, India). The standard titration procedure determined titratable acidity according to the method of AOAC [16]. 10 g of sample was dissolved in 90 ml of carbon-dioxide-free distilled water and then titrated by 0.1 N NaOH using phenolphthalein (0.1% w/v in 95% ethanol) as an indicator. The percentage of titratable acidity was calculated as a percentage (% w/v) of lactic acid content, according to the following formula:

$$\begin{aligned} \text{Total titratable acidity (\% of lactic acid)} \\ = \text{ml of 0.1 N NaOH} \times 0.009 \times 100 / \text{sample amount (g)}. \end{aligned}$$

### Proximate analysis

Analysis of moisture, protein, fat, carbohydrate, and ash content of the fermented samples was done using the method employed by AOAC [16].

Moisture content was analyzed by weight difference method (Method No. 925.10)

Ash content was analyzed by combustion method (Method No. 930.05).

Crude protein ( $N \times 6.25$ ) contents were estimated in following the Kjeldahl method (Method No. 978.04).

Crude fat was determined in accordance with the Soxhlet extraction method (Method No. 930.09).

Fibre content was analyzed by method no. 962.09.

Total carbohydrate content was calculated from the above parameters, and total caloric value was evaluated using the “Atwater factor.”

### Analysis of oligosaccharides by TLC and HPLC

The method described by Dreisewerd et al. [17] was employed with slight modification to detect oligosaccharides using thin-layer chromatography (TLC). A commercially available silica gel TLC plate (Merck, India) of 0.2 mm in diameter was used. The sample/standard of 5  $\mu$ l was charged onto the plate, and a solvent system (mobile phase) consisting of *n*-propanol/acetic acid/water in the ratio of 3:2:2 was run on it. After that, 1% arsenal (50%  $H_2SO_4$ , v/v) was sprayed and kept at 110 °C for 15 min for color development. The quantities of malto-oligosaccharides in the food samples were also determined through HPLC described by Ghosh et al. [10]. Food samples were diluted with 50 mM Tris HCl (pH 8.8) at the ratio of 1:3 (W/V) and kept at 4 °C for 1 h followed by centrifugation at 10,000 rpm for 20 min. The collected supernatant was filtered in a 0.22 mm pore size filter and then used for HPLC analysis (Agilent Technology, 1200 infinity series, USA). For this purpose, the carbohydrate- $NH_2$  column was used. The mobile phase was acetonitrile and water at the ratio of 75:25 with a constant flow rate of 1 ml/min. The temperature of the column was maintained at 30 °C. Different concentrations of commercial malto-oligosaccharides (Sigma) and glucose were used as standards in both the chromatography.

### Analysis of fatty acids

Control and test samples (5 g) were mixed intimately with 30 ml of hexane for 5 min and sonicated for 5 min at room temperature. The samples were then vortex for a few seconds and centrifuged at 7000 rpm for 5 min. After that, the upper hexane layer was separated and kept in another tube, and the remaining part was again mixed with hexane, and a similar step was followed. All collected supernatants were next evaporated under a stream of nitrogen. The dry product was dissolved in 1 ml of hexane solution and again kept for drying under the stream of nitrogen. Using triethylamine

and 2-bromo-2'-acetophenone solutions, free fatty acids were derivatized to fatty acid vinyl ester [18]. The reaction was done by incubating the reaction mixture for 15–20 min at 85 °C, followed by the addition of acetone. The extracted fatty acid was separated using an RP-HPLC column (5  $\mu$ m, 250  $\times$  4.6 mm) that was thermostated at 35 °C [18]. The mobile phase was prepared with a combination of methanol and water at a ratio of 75:25. The flow rate was set to 1 ml/min, and the wavelength was set to 250 nm. Fatty acid standards (Sigma) were also run.

### Analysis of amino acids by HPLC

The amino acid composition in the rice fermented product was analyzed following the method of Das et al. [19] with some modification. At first, food samples were kept in a hydrolysis tube mixed with 6 M HCl containing 0.1% phenol and held at 110 °C for 24 h. After that, the residual acid was dried off in a vacuum oven. Then the samples were again suspended in 100 mM HCl and filtered (0.22  $\mu$ m size syringe filter). Next to that, 100  $\mu$ l sample, 900  $\mu$ l of borate buffer (1 M, pH 6.2), and 1 ml of fluorenyl-methyl-oxycarbonyl chloride were mixed for derivatization. The mixture was kept for 2 min, and then 4 ml of *n*-pentane was added by vortexing for 4–5 min. The upper layer was separated and discarded. The lower layer was collected and filtered (0.22  $\mu$ m size syringe filter).

Amino acids were analyzed using the HPLC system (Agilent Technology, 1200 infinity series) equipped with a Zorbax SB-C18 column (5  $\mu$ m beads size; 4.0  $\times$  250 mm). The injection volume was 20  $\mu$ l, and the wavelength detector was used at 265 nm (UV range). The column oven temperature was maintained at 30 °C. The mobile phase used was (A) acetate buffer–acetonitrile (9:1) and (B) acetate buffer–acetonitrile (1:9) with a flow rate of 1.0 ml/min. Different commercially available amino acid standards (SRL, India) were prepared (1 mg/ml) using methanol as a solvent and detected side by side to estimate the unknown concentration [19].

### Organic acid content

Water/salt-soluble extracts of fermented samples were prepared by slightly modifying the method described by Hor et al. [20]. Briefly, 10 g of product was diluted with 30 ml of 50 mM Tris-HCl (pH 8.8), kept at 4 °C for 1 h, and centrifuged at 16,000g for 20 min. The supernatant containing the water/salt-soluble fraction was filtrated through a 0.22-mm pore size filter. The extracts were then analyzed by High-Performance Liquid Chromatography (HPLC) system (Agilent Technology, 1200 infinity series) equipped with a Zorbax SB-C18 column. The elution was carried out using

10 mM H<sub>2</sub>SO<sub>4</sub> as a mobile phase with a flow rate of 0.5 ml/min at 60 °C.

### Estimation of hydrosoluble vitamins

Hydrosoluble vitamins present in the fermented product were extracted following the method of Hor et al. [20]. The extracted water soluble vitamins were then analyzed by reverse-phase High-Performance Liquid Chromatography (RR-HPLC) system (Agilent Technology, 1200 infinity series) equipped with a Zorbax SB-C18 column [20] and the mobile phase was 0.05 M KH<sub>2</sub>PO<sub>4</sub> (pH 2.5) and acetonitrile [20]. The temperature was kept at 15 °C, and with a constant flow rate of 1 ml/min. The effluent from the column was monitored by a UV detector at 250 nm.

### Analysis of volatile compounds

Volatile compounds in the fermented food were extracted by dichloromethane and analyzed by Gas chromatography (Agilent Technology) that equipped a manual injector and a flame ionization detector (FID) [20]. A capillary column HP 5 (30 m × 0.25 mm i.d., 0.25 μm film thickness) was used. The temperature of the injector and detector was both set to 250 °C. The oven temperature was fixed at 50 °C for 5 min, then rise from 50 to 220 °C, at 3 °C/min, and finally 250 °C for 10 min. Nitrogen was used as a carrier gas, and the split vent was set to 13 ml/min. Quantification of volatiles was made by comparing retention time indices of pure standard compounds using Chem Station software [20].

### Free mineral content

The contents of free minerals in the water extracts of the fermented rice product were measured by atomic absorption spectrophotometer (AAS) [Shimadzu Analytical (India) Pvt. Ltd]. Briefly, 5 g of fermented rice sample was dissolved in 25 ml deionized distilled water and homogenized, followed by centrifugation at 12,000 rpm for 10 min. The clear water extracts of the product (supernatant) were subjected to metal analysis by following the standard protocol [21] with some modifications.

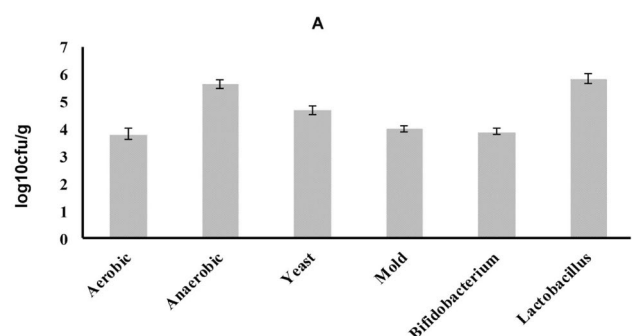
### Statistical analysis

Each experiment was carried out in triplicate, and data were represented as mean ± SE. The significant difference was analyzed by one-way ANOVA, and the t-test was done in all possible pairs using Sigma plot 11.0 (USA) statistical software.

## Results and discussion

### Microbial loads in the fermented rice

For standardization of the batch fermentation, few unique parameters (for traditional food preparation considering the age-old practice) like the amount of starter, incubation period, and location of collected herbal residues were selected and optimized. The sensory attributes, particular flavor, and aroma (mild acid-alcoholic) of rice fermented beverages are good indicators of mature fermented products [2]; therefore, this property has been selected to optimize fermentation conditions. It was noted that 0.5% (w/w) herbal starter and 4th day of fermentation at room temperature were most suitable for rice fermentation. The starter plant from different locations had no significant impact on the quality and consistency of the final food product. The herbal starter instigated rice fermented food was enriched with a group of indicator microbes including total anaerobes ( $3.79 \pm 0.20 \log_{10}$  cfu/g), total aerobes ( $5.64 \pm 0.16 \log_{10}$  cfu/g), yeast ( $4.66 \pm 0.15 \log_{10}$  cfu/g), mold ( $4.01 \pm 0.11 \log_{10}$  cfu/g), LAB ( $3.89 \pm 0.10 \log_{10}$  cfu/g), and *Bifidobacterium* sp. ( $5.82 \pm 0.17 \log_{10}$  cfu/g) after 4th day of fermentation at room temperature with 0.5% (w/w) herbal starter (Fig. 1). It was observed that no notable textural and sensorial changes as well as microbial growth in the control (Cs) sample. After the 4th day of fermentation, the number of anaerobic organisms like yeast, total anaerobes, LAB, and *Bifidobacterium* sp. was surprisingly higher, and these are populated due to the establishment of anaerobic conditions that may create by molds decomposing the rice [10]. Tamang et al. [22] proposed that fermented food containing both functional and non-functional microbes and functional microbes participate in the itemization of the substrate, facilitated the bioavailability of nutrients, produced a mine of bioactive substances that alter the sensory qualities, extended the shelf-life, and promoted the probiotic functions. Thus, fermented food not



**Fig. 1** Enumeration of dominant microbes in the 4th day of fermented rice. Selective media were employed for cultivating the microbes

only provides nutrients to meet hunger but is also curative to many diseases as natural medicine. The abundance of yeast, LAB, and *Bifidobacterium* sp., generally regarded as health-supporting microbes, makes this aforesaid a probiotic food. The succession of these organisms from the plant phyllosphere [23] to the food was first established by this study. These microbes are wild, biologically active, and not altered by the atmospheric agents [24]. Consumption of a large number of microbe (“live and active”)-containing rice fermented food may support and improve health through modulation of gut microbiota.

### pH and titratable acidity and organic acid content

Both titratable acidity (TA) and pH are the measures of acidity of any food. It has been estimated that the pH and TA of the newly formulated rice fermented food were 4.9 and 0.56%. The lowering of pH during fermentation enables the creation of anaerobiosis that facilitated the growth of anaerobic microbes [10, 12] and the induction of a group of enzymes that can disintegrate the food matrix and even dietary fibers [25]. Concerning this, the content of lactic acid and acetic acid were also measured (Table 1), and it indicated that the participatory microbes supported heterolactic fermentation within the rice substrate. Many evidence supported that the produced lactic acid and other metabolites in food could inhibit the growth of intestinal pathogens, immune-stimulatory, cholesterol-lowering, anti-ulcer, anti-tumor, and have anti-allergic activities [10, 11, 25]. Besides, lactic acid has wide applications in food, pharmaceutical, textile, leather industries, and chemical feedstock [26].

### Proximate composition of rice fermented food

The proximate composition includes moisture, fat, protein, crude fiber, carbohydrate, and energy content are essential for dietitians and other health professionals to promote the food. The proximate composition of the rice fermented food was evaluated (Table 1). The content of protein (10.25 g%) and fat (1.3 g%) was notably higher than unfermented milled rice (protein 6.3–7.1 g%, fat 0.3–0.7 g%) [27]. The increase in protein may partly be attributed to simultaneous enhancement of microbial biomass and loss of dry matter of substrate during fermentation [28], while hydrolysis of starch-lipids complex (lipids associated with starch granules) and synthesis of phospholipids content of the cell membrane of fungus are possibly related to the enhancement of fat content in the fermented rice [29]. In contrast, carbohydrate content was lower (62.32 g%) in fermented food than the unfermented (77–89 g%) [27], and this is due to enzymatic decomposition. Evidence also supported that acidification (lactic acid) during fermentation leads to the transformation of rapidly digestible starch (RDS) into slowly digestible

**Table 1** Proximate composition, vitamin, mineral and volatile components in 4th day of rice fermented food

Parameters	Fermented rice sample	
Proximate composition	pH	4.9 ± 0.03
	Moisture (%)	25.05 ± 1.52
	Protein (g%)	10.25 ± 0.76
	Carbohydrate (g%)	62.34 ± 2.73
	Fat (g%)	1.3 ± 0.02
	Crude fiber (g%)	1.06 ± 0.02
	Total energy (kcal/100 g)	295
Acidity	Titratable acidity (%)	0.65 ± 0.03
	Lactic acid (mg/g)	0.21 ± 0.06
	Acetic acid (mg/g)	0.13 ± 0.04
Vitamins	Vitamin B <sub>12</sub> (mg/g)	0.25 ± 0.02
	Folic acid (mg/g)	1.29 ± 0.65
	Riboflavin (mg/g)	0.03 ± 0.006
	Thiamine (mg/g)	0.41 ± 0.06
	Vitamin C (mg/g)	0.98 ± 0.04
Volatile compounds	Methanol (ml/g)	0.004 ± 0.001
	Propan-2-ol (ml/g)	0.032 ± 0.02
	Butan-1-ol(ml/g)	0.08 ± 0.004
	Fatty alcohol (ml/g)	0.65 ± 0.007
Minerals	Ca <sup>2+</sup> (ppm)	5.12 ± 0.34
	Mg <sup>2+</sup> (ppm)	4.08 ± 0.22
	Fe <sup>2+</sup> (ppm)	0.11 ± 0.02
	Zn <sup>2+</sup> (ppm)	0.68 ± 0.04
	Mn <sup>2+</sup> (ppm)	0.15 ± 0.02

starch (SDS), thereby, improved glycemic index [20]. The crude fiber content was unaffected, but the total energy content was lower in fermented rice than the milled rice. The lowering of the energy content is very related to the content of the carbohydrate. Thus, the microbial interplay makes the fermented rice more nutritious and healthy than the unfermented.

### Analysis of vitamins, minerals, and volatile compounds

Vitamins like vitamin B<sub>12</sub>, folic acid, riboflavin, thiamine, and vitamin C were present in a considerable amount in rice fermented food (Table 1). The content of vitamins mentioned above in the fermented rice was substantially higher than boiled rice [30] and more appropriate than the Recommended Daily Allowance (RDA) level for Indian people [31]. Among them, the content of folic acid and vitamin C was notably improved in the fermented rice. Folic acid (Vit 9) is involved in the DNA synthesis of growing cells and the repairing process. The pregnant women of developing countries are mostly deficient in folic acid, and its RDA is 75–150 µg, and this deficiency can be easily compensated by

the consumption of fermented rice (as it contains 1.29 mg/g). Different strains of LAB and yeasts are the natural producer of water-soluble vitamins, and most of the reported rice fermented foods are enriched with this type of vitamin [20, 21].

The contents of free calcium, magnesium, iron, zinc, and manganese in the rice fermented food were analyzed and represented in Table 1. Considering the data, this newly formulated herbal starter-based rice fermented food can be designated as calcium–magnesium-rich food. Fermentation of rice leads to dephytinization (by microbial enzyme phytase) and bioaccessibilities of minerals [29, 32, 33].

The aroma of a food principally depends upon the content of volatile organic compounds (VOCs) [20]. Different alcohol-based volatile compounds were analyzed in both foods and found that only methanol, propan-2-ol, butan-1-ol, and fatty alcohol were present in very little quantity in fermented food. Banik et al. [33] described that a yeast *Saccharomyces cerevisiae* isolated from fermented rice beverage was able to synthesize 38 volatile compounds including 15 acids, 6 alcohols, 7 ketones, 1 aldehyde, 1 sterol, 3 esters, alkane, and 4 others.

### Carbohydrate fractions in the fermented food

After 4 days of fermentation, accumulation of only maltooligosaccharides (G3/maltotriose) was detected in the fermented food (Ts), but no such oligosaccharide was found in the unfermented sample (Fig. 2). The fraction was also confirmed by HPLC analysis, and it remained in 1.7 mg/g of ferment. In a starchy environment, participating microbes probably liberated a group of amylolytic or carbohydrate-active enzymes (CAZymes), which sequentially hydrolyzed the rice starch and produced simple sugars such as mono disaccharides were assimilated by the microbes leaving behind

the comparatively larger ones. Ghosh et al. [10] also noted maltooligosaccharides (G3 and G4) in the end products of rice fermented beverages. These maltooligosaccharides have multifaceted health benefits, particularly promoted the growth of indigenous gut flora whose metabolic end product is a short-chain fatty acid (SCFA), induced mucine production, stimulated gut-associated lymphoid tissues (GALTs), etc. [10, 34].

### Fatty acid composition

A comparative account of the fatty acid profile of fermented (Ts) and unfermented (Cs) samples has been given in

**Table 2** Analysis of fatty acid component of unfermented (Cs) and fermented rice (Ts) sample ( $\mu\text{g/ml}$ )

Fatty acids	Cs	Ts
Methyl octanoate	Not detected	67.90 $\pm$ 0.73
Methyl dodecanoate	Not detected	45.22 $\pm$ 0.92
Methyl myristate	69.83 $\pm$ 1.32 <sup>a</sup>	58.75 $\pm$ 1.19 <sup>b</sup>
Methyl pentadecanoate	Not detected	15.79 $\pm$ 0.69
Methyl palmitate	892 $\pm$ 1.70 <sup>b</sup>	1022.87 $\pm$ 3.14 <sup>a</sup>
Methyl palmitoleate	14.88 $\pm$ 0.31 <sup>b</sup>	35.42 $\pm$ 1.01 <sup>a</sup>
Methyl heptadecanoate	18.35 $\pm$ 0.78 <sup>b</sup>	60.76 $\pm$ 1.34 <sup>a</sup>
Methyl stearate	76.83 $\pm$ 0.63 <sup>b</sup>	95.83 $\pm$ 0.34 <sup>a</sup>
Methyl octadecenoate	16.59 $\pm$ 0.60 <sup>b</sup>	46.04 $\pm$ 0.68 <sup>a</sup>
Methyl oleate	17.92 $\pm$ 0.29 <sup>b</sup>	842.10 $\pm$ 1.93 <sup>a</sup>
Methyl linoleate	996.23 $\pm$ 1.68 <sup>b</sup>	2108.14 $\pm$ 3.66 <sup>a</sup>
Methyl eicosenoate	Not detected	25.02 $\pm$ 0.96
Methyl linolenate	37 $\pm$ 1.37 <sup>b</sup>	120.34 $\pm$ 1.17 <sup>a</sup>

Data in same row with different superscript (alphabets) indicated the significant difference ( $p < 0.05$ )

**Fig. 2** Analysis of maltooligomers (G1: glucose, G2: maltose, G2: malto-triose, G3: malto-tetraose) using TLC method where sample wells were marked as standard, 'Cs' and test 'Ts' (A). HPLC chromatogram of standard maltooligomers (B). The concentration (1.7 mg/g extracts) of maltooligosaccharide in the 'Ts' sample was analysed from of area of HPLC chromatogram using the corresponding standards

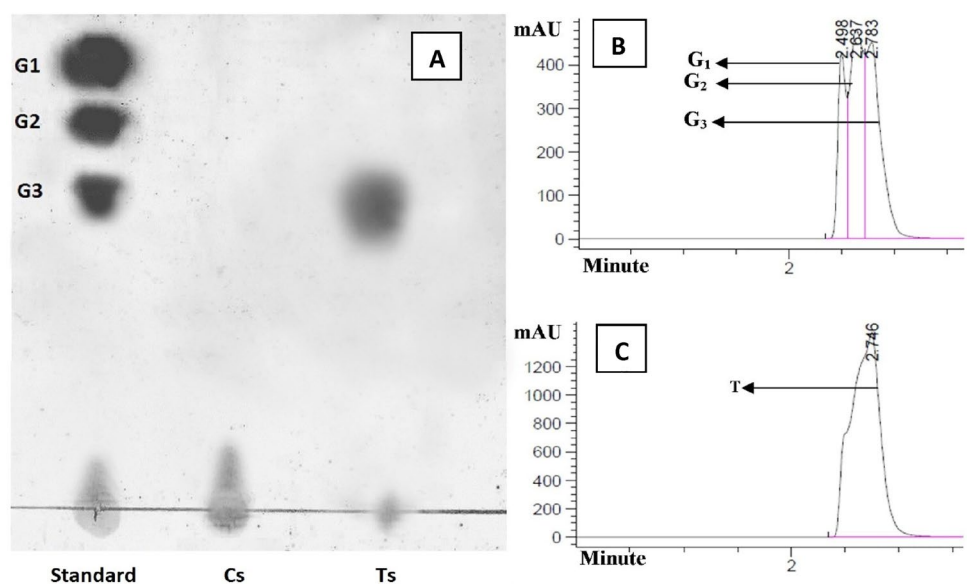


Table 2. A group of fatty acids was newly evolved, and concentrations of many healthy fatty acids were also improved due to fermentation. The fatty acids such as octanoate, dodecanoate, pentadecanoate, and eicosenoate were absent in the Cs, but their concentrations increased in many folds in the fermented product. Interestingly, the concentrations of health-beneficial unsaturated fatty acids such as linolenate ( $\omega 3$ ), linoleate ( $\omega 6$ ), palmitoleate ( $\omega 7$ ), and eicosenoate ( $\omega 9$ ) were improved 3.2, 2.1, 2.3, and 25 folds, respectively, during fermentation of rice. dos Santos Oliveira et al. [29] mentioned that fermentation of whole rice bran leads to enhancement of phospholipids with a reduction in saturated fatty acid (20%) and enhancement of unsaturated fatty acids (5%) that enriched with  $\omega 3$  (1.3 folds),  $\omega 6$  (1.18 folds) and PUFA (1.07 folds). A perusal of literature also revealed that mold and yeast could synthesize fatty acids, and it is encumbered by slow growth and strictly anaerobic culture conditions [12, 35]. It was also suggested that fatty acids of various chain lengths are important nutraceuticals that improve glycemic and lipid profile, promote the function of the brain and nervous system [36]. Besides, unsaturated fatty acid could intensify the production and secretion of intestinal alkaline phosphatase, which might suppress lipopolysaccharides (LPS; a component of the Gram-negative bacterial cell wall)—producing microbes such as *Proteobacteria*, therefore, prevented metabolic endotoxemia and systemic inflammation, relevant to obesity and diabetes like metabolic diseases [37]. Recently, Aryan et al. [38] mentioned that arachidonic acid, eicosapentaenoic acid, and docosahexaenoic acid are enigmatic chemicals that could prevent the multiplication of coronavirus. Our results demonstrate that the enrichment of different nutraceuticals in rice was due to the interaction of active microbes and not by the phytochemicals from plant residue (Cs).

### Amino acid enrichment

The fermented rice was enriched with a pool of essential (leucine, histidine, lysine, methionine, phenylalanine, and valine) and non-essential (arginine, serine, aspartic acid, glutamic acid, glycine, alanine, tyrosine, and proline) amino acids. Their quantity surprisingly improved during fermentation of rice with the herbal starter (Table 3). A significant portion of the rural population of least developed and developing countries are at risk of quality protein inadequacy. Lysine is now being considered as an indicator of the quality of protein. Considering this point of view, the studied fermented rice can be categorized as a quality food product with a balanced composition of essential and dispensable amino acids. Holzapfel [39] mentioned that LAB fermentation in cereal-based foods improves concentrate and enhances nutrients like minerals, vitamins, and essential amino acids. Similarly, yeast-enriched food also showed

**Table 3** Amino acid profile of unfermented and fermented rice

Amino acids	Concentration (mg/g)	
	Unfermented (Cs)	Fermented (Ts)
Arginine	0.56 ± 0.04 <sup>a</sup>	1.23 ± 0.36 <sup>b</sup>
Serine	0.13 ± 0.02 <sup>a</sup>	0.46 ± 0.02 <sup>b</sup>
Aspartic acid	1.29 ± 0.26 <sup>a</sup>	4.12 ± 0.65 <sup>b</sup>
Glutamic acid	0.67 ± 0.15 <sup>a</sup>	1.45 ± 0.28 <sup>b</sup>
Glycine	0.22 ± 0.02 <sup>a</sup>	0.87 ± 0.03 <sup>b</sup>
Alanine	Not detected	0.14 ± 0.02
Tyrosine	1.12 ± 0.19 <sup>a</sup>	5.34 ± 0.52 <sup>b</sup>
Proline	0.065 ± 0.002 <sup>a</sup>	0.45 ± 0.03 <sup>b</sup>
Methionine	Not detected	0.19 ± 0.02
Valine	0.091 ± 0.002 <sup>a</sup>	0.55 ± 0.06 <sup>b</sup>
Phenylealanine	Not detected	0.26 ± 0.04
Leucine	0.082 ± 0.003 <sup>a</sup>	0.38 ± 0.02 <sup>b</sup>
Histidine	0.40 ± 0.02 <sup>a</sup>	1.12 ± 0.56 <sup>b</sup>
Lysine	0.032 ± 0.001 <sup>a</sup>	0.29 ± 0.02 <sup>b</sup>

Data in same row with different superscript (alphabets) indicated the significant difference ( $p < 0.05$ )

positive nitrogen balance with a large number of free amino acids [28]. Our results are consistent with other traditional rice fermented beverages like apong, jou, judima, etc. [32].

### Conclusion

The most extended lifespan of the people of Okinawa islands (islands known as the “land of the immortals”) of Japan is primarily due to their traditional healthy food culture. Conventional fermented food occupied a leading part of their dishes. Native people have the traditional knowledge about the preparation process, nutrient and medicinal values of the traditional fermented food. In food processing, plant parts (as substrate or adjunct) serve as the source of wild, bioactive, and healthy microbes, which facilitated the fermentation by liberating a group of enzymes and bio-embolden the substrate with varieties of health-promoting micronutrients, phytochemicals, and other functional components. This experimental study explored that rice fermentation with the selective herbal starter (rhizome of *Asparagus racemosus*) enriched rice with mine of nutraceuticals like vitamins, minerals, oligosaccharides, essential fatty acids, and a pool of amino acids. Thus, fermentation is a useful process strategy to improve the nutritional quality of this low-cost and abundant substrate. The technological advancement of this experiment is that the fermentation was controlled as a precise quantity of starter (selected amount of herbal residues) was inoculated, and processing was optimized towards a large-scale basis. However, experimental trials and validation of



the health impacts are essential to explore this nutrient-rich rice fermented food into a functional one.

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## Declarations

**Conflict of interest** The authors do not have any conflict of interest to declare.

**Consent for publication** The trial was conceived by PH, DG and KM. KM designed the study. PH, DG, and MT conducted the research and analyzed the data. SKM and KM prepared the manuscript. KM had the primary responsibility of the final content. All authors read and approved the final manuscript. All authors have agreed to submit the manuscript to SMAB for eventual publication.

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