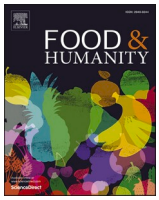
Food and Humanity 5 (2025) 100707

Contents lists available at ScienceDirect

Food and Humanity

journal homepage: www.editorialmanager.com/foohum/journal_overview.html



Development of a fermented rice product (Pachoi): Evaluation of vitamin and mineral composition, microbiological investigation and shelf-life stability

Laky Khatun [a,1], Subhajit Ray [a,*,2], Rangina Brahma [a,3], Dulal Chandra Boruah [b,4]

[a] Department of Food Engineering &Technology, Central Institute of Technology Kokrajhar, Kokrajhar, BTAD, Assam 783370, India
[b] Department of Botany, Goalpara College, Goalpara, Assam 783121, India

ARTICLE INFO

Keywords:
Fermentation
Minerals
Vitamin B
Microbial count
Shelf-life
Lactic acid bacteria

ABSTRACT

In Northeast India, fermented rice consumption is very much popular. However, there is an absence of proper scientific literature dealing with this rice product in particular. Pachoi is a traditional fermented rice product commonly consumed by Bengali Muslim community in Assam, India. In this study, Pachoi was prepared in two ways utilizing ahu kalogoria rice and were assigned as FRP1(traditional) and FRP2(optimized). This study was aimed to examine the minerals, vitamin B levels, biochemical constituents, microbiological count, and shelf-life of the rice products viz. FRP1 and FRP2. It is examined that FRP2 exhibits greater shelf stability than FRP1. Both varieties showed significantly higher magnesium concentrations after fermentation, with values of 23.66 ± 0.47 ppm and 23.29 ± 0.06 ppm, compared to the control samples FRP1 and FRP2 before fermentation, which had magnesium levels 14.04 ± 1.04 and 14.37 ± 0.56 ppm respectively. Among the B-complex vitamins, Vitamin B5 exhibited the highest concentration, with values of 90.171 ± 0.21 ppm and 92.568 ± 1.43 ppm respectively in both fermented samples. Magnesium plays a crucial role in muscle and nerve function, energy production, and bone health, while vitamin B5 (pantothenic acid) is essential for synthesizing coenzymes, supporting metabolism, and maintaining healthy skin and hormone levels. In FRP1 and FRP2, lactic acid bacterial count after 24 h period was observed as 6.16 ± 0.16 $\log_{10}$CFU/g and 10.65 ± 0.20 $\log_{10}$CFU/g. The highest total plate count was reported on the 12th day in both samples, 9.43 ±.0.20 $\log_{10}$CFU/g in FPR1 and 9.38 ± 0.13 $\log_{10}$CFU/g in FRP2. Experimental study revealed that the amount of protein, fat, and carbohydrates decreased with the increase of fermentation period. After increasing for a while, the total microbial, yeast, and mold counts begins to decline. The coliform count was detected in FRP1 and FRP2 on day 36th and day 39th respectively.

1. Introduction

Pachoi is a traditional rice-based fermented beverage consumed in various regions of India, particularly among tribal and rural communities in Jharkhand, Odisha, and West Bengal. It holds cultural significance as both a ceremonial and everyday drink, often produced using indigenous starter cultures comprising a complex microbial consortium (Tamang et al., 2016). Despite its widespread traditional use, *Pachoi* remains largely unexplored in scientific literature, especially in terms of its microbial composition, nutritional properties, and potential as a functional food.

Fermented rice products like *Tape Ketan* (Indonesia) and *Haria* (India) have been studied for their probiotic potential, organic acid content, and antioxidant properties (Nuraida, 2015; Das et al., 2020),

Abbreviations: FRP, fermented rice product; HPLC-DAD, high performance liquid chromatography diode array detector; KH_2PO_4, potassium dihydrogen phosphate; PH, the negative log of the hydrogen ion concentration; FRP1, Fermented rice product 1; FRP2, Fermented rice product 2.

* Corresponding author.
E-mail address: subhajit@cit.ac.in (S. Ray).
[1] https://orcid.org/0000-0003-2906-6698
[2] https://orcid.org/0000-0003-0081-9836
[3] https://orcid.org/0000-0002-5707-0072
[4] https://orcid.org/0000-0001-7423-1555

https://doi.org/10.1016/j.foohum.2025.100707
Received 17 April 2025; Received in revised form 1 July 2025; Accepted 7 July 2025
Available online 8 July 2025

yet similar scientific attention has not been directed toward *Pachoi*. A preliminary literature search reveals a lack of peer-reviewed studies specifically characterizing *Pachoi*, highlighting a clear research gap in the documentation and evaluation of this traditional food. Investigating such indigenous fermented food is essential not only for cultural preservation but also for unlocking new nutritional, functional and commercial opportunities.

Fermentation is an ancient food processing method that has been used across cultures for centuries, especially in developing countries (Adebo et al., 2022). It is a natural biochemical process that alters the composition of food through the action of microorganisms under controlled conditions. Proteins are broken down into peptides and amino acids by microbial enzymes, while carbohydrates such as starch and sugars are converted into simpler compounds, including alcohols and organic acids (Sharma et al., 2020). This transformation not only enhances the organoleptic properties of food—such as taste, smell, and appearance—but also improves its nutritional value, digestibility, and shelf life (Sharma et al., 2020).

Cereal grains such as rice, wheat, corn, and sorghum are staple foods worldwide, particularly in the Asia-Pacific region. These grains serve as a major source of energy, protein, and micronutrients. Fermentation of cereals has been shown to increase their nutritional content and reduce antinutritional factors, thereby improving their health-promoting properties. Traditional fermented cereal products are widely consumed in many Asian communities. Among them, fermented rice-based foods and beverages hold cultural and nutritional importance (Ghosh et al., 2015).

Fermentation plays a critical role in improving the bioavailability of nutrients such as magnesium, iron, calcium, and zinc by reducing phytate levels, a common antinutritional factor in cereals and legumes (Nkhata et al., 2018). In addition, fermentation can enhance the content of vitamins, amino acids, and antioxidants. Whole cereals also contain phytochemicals—such as phenolic acids, flavonoids, fiber, and vitamins—that contribute to reducing the risk of chronic illnesses like diabetes, cardiovascular disease, and metabolic syndrome (Wang et al., 2014).

Lactic acid bacteria (LAB) are key players in the spontaneous and controlled fermentation of plant-based foods, including cereals. These gram-positive bacteria contribute significantly to food preservation by lowering pH through lactic acid production, which inhibits the growth of spoilage and pathogenic microorganisms. LAB are also known for their probiotic potential and health benefits, including enhancing gut microbiota, boosting immune function, and producing bioactive compounds (Mangang et al., 2017).

In cereal fermentations, especially those involving rice, LAB dominate the microbial community and drive the transformation of raw materials into nutritionally superior products. They contribute to the release of free amino acids, improvement in mineral bioavailability, and synthesis of B-complex vitamins, notably B5 and B12 (Ilango & Antony, 2021; Mishra et al., 2022).

This study investigates two fermented rice products—FRP1 and FRP2. FRP1 was prepared under natural fermentation conditions, allowing the native microflora, primarily LAB and yeast, to drive the fermentation process. In contrast, FRP2 was produced under specified conditions to ensure more controlled microbial activity. Both products were subjected to biochemical and microbiological analysis to assess their nutritional quality and safety. In addition to biochemical profiling, the shelf life of both FRP1 and FRP2 was evaluated through bacterial count and storage stability. The focus was placed on assessing LAB populations due to their crucial role in preserving the products and enhancing their functional properties. The microbial counts and nutrient levels over time provided insights into the comparative stability and nutritional advantages of naturally versus controlled fermented rice products.

This study aims to develop and compare two Pachoi variants for their nutritional, functional, microbiological, sensorial and shelf-life properties.

2. Materials and method

Ranjit rice and Ahu Kalogoria Paddy rice was collected and then followed by germination of Ahu kalogoria paddy.

2.1. Germination of Ahu Kalogoria Rice and preparation of flour

The Ahu Kalogoria paddy was cleaned with water and then steeped in distilled water (1:2) for 12 h at a room temperature (30–32 °C). After 12 hrs the paddy separated from water and put on a damp cloth that was kept in the dark. Next, to keep it wet, cover it with the jute bag and lay the banana leaves on top of it. Water was sprayed on the grains during the germination phase to keep them in a humid environment (Khatun et al., 2023) The roots and shoots grew to be 3–5 cm and 0.5–1.5 cm in length after 5 days of germination. Grain germination was then stopped by sun drying. To remove the outer layer and split the roots and shoots of rice grains, ahu grains that have germinated has been processed in a husking peddle, or Dheki, are used for threshing. Then, using a bamboo-built winnowing fan known as a Kula in Assamese, the rice was separated from the mixture. Then the rice powdered into flour and the mixture of husk kept for further use.

2.2. Preparation of fermented rice product FRP1 and FRP2

To prepare the fermented rice product FRP1, 5 g of flour made from powdered germinated Ahu Kalogoria rice, 0.5 g of salt, 7 g of sugar, and 100 g of cooked Ranjit rice were thoroughly mixed. The use of germinated Ahu Kalogoria rice flour aimed to enhance the nutritional value and fermentability of the mixture by providing additional carbohydrates and bioactive compounds.

Separately, 8 g of husk derived from germinated Ahu Kalogoria rice were soaked in 30 millilitres of distilled water for 30 min. This soaking process allowed the release of water-soluble nutrients and microbial metabolites from the husk. The mixture was then filtered to obtain a nutrient-rich husk filtrate, which was combined with 3 millilitres of homogenized toned milk (commercially procured in the Goalpara district of Assam, NER, India). The resulting filtrate–milk mixture was then added to the initial blend of flour, salt, sugar, and cooked rice. The complete mixture was stirred well, transferred to a 500 ml beaker, covered with a petri plate, and allowed to ferment at a temperature of 30–32 °C for 24 h. The product obtained was designated as FRP1.

For the preparation of FRP2, all ingredient quantities and procedures remained the same as those used for FRP1, except for the fermentation conditions. In this case, the mixture was fermented at a lower temperature of 25 °C, and the pH was carefully adjusted to 5.4 prior to fermentation. To achieve this, food-grade lactic acid was added dropwise to the mixture while continuously monitoring the pH with a pH meter. This controlled acidification step helped to establish optimal conditions for the selective growth and metabolic activity of lactic acid bacteria (LAB). The mixture was then allowed to ferment for 24 h under these conditions to produce FRP2. This pH level was selected based on existing literature indicating that lactic acid bacteria (LAB), particularly Lactobacillus spp., exhibit optimal growth and metabolic activity in slightly acidic conditions, typically between pH 5.0 and 6.0. Maintaining pH at 5.4 helps to both favor the growth of beneficial LAB and inhibit the proliferation of undesirable spoilage microorganisms.

The temperature of 25°C was chosen to simulate ambient fermentation conditions common in traditional food fermentation processes, especially in resource-limited settings. This temperature is also within the optimal growth range for many mesophilic LAB strains, balancing microbial activity with energy-efficient processing. Other environmental parameters, such as humidity, were not specifically controlled, as the fermentation was conducted in a laboratory incubator under static conditions.

2.3. Determination of minerals

The mineral content of the samples was determined using Atomic Absorption Spectrometry (AAS), following acid digestion. The digestion process was carried out using a mixture of nitric acid and hydrochloric acid in a 2:1 ratio, as described by Uddin et al. (2006). Specifically, 5 millilitres of 65 % nitric acid were added to each sample, and the mixture was gently heated for 30–45 min. After cooling, 2.5 millilitres of 70 % hydrochloric acid were added, and the solution was again heated until the appearance of dense white fumes indicated complete reaction.

Once cooled, 10 millilitres of deionized water was added to the mixture, which was then reheated to boiling until all vapours were released. The digested samples were filtered using Whatman No. 42 filter paper (2.5 μm particle retention). To minimize sample loss, the inner walls of the digestion beakers were rinsed with 2 millilitres of deionized water before filtration. The filtrate was then diluted with deionized water to a final volume of 50 millilitres.

Mineral determination was carried out using a PerkinElmer AAnalyst 400 Atomic Absorption Spectrometer (or specify the model used), calibrated with standard solutions of known metal concentrations.

2.4. Estimation of Vitamin B

For sample extraction5 g of ground powder, precisely weighed, should be added to 25 ml of water. Samples were ultrasonically extracted for 45 min, and then centrifuged at 4500 rpm. Following the filtration of the supernatant using a 0.4μm filter, the samples were introduced into the HPLC apparatus (Hor et al., 2021). Using an HPLC-DAD with a column Shim-pack 4.6 mm × 150 mm, 3μm, the vitamin B complex content of sample was examined. The mobile phase contained 25 mM KH2PO4 (pH-7) (solvent A) and 50:50 acetonitrile: methanol (solvent B), with an injection volume of 20 μl and a flow rate of 0.45 ml/min. The temperature of the column was kept constant at 35°C.A UV detector operating at 250 nm monitored the column's effluent (Hor et al., 2022). Quantification of Vitamin B complex (B1, B5, B6, and B12) in samples FRP1 and FRP2 was performed using High Performance Liquid Chromatography (HPLC). Each sample underwent three independent extractions, followed by individual HPLC injections and analysis for each replicate. The vitamin concentrations reported in Table 2 represent the mean ± standard deviation (SD) of these triplicate measurements. This approach ensured statistical reliability and reproducibility of the data.

2.5. pH analysis

The pH scale serves as a crucial measurement in analytical chemistry, defining the level of acidity or alkalinity across a range from 0 to 14, with neutrality indicated by a central value of 7. Acidic substances have pH readings below 7, whereas alkaline substances possess values exceeding 7. Using an advanced digital pH meter like the OAKTON pH Testr30 from Vernon Hills, Illinois, pH readings are precisely obtained after thorough calibration with a series of buffer solutions covering pH 4 and 7. Following standardized procedures with little modifications, as outlined by Vivek et al. (2018) guarantees precision and reliability in measurements. The glass sensor of the pH device was immersed in the sample, which was poured into a sterile glass container at room temperature, and left to equilibrate for a while. Prior to taking measurements, the digital pH meter was validated for accuracy using standard buffer solutions at pH 4.0 and 7.0. The glass probe was thoroughly rinsed with distilled water and carefully dabbed dry with a lint-free cloth. It was then submerged into the prepared sample mixture, ensuring it did not come into contact with the walls or bottom of the container. The pH reading was recorded after it stabilized, noted with a precision of up to two decimal places. All samples were analyzed in triplicate, and the mean value was used for final reporting.

Table 2
Vitamin B complex analysis in FRP1 and FRP2.

Sample name	Vitamin B	Ret.time	Area	Amount (ppm)
FRP1	B1	4.827	1624034	21.539 ± 0.08
	B5	4.220	4082712	90.171 ± 0.21
	B6	4.827	13273333	79.259 ± 0.36
	B12	4.827	12948796	68.380 ± 0.23
FRP2	B1	4.840	1517779	20.129 ± 0.14
	B5	4.247	4191233	92.568 ± 1.43
	B6	4.840	11059449	66.039 ± 0.56
	B12	4.840	12127318	64.042 ± 0.69

Vitamin B complex concentrations are presented as mean ± standard deviation (SD), calculated from triplicate independent measurements.

Table 3
Microbial counts for FRP1 and FRP2.

Sample Code	Plate counts (log $_{10}$CFU/g)	Yeasts (log $_{10}$CFU/g)	Molds (log $_{10}$CFU/g)	Lactic acid Bacteria (log $_{10}$CFU/g)
FRP1	6.44 ± 0.14	6.27 ± 0.17	Not Detected	6.16 ± 0.16
FRP2	5.80 ± 0.20	4.55 ± 0.15	3.77 ± 0.17	10.65 ± 0.20

Microbial count is presented as mean ± standard deviation (SD), calculated from triplicate independent measurements

2.6. Estimation of alcohol content of FRP1 and FRP2

The quantitative determination of ethanol was conducted using the potassium dichromate technique (Crowell & Ough, 1979). 10–50 microliters of pure alcohol were placed in separate test tubes as a standard, and the total volume was adjusted to 500 microliters by adding distilled water. 30 microliters of test samples (juice) were measured, and the volume was brought to 500 microliters by incorporating distilled water. 1 milliliter of potassium dichromate solution was introduced into each test tube, followed by 2 milliliters of sodium hydroxide solution, and the mixture was incubated at 50°C for 30 min. After incubation, the absorbance was determined at a wavelength of 600 nanometers using a spectrophotometer. Finally, a graph was plotted to calculate the ethanol concentration.

Table 1
Mineral contents in FRP1 and FRP2.

Control sample (Before fermentation)	Fe	Mg	Zn	Ca
FRP1	0.07 ± 0.015^{a}	14.04 ± 1.035^{a}	0.37 ± 0.01^{a}	3.53 ± 0.50^{a}
FRP2	0.082 ± 0.025^{a}	14.37 ± 0.560^{a}	0.394 ± 0.01^{a}	3.49 ± 0.46^{a}
Sample (Fermented)	Fe (ppm)	Mg(ppm)	Zn (ppm)	Ca(ppm)
FRP1	0.11 ± 0.01^{a}	23.66 ± 0.47^{a}	0.48 ± 0.02^{a}	5.07 ± 0.01^{b}
FRP2	0.16 ± 0.02^{b}	23.29 ± 0.06^{a}	0.65 ± 0.02^{b}	4.15 ± 0.005^{a}

The values are expressed as means ± SD of triplicate assays, and the values with different superscripts (a,b,) indicates that they are significantly different ($p < 0.05$).

2.7. Sensory qualities of FRP1 and FRP2

Rice product sample FRP1 and FRP2 were subjected to sensory analysis by 10 panelists. The judges rated the quality characteristics of each sample on a nine-point hedonic rating scale with 9 = like extremely, 8 = like very much, 7 = like moderately, 6- like slightly, 5 = neither like nor dislike, 4 = dislike slightly, 3 = dislike moderately, 2 = dislike very much and 1 = dislike extremely. The judge evaluated randomly coded samples in terms of appearance, colour, acidity, taste, flavour and overall acceptability. Product evaluation was carried out under daylight illumination (Stone et al.,2021)

2.8. Analysis of microbial community

2.8.1. Total plate count

The total plate count, sometimes also referred to as the total microbial count, is probably the most widely used technique for evaluating microorganisms in foods. The purpose, as its name implies, is to estimate the number of viable microorganism cells in a given sample of food.A flask containing 225 ml of sterile water was homogenized after 25 ml of well-mixed samples were added individually. Following homogenization, 1 millilitre of each sample was carefully placed into 9 millilitres of sterile water, completely mixed with a vortex mixer, and labelled as 10^{-1}. Subsequent dilution was carried out, and all test tubes were labelled in the same way. One millilitre of suspension was extracted from each dilution, put into sterile Petri plates, and labelled. After that, addition of roughly 15 ml of nutritional agar medium that had been melted and chilled to 45°C then letting it solidify. Place these plates inverted and incubate them at 37°C for 24–48 h. Only the plates with between 30 and 300 colonies after incubation incubate the colony counts were taken, and expressed as $\log_{10}$ CFU/g. (Mangang et al., 2017).

2.8.2. Yeast and mold count

A very approximate estimate of the amount of mold in the air or food is given by the yeast and mold count. Although various media and incubating conditions are utilized, the process is similar to the normal plate count. From appropriate dilutions, 0.1 ml aliquot was spread-plated on Potato Dextrose Agar supplemented with 0.1 % tartaric acid and incubated at ambient temperature for 2–5 days and the colony counts were taken, and expressed as $\log_{10}$ CFU/g. (Mangang et al., 2017).

2.8.3. Lactic acid bacteria count

MRS is frequently employed in the culture of lactic acid bacteria. In an anaerobic environment, place the inoculated MRS plates in an incubator set to 30–37°C. For 24–48 h, incubate the colony counts were taken, and expressed as $\log_{10}$ CFU/g. (Mangang et al., 2017).

2.9. Shelf-life study

For the shelf-life study, the microbial count and biochemical analysis was selected.

2.9.1. Protein analysis

The total nitrogen was determined using the Kjeldahl method, and the crude protein was then estimated. To find the protein amount, multiply the N% by 5.95 (AOAC, 2005).

2.9.2. Carbohydrate analysis

With just minor modifications, the phenol sulphuric acid technique (Guo et al., 2014) was used to analyse carbohydrates. D-glucose was employed as the standard solution in this procedure. After heating the 500 mg sample in 5 millilitres of 2.5 N HCL for three hours in a water bath, the volume was topped off with 50 millilitres of distilled water. The standard was taken in five test tubes with varying concentrations, then adding 5 % phenol followed by 96 % sulfuric acid. Phenol and sulfuric acid were then added to various test tubes containing various samples. Using a spectrophotometer, the absorbance at 490 nm was measured, and a graph was created. The graph was used to calculate the sample's carbohydrate content.

2.9.3. Fat analysis

The sample was extracted using petroleum ether in a Soxhlet device for eight hours in order to estimate the lipid content. The amount of lipid was then calculated following the removal of the petroleum ether (AOAC, 2005). The flask's initial weight, or W1, is its weight. W2 is the flask's final weight, or the weight after the ether has evaporated.

$$\text{Fat\%} = \text{W2} - \text{W1/sample weight} \times 100$$

Total plate count, Total yeast & mold count was done according to the method mentioned above in the section microbial analysis.

2.9.4. Colliforms count

Double layer pour plate technique was used for enumeration of Colliforms in the sample for this purpose 100 microliter of the diluted sample was pipetted onto sterilized petri plates and about 10–15 ml of VRBA sterilized media at about 45–50°C was poured onto the plates and spread evenly. After solidification of the media, another 10 ml of liquid media was poured on top of the solidified media and spread evenly. The plates were incubated in inverted position in anaerobic condition at 37 °C incubate the colony counts were taken, and expressed as $\log_{10}$ CFU/g (APHA, 2015).

2.10. Statistical analysis

The experimental data were reported as the mean values and standard deviations. Mean comparisons among the treatments were performed using Tukey's test at a significance level of $p < 0.05$. The analyses were performed using OriginPro 9.0 software and were performed in triplicate.

3. Result and discussion

3.1. Estimation of mineral content

Calcium, magnesium, iron, zinc, and manganese contents of fermented rice products were measured and shown in Table 1. It was found that magnesium is in higher quantity in both the sample and then followed by calcium. The increase in mineral content was evaluated relative to the control sample. Goswami et al. (2016) also reported that Magnesium is in higher quantity in Poita Bhat then calcium. Based on the information, this newly developed rice fermented meal qualifies as a calcium-and magnesium-rich food. Rice fermentation results in the microbial enzyme phytase deoptimizing the grain and making minerals more bioavailable (Hor et al., 2022). The increase and decrease in the mineral linked to metabolic activities of fermenting organisms which hydrolysed metal-phytate complexes to release free minerals (Adebo et al., 2022). According to Pranoto et al. (2013), magnesium, iron, calcium, and zinc content increases in fermented food. After fermentation, it was discovered that the magnesium content of various cereals and foods based on pulses, such as idli, dosa, chikni papad, etc., increased. It might have resulted from microbial action reducing the phytate content (Goswami et al., 2016)

3.2. Estimation of vitamin B content

Vitamin B1, B5, B6, B12 were analysed in both the sample and represented by Table 2. Experimental investigation revealed that vitamin B5 was in highest amount in both FRP1 (90.171 ± 0.21) in ppm and FRP2 (92.568 ± 1.43) ppm. Among both the sample FRP1 contains

more Vitamin B1, B6 and B12 content except B5 the sample. During fermentation Vitamin B increases may be due to Lactic acid bacteria which have the ability to hydrolyse and degrade antinutritional elements such as phytic acid during fermentation, which improves the accessibility of vitamin B compounds and micronutrients. (Mishra et al., 2022; Sharma et al., 2020). Also, Enzyme interactions and the release of the vitamins' bound forms are the reasons for the rise in vitamin B.

3.3. pH analysis

The pH of FRP1 end product is 4.1 ± 0.02. The pH of pachoi FRP1 decreased after 24 h of fermentation, dropping from 5.9 to 4.1. and in FRP2 5.4–4.5. Studies have shown that pH reduction is common in various fermented foods during cereal fermentation. Lactic acid bacteria break down free sugars in cereals to produce lactic acid, leading to this pH decline. A lower pH enhances the activity of microbial enzymes, providing additional benefits to fermented foods. Moreover, acidic conditions help in food preservation by slowing the growth of harmful microorganisms (Magala et al., 2015; Goswami et al., 2016; Borah et al., 2021).

3.4. Alcohol content

The alcohol content differed between the two fermented rice products. FRP1 recorded an average alcohol level of 1.37 ± 0.06 % (v/v), while FRP2 had a comparatively lower average of 1.09 ± 0.11 % (v/v)

There was a variation in the levels of alcohol among the different beers, which might be due to the varying fermentation abilities of the respective microorganisms. In drinks, the alcohol percentage significantly affects the formation of haze; however, the primary factor responsible is the interaction between proteins and polyphenols. (Bamforth, 2002) analyzed the alcohol concentration in numerous beers and recorded values ranging between approximately 3 % and 6 % (v/v). Beer is regarded as the most widely available and frequently consumed alcoholic drink, with its alcohol and polyphenol components offering protection against multiple cardiovascular ailments and helping to alleviate oxidative stress caused by diverse metabolic functions and stressful conditions (Arranz et al., 2012). Traditional sorghum-based beer, Ikigage available in Rwanda, underwent physicochemical assessment, revealing an ethanol concentration of 2.2 % (v/v) and a total acidity of 1.7 % (Lyumugabe et al., 2010)

At the start of the fermentation process, starch-breaking fungi break down starch into dextrin with the help of α-amylase, which is then further converted into glucose by glucoamylase. After that, ethanol-producing yeast (primarily *Saccharomyces cerevisiae*) takes control of the fermentation, also enriching the final product with various vitamins, amino acids, and contributing to the taste and scent (Ghosh et al., 2015).

3.5. Microbiological examination

The total plate count, total yeast and mold and total Lactic acid bacteria after 24 hrs of fermentation in each sample was shown in Table 2. Total plate count and total yeast was higher in FRP2 sample then FPR1. In FRP1 there was no detection of molds but in FRP2 it was found 3.77 ± 0.17 $\log_{10}$CFU/g. Mold detection in high amount is may be due to contamination from sugar used in sample preparation (Yonzan & Tamang, 2010). Lactic acid bacteria were found more in FRP2 (10.65 ± 0.20 $\log_{10}$CFU/g) then FRP1 (6.16 ± 0.16 $\log_{10}$cfu/g). A substantial population of lactic acid bacteria (LAB) was detected in the fermented rice sample, reflecting a microbiological condition typically linked with advantageous fermentation activity. LAB are extensively acknowledged for their contribution to improving nutritional properties and supporting digestive health. Through their metabolic processes, LAB can enhance the absorption of micronutrients and aid in extending the product's shelf life (Cichońska et al., 2022). Research on traditional rice-based fermented beverages shows significant variation in microbial populations. For example, Haria, a rice-based drink from West Bengal, exhibited a high initial count of aerobic bacteria, approximately 10.51 $\log_{10}$ CFU/g, which remained prominent until the third day of fermentation (Ghosh et al., 2015).

Additionally, fermentation produces the ideal pH range for phytate's enzymatic breakdown and releases minerals including calcium, iron, zinc, and manganese—all of which are crucial for the growth of Lactic acid bacteria. Lactic acid bacteria are essential for agriculture, food production, and health. The bacteria that comprise this group are typically described as gram-positive, non-sporing, cocci or rods that produce lactic acid as the primary byproduct during the fermentation of carbohydrates (Hayek and Ibrahim, 2013). A product's shelf life can be increased by Lactic acid bacteria by producing lactic acid and other antimicrobial substances that stop dangerous germs from growing and reduce the quantity of sugar in the product (Patel et al., 2023).

3.6. Estimation of biochemical constituents during shelf-life studies

Quantitative levels of protein, carbohydrate and fat contents of both fermented rice varieties were represented by Fig. 1.

The carbohydrates decrease along with the time period. Highest decrease upto 3rd day in both sample but after 24 days it becomes almost constant. Starch, the primary carbohydrate found in cereals and legumes, supplies the majority of calories in underdeveloped nations (Day & Morawicki, 2018). Enzymes that hydrolyze starch, such as maltase and α-amylase, are activated during fermentation and cause starch to break down into simple sugars and maltodextrins, respectively. Research has indicated that an elevation in glucose occurs in the initial phases of fermentation as a result of activated maltase and α-amylase hydrolyzing starch. A portion of the reason for the decline overall carbohydrates after 24 h of fermentation may be attributed to the glucose produced during fermentation, which is a desired nutrient for the microbes making the food (Nkhata et al., 2018).

After 3rd day, the protein level in both samples slightly decrease. Due to variations in the original protein or amino acid profile of meals, different experimental designs, and varied study durations, the effect of fermentation on proteins has produced conflicting results. While some research found a decline (Pranoto et al., 2013), others claimed an increase (Chavan et al., 1989; Duodu et al., 2003).

Proteins rise as a result of fungal metabolism, but fall as a result of protein decomposition to promote fungi to developed (Suarti et al., 2021). Amino acid and protein loss occurs as a result of fermenting microflora's ability to use these substances during fermentation (Nkhata et al., 2018). Anyiam et al. (2023) study on the fermented cassava product, mahewu, similarly revealed that protein levels increase during the initial 42 h of fermentation before declining over the subsequent 48 h. According to Sharma et al. (2020) the surge in protein content may be attributed to the possible secretion of extracellular enzymes—naturally occurring proteins aimed at breaking down starch for energy utilization.

The reduction in protein levels after the third day in this research could result from the breakdown of protein into smaller components by fermenting microorganisms over an extended fermentation period. The possible reason could be the release of protein into the surrounding liquid environment during fermentation (Sharma et al., 2020). Microbial activity during fermentation can degrade protein into smaller fragments and utilize the amino acids produced throughout the prolonged process (Osman, 2011)

There is a little drop in fat content but no significant change in both the sample. Utilizing components related to fat for mycelial synthesis resulted in a reduction in lipid (Kupski et al.,2012). According to Nnam and Obiakor (2003), Reduction in fat correlated with elevated lipase activity. (Adebo et al., 2022), reported that decrease in fat due to fermentation because of the process by which the fermenting microorganisms metabolize fats and release soluble inorganic ions has been linked to the decline in the amount of fat.

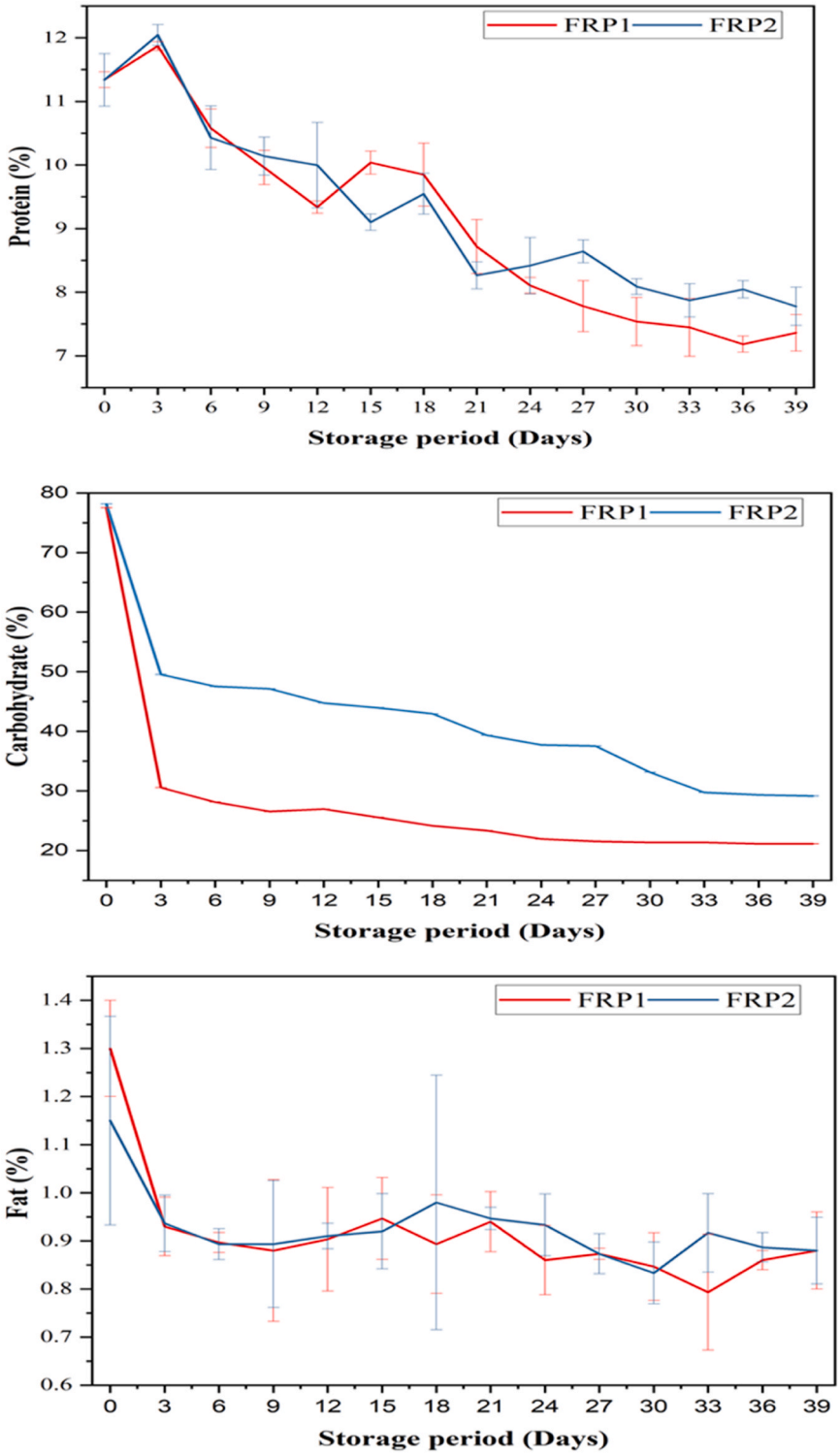


Fig. 1. Protein, carbohydrate and fat content during storage period of FRP1 and FRP2.

Anyiam et al. (2023) reported a decline in fat content over time during their research on fermentation. This may be due to an increase in the production of lipolytic enzymes by fermenting microorganisms, which break down lipid molecules into glycerol and fatty acids. The reduction in fat content results from the fermenting organisms utilizing the liberated fatty acids as an energy source. Several researchers have linked microbial lipid metabolism to the observed decreases in fat levels. Likewise, (Nnam & Obiakor, 2003) observed that rice subjected to fermentation for 72 h exhibited an approximately 18 % reduction in fat content. A lower fat percentage, as recorded in this study, contributes to an extended shelf life of the final product by minimizing the likelihood of spoilage and the development of undesirable odors and flavors.

Adebo et al. (2022) suggested that the breakdown of fats by fermenting microbes, accompanied by the release of soluble inorganic ions, could be associated with the fat reduction occurring during fermentation.

3.7. Sensory evaluation

Both the Fermented rice product obtained good ratings in sensory evaluation shown in Fig. 2. In terms of overall flavour, taste and overall acceptability FRP2 in day 1 was rated with highest score. In both FRP1 and FRP2 shows lower overall acceptability on 3rd day. In case of revers rating for acidity the highest score was found in FRP2 on day 3rd. Therefore, it has been observed that the qualitative acceptability of the fermented rice product pachoi was decreased with the increase in storage life in terms of flavour, taste and overall acceptability. Based on the hedonic scale, the total acceptability was measured at 6.56 for FRP1 and 7 for FRP2. The evaluators noted that the beverage's initial taste upon consumption was its sweetness and subtle fruity (banana-like) fragrance. They also reported experiencing a smooth texture with a well-balanced level of astringency. Consequently, the evaluators described the overall mouthfeel sensation as "pleasing."

On the third day, both FRP1 and FRP2 showed a decline in overall acceptance. On this same day, FRP2 recorded the highest score in the acidity reversal assessment. As a result, it was observed that with prolonged storage, the qualitative acceptability of the fermented rice product pachoi—including its flavor, taste, and overall appeal—diminished. Fermented rice products typically exhibit three primary tastes: sweetness, sourness, and bitterness. The sweetness originates from glucose, while tannin-associated phenolic compounds contribute to the bitter taste and enhance the astringent sensation (Jian-guo 2004).

The tartness has been associated with acids such as tartaric, lactic, and acetic acid, while the umami sensation has been connected to glutamic acid. Conversely, ethanol was discovered to influence mouthfeel perceptions (warmth, richness, and thickness) as well as taste (sweetness and bitterness). In general, the balance of sugars, glycerol, ethanol, and organic acids significantly affected the refined flavor and texture of the fermented rice beverages. (Liu, 2014)

Consumer acceptance based on sensory evaluation may not always align with microbiological safety, chemical integrity, or the preservation of nutrients. The shelf-life study of the fermented rice products (FRP1 and FRP2) was conducted by assessing both microbial load and biochemical stability over time. Monitoring microbial growth provides a direct indication of product safety and spoilage, while changes in key nutritional components (protein, fat, and carbohydrates) serve as indicators of biochemical degradation or stability during storage. The retention or decline of these macronutrients helps evaluate the product's nutritional quality and shelf stability, as spoilage and microbial metabolism can lead to significant alterations in these components.

3.8. Microbial analysis during shelf life study

3.8.1. Total plate count

The FRP1 sample showed a higher microbial count than FRP2 (Table 4), with initial values of 3.07 $\log_{10}$ CFU/g and 2.77 $\log_{10}$ CFU/g, respectively, on day 0. In FRP1, microbial growth increased sharply, reaching a peak of 9.43 $\log_{10}$ CFU/g by day 12, followed by a decline to 6.77 $\log_{10}$ CFU/g. In contrast, FRP2 exhibited a more gradual rise in microbial load, peaking at 9.38 $\log_{10}$ CFU/g by day 15. The count then steadily decreased, reaching 7.35 $\log_{10}$ CFU/g by day 39. This reduction in microbial numbers over time could be attributed to nutrient depletion as fermentation progressed.

3.8.2. Total yeast and mold count

In the FRP2 sample, the yeast count began at 2 $\log_{10}$ CFU/g and rose rapidly to 5.30 $\log_{10}$ CFU/g by day 3. It continued to increase, peaking at 10.42 $\log_{10}$ CFU/g on day 15 before dropping significantly to 6.88 $\log_{10}$ CFU/g by the final stage. In comparison, FRP1 showed a sharp rise in yeast population from 3.20 $\log_{10}$ CFU/g to 9.30 $\log_{10}$ CFU/g within six days. The count then continued to grow steadily, reaching a peak of 10.46 $\log_{10}$ CFU/g on day 18, followed by a decline to 6.47 $\log_{10}$ CFU/g, as presented in Table 4. These trends reflect typical microbial growth patterns, characterized by exponential increase during the availability of nutrients, followed by a decline as substrates become depleted.

3.8.3. Total colliform

Coliform bacteria were first detected in FRP1 on the 33rd day of storage, whereas signs of spoilage in FRP2 appeared slightly later, on the 36th day, as shown in Table 4.3 and Figure 4.6. In FRP2, microbial analysis indicated a coliform count of 3.69 $\log_{10}$ CFU/g on day 36, signalling the beginning of spoilage. In contrast, FRP1 showed earlier contamination, with a coliform level of 3.57 $\log_{10}$ CFU/g recorded on day 33. These findings suggest that FRP2 maintained microbial stability for a longer duration than FRP1. Nevertheless, when spoilage occurred,

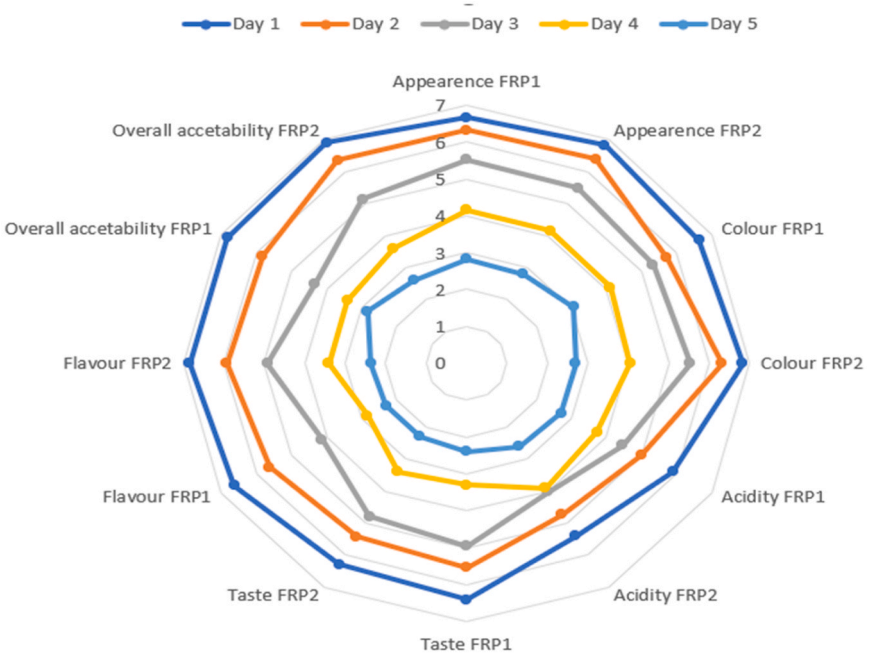


Fig. 2. Sensory quality of FRP1 and FRP2 up to 5 days.

Table 4
Microbial count of FRP1 and FRP2.

Microbial count log_{10}CFU/g						
Days	Total plate count		Yeast and Mold count		Total colliform	
	FRP1	FRP2	FRP1	FRP2	FRP1	FRP2
0	3.07 ± 0.13	2.77 ± 0.12	3.20 ± 0.12	2 ± 0.12	ND	ND
3rd	8.48 ± 0.28	6.85 ± 0.13	6.90 ± 0.14	5.30 ± 0.28	ND	ND
6th	9.91 ± 0.35	8.35 ± 0.10	9.30 ± 0.12	8.36 ± 0.14	ND	ND
9th	9.32 ± 0.21	9.10 ± 0.15	10.29 ± 0.14	9.31 ± 0.28	ND	ND
12th	9.43 ± 0.20	9.26 ± 0.14	10.43 ± 0.12	10.33 ± 0.13	ND	ND
15th	9.41 ± 0.08	9.38 ± 0.13	10.45 ± 0.12	10.42 ± 0.34	ND	ND
18th	9.37 ± 0.26	9.06 ± 0.16	10.46 ± 0.10	10.38 ± 0.11	ND	ND
21th	8.76 ± 0.16	9.04 ± 0.09	9.78 ± 0.16	9.37 ± 0.38	ND	ND
24th	8.17 ± 0.25	8.98 ± 0.13	9.69 ± 0.12	9.24 ± 0.09	ND	ND
27th	7.94 ± 0.26	8.40 ± 0.12	9.19 ± 0.14	9.10 ± 0.20	ND	ND
30th	7.35 ± 0.28	8.34 ± 0.14	7.36 ± 0.14	8.83 ± 0.31	ND	ND
33th	7.11 ± 0.29	7.94 ± 0.14	6.93 ± 0.15	8.23 ± 0.08	ND	ND
36th	6.96 ± 0.23	7.36 ± 0.16	6.74 ± 0.21	7.18 ± 0.30	3.57 ± 0.13	ND
39th	6.77 ± 0.28	7.35 ± 0.13	6.47 ± 0.26	6.88 ± 0.13	3.94 ± 0.14	3.69 ± 0.14

Microbial count in different storage period is presented as mean ± standard deviation (SD), calculated from triplicate independent measurements

the bacterial load in FRP2 was slightly higher than that in FRP1 at the time of detection. This variation in spoilage onset and microbial growth could be attributed to factors such as storage environment, sample composition, or the initial microbial load present in each sample.

In this study, the total plate count and yeasts indicated a common trend of increase up to the peak point with the passage of time and a slight decrease at the end point in both of the samples. In their study on the shelf life of beer using microbiological analysis, (Mangang et al., 2017).discovered that total aerobes, yeast, and Lactic acid bacteria rise until the point of fermentation and subsequently drop. The microbial populations of 46 sorghum beer samples were counted by Pattison et al. (1998) who also got aerobic and Lactic acid bacteria plate counts of 9.12×10^5 and 2.75×10^6 CFU/ml. Additional microbiological investigation of beer samples revealed the presence of yeast, Staphylococcus aureus, total aerobic mesophilic bacteria, Lactic acid bacteria, and molds with CFU/ml values of 33.6×10^6, 35.4×10^4, 10.2×10^6, 16.0×10^3, and 12×10^4, respectively.

According to the results of other experiments, the number of yeast and molds increases until a period of fermentation, but eventually starts to decline and the mold in haria, bhatijaanr, and tape ketan vanishes. Aerobic conditions are what cause a lot of mold and yeast to grow during the first stage of fermentation. These microbes released several hydrolytic enzymes that aided in the quick breakdown of the starch that was available in rice grains. (Ghosh et al., 2015; Tamang & Thapa, 2006). The progression of microbial communities affects the evolution and results of the fermentation process over time. In research on the fermentation of Haria, a traditional rice beer from eastern India, fungi and yeasts reached their highest levels on the second day of fermentation, while lactic acid bacteria (LAB) and Bifidobacterium rose concurrently (Ghosh et al., 2015). While coliform detection is a widely used indicator of general hygiene and potential fecal contamination, it does not provide pathogen-specific information. The absence of targeted testing for pathogens such as *Escherichia coli* O157:H7, *Salmonella spp.*, or *Listeria monocytogenes* limits the ability to definitively assess microbiological safety. Thus, relying solely on coliform counts may overlook the presence of specific harmful microorganisms, and this represents a significant limitation in fully addressing food safety (Martin et al., 2016)

Numerous studies have shown that yeast and mold populations typically increase during the early stages of fermentation but decline as the process progresses. In traditional fermented products like Haria, Bhatijaanr, and Tape Ketan, mold populations eventually disappear. This initial aerobic phase of fermentation supports the proliferation of yeasts and molds, which play a key role by secreting hydrolytic enzymes that aid in the efficient breakdown of rice starches (Ghosh et al., 2015; Tamang & Thapa, 2006).

Regarding the industrial potential, the study presents a preliminary exploration of pachoi's viability in functional food applications. While detailed economic modeling or scale-up logistics were beyond the scope of this initial investigation, the mention of industrial potential serves to highlight future applicability rather than assert commercial readiness. The acknowledgment of this potential was grounded in the product's sensory acceptability and nutritional profile, which form the basis for further investment in practical studies, including cost analysis, packaging design, and regulatory assessment. These aspects are identified as crucial future steps in the conclusion and are intentionally left open for future research rather than addressed speculatively in the current scope.

4. Conclusion

The study's findings indicated that both fermented foods have high mineral and vitamin B1, B5, B6, and B12 content. Vitamin B5 is present in greater amounts in both samples in FRP1 (90.171) and in FRP2 (92.568). It is observed in both samples that the total plate count and the total yeast and mold count rise for a while before declining during the duration of storage. Due to the innovative preparation method and its high bacterial count and nutritional value, it may be produced on an industrial scale for marketing purposes as well. The development of a fermented pachoi-based food product demonstrated promising sensory acceptability and nutritional enhancement, supporting its potential as a functional food. Fermentation not only improved palatability but also suggested increases in beneficial compounds, positioning pachoi as a viable ingredient in health-oriented food innovation. However, certain limitations must be acknowledged. The absence of pathogen-specific microbiological testing (e.g., for *E. coli*, *Salmonella sp*) restricts definitive conclusions about product safety. Additionally, the small sensory panel and lack of trained evaluators may affect the reliability and generalizability of consumer acceptability data. Future research should focus on pilot-scale production of FRP2 to evaluate feasibility for industrial manufacturing. This includes assessing consistency in product quality, cost-effectiveness, packaging technologies, and consumer acceptability in broader markets.

A detailed metagenomic analysis of the microbial communities present during fermentation can provide insights into the specific strains contributing to flavor, shelf life, and probiotic benefits. Isolating and identifying dominant lactic acid bacteria could facilitate the development of a standardized starter culture. While the claim of industrial potential is encouraging, it requires substantiation through cost-benefit analysis and market feasibility studies. Factors such as raw material availability, production scalability, consumer demand, and regulatory compliance must be thoroughly evaluated to ensure successful product development and market integration.

Ethics approval statement

The plants utilised in this investigation were collected following local or national guidelines. Plant safety guidelines were meticulously adhered during the harvesting process. Authors hereby declare that the sensory panelists who participated in sensory analysis in sensory evaluation of Pachoi has given their full consent. Therefore, the objective of

this organoleptic evaluation is carried out ethically. We confirm that appropriate protocols for protecting all participants' rights and privacy were followed during the research (no coercion to participate, full disclosure of study requirements and risks, verbal consent of participants, no release of participant data without their knowledge, and the ability to withdraw from the study at any time).

Funding statement

None.

CRediT authorship contribution statement

Laky Khatun: Writing – original draft, Methodology, Formal analysis, Data curation, Conceptualization. **Rangina Brahma:** Writing – review & editing. **Subhajit Ray:** Visualization, Supervision. **Dulal Chandra Boruah:** Writing – review & editing.

Declaration of Competing Interest

The authors declare that there are no competing interests.

Acknowledgements

Authors are grateful to the Department of Food Engineering & Technology, Central Institute of Technology Kokrajhar, Kokrajhar, Assam, India.

Clinical trial registration

Not applicable

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.foohum.2025.100707.

Data availability statement

All data underlying the results is available as part of the article.

References

Adebo, J. A., Njobeh, P. B., Gbashi, S., Oyedeji, A. B., Ogundele, O. M., Oyeyinka, S. A., & Adebo, O. A. (2022). "Fermentation of cereals and legumes: impact on nutritional constituents and nutrient bioavailability". *Fermentation, 8*(2), 63. https://doi.org/10.3390/fermentation8020063

Anyiam, P. N., Nwuke, C. P., Uhuo, E. N., Ije, U. E., Salvador, E. M., Mahumbi, B. M., & Boyiako, B. H. (2023). Effect of fermentation time on nutritional, antinutritional factors and in-vitro protein digestibility of macrotermes nigeriensis-cassava mahewu. *Measurement: Food*.

AOAC. (2005). *Official Methods of Analysis of the Association of Analytical Chemists International* (18th ed.).). Maryland, USA: AOAC Gaithersburg.

APHA. (2015). *Standard Methods for the Examination of Water and Wastewater* (23rd ed.).). American Public Health Association.

Arranz, S., Chiva-Blanch, G., Valderas-Martínez, P., Medina-Remón, A., Lamuela-Raventós, R. M., & Estruch, R. (2012). Wine, beer, alcohol, and polyphenols on cardiovascular disease and cancer. *Nutrients, 4*(7), 759–781. https://doi.org/10.3390/nu4070759

Bamforth, C. (2002). Great brewing debates: Part 5. Beer freshness: is the maltster to blame? *Brewers Guardian, 131*, 22–24.

Borah, V. V., Choudhury, M. G., & Phanjom, P. (2021). Preparation and health benefits of rice beverages from ethnomedicinal plants: Case study in North-East India. *Plant-based Functional Foods and Phytochemicals* (p. 36). Apple Academic Press. https://doi.org/10.1201/9781003055419

Chavan, J. K., Kadam, S. S., & Beuchat, Larry R. (1989). Nutritional improvement of cereals by fermentation. *Critical Reviews in Food Science and Nutrition, 28*(5), 349–400. https://doi.org/10.1080/10408398909527507

Cichońska, P., Ziębicka, A., & Ziarno, M. (2022). Properties of rice-based beverages fermented with lactic acid bacteria and Propionibacterium. *Molecules, 27*(8), 2558. https://doi.org/10.3390/molecules27082558

Crowell, E. A., & Ough, C. S. (1979). A modified procedure for alcohol determination by dichromate oxidation. *American Journal of Enology and Viticulture*. https://doi.org/10.5344/ajev.1979.30.1.61

Das, A. J., Deka, S. C., & Miyaji, T. (2020). Fermentation of rice beverage Haria: microbial diversity and nutritional profile. *Journal of Ethnic Foods, 7*(1), 1–7. https://doi.org/10.1186/s42779-019-0034-z

Day, C. N., & Morawicki, R. O. (2018). "Effects of fermentation by yeast and amylolytic lactic acid bacteria on grain sorghum protein content and digestibility. *Journal of Food Quality*, (1), 3964392.

Duodu, K. G., Taylor, J. R. N., Belton, P. S., & Hamaker, B. R. (2003). "Factors affecting sorghum protein digestibility. *Journal of Cereal Science, 38*(2), 117–131.

Ghosh, K., Ray, M., Adak, A., Dey, P., Halder, S. K., Das, A., Jana, A., Parua, S., Vágvölgyi, C., Mohapatra, P. K. Das, et al. (2015). "Microbial, saccharifying and antioxidant properties of an Indian rice based fermented beverage.". *Food Chemistry, 168*, 196–202.

Goswami, G., Baruah, H., Boro, R. C., & Barooah, M. (2016). Fermentation reduces anti-nutritional content and increases mineral availability in poitabhat. *Asian Journal of Chemistry, 28*, 1929–1932. https://doi.org/10.14233/ajchem.2016.19820

Guo, Q., Cui, S. W., & Kang, Ji (2014). Classical methods for food carbohydrate analysis. *Food Oligosaccharides: Production, Analysis Bioactivity*, 284–299. https://doi.org/10.1002/9781118817360.ch16

Hayek, Saeed A., & Salam, A. Ibrahim (2013). "Current limitations and challenges with lactic acid bacteria: a review.". *Food and Nutrition Sciences 4, 11*, 73–87.

Hor, P. K., Ghosh, K., Halder, S. K., Soren, J. P., Goswami, D., Bera, D., Singh, S. N., Dwivedi, S. K., Parua, S., Hossain, M., et al. (2021). Evaluation of nutrient profile, biochemical composition and anti-gastric ulcer potentialities of khambir, a leavened flat bread. *Food Chemistry, 345*, Article 128824. https://doi.org/10.1016/j.foodchem.2020.128824

Hor, P. K., Goswami, D., Ghosh, K., Takó, M., Halder, S. K., & Mondal, K. C. (2022). Preparation of rice fermented food using root of Asparagus racemosus as herbal starter and assessment of its nutrient profile. *Systematics Microbiology and Biomanufacturing, 2*, 147–156. https://doi.org/10.1007/s43393-021-00046-8

Ilango, S., & Antony, U. (2021). Probiotic microorganisms from non-dairy traditional fermented foods. *Trends Food Science and Technology, 118*, 617–638. https://doi.org/10.1016/j.tifs.2021.05.034

Jian-guo, W. (2004). Analysis of composition and source of color, aroma, taste, type in rice wine. *China Brew, 4*, 6–10.

Khatun, L., Ray, S., & Baruah, D. C. (2023). Impact of germination versus non-germination on nutritional and functional potentials of two rice varieties (Ranjit and Ahu Kalogoria) available in three different districts of Assam, India. *Journal of Food Technology Research, 10*, 103–121. https://doi.org/10.18488/jftr.v10i4.3567

Kupski, L., Cipolatti, E. P., Rocha, M. D., Oliveira, M. D., Souza-Soares, D. A. Leonor, & Badiale-Furlong, E. (2012). Solid-State Fermentation for the Enrichment and Extraction of Proteins and Antioxidant Compounds in Rice Bran by Rhizopus oryzae. *Brazilian Archieves of Biology and Technology, 55*, 937–942. https://doi.org/10.1590/S1516-89132012000600018

Liu, Q., 2014, Analysis of volatile compounds and their changes during liquor aging of Chinese liquor 'gujing gongjiu'. ⟨https://tigerprints.clemson.edu/all_theses/1888⟩.).

Lyumugabe, F., Kamaliza, G., Bajyana, E., & Thonart, P. H. (2010). Microbiological and physico-chemical characteristics of Rwandese traditional beer 'Ikigage'. *African Journal of Biotechnology, 9*(28), 4241–4246.

Magala, M., Kohajdová, Z., Karovičová, J., Greifová, M., & Hojerová, J. (2015). Application of lactic acid bacteria for production of fermented beverages based on rice flour. *Czech Journal of Food Sciences, 33*(5), 458–463. https://doi.org/10.17221/74/2015-CJFS

Mangang, K. C., Das, A. J., & Deka, S. C. (2017). Comparative shelf-life study of two different rice beers prepared using wild-type and established microbial starters. *Journal of the Institute of Brewing, 123*, 579–586. https://doi.org/10.1002/jib.446

Martin, N. H., Trmčić, A., Hsieh, T. H., Boor, K. J., & Wiedmann, M. (2016). The evolving role of coliforms as indicators of unhygienic processing conditions in dairy foods. *Frontiers in Microbiology, 7*, 1549. https://doi.org/10.3389/fmicb.2016.01549

Mishra, S.,K., Mithul, S. A., Charpe, P., Ajlouni, S., Ranadheera, C. S., & Chakkaravarthi, S. (2022). Traditional rice-based fermented products: Insight into their probiotic diversity and probable health benefits. *Food Bioscience, 50*, Article 102082. https://doi.org/10.1016/j.fbio.2022.102082

Nkhata, S. G., Ayua, E., Kamau, E. H., & Shingiro, J. B. (2018). Fermentation and germination improve nutritional value of cereals and legumes through activation of endogenous enzymes. *Food Science and Nutrition, 6*, 2446–2458. https://doi.org/10.1002/fsn3.846

Nnam, N. M., & Obiakor, P. N. (2003). effect of fermentation on the nutrient and antinutrient composition of baobab (Adansonia digitata) seeds and rice (Oryza sativa) grains. *Ecology of Food and Nutrition, 42*, 265–277. https://doi.org/10.1080/03670244.2003.9657684

Nuraida, L. (2015). A review: Health promoting lactic acid bacteria in traditional Indonesian fermented foods. *Food Science and Human Wellness, 4*(2), 47–55. https://doi.org/10.1016/j.fshw.2015.06.001

Osman, M. A. (2011). Effect of traditional fermentation process on the nutrient and antinutrient contents of pearl millet during preparation of Lohoh. *Journal of the Saudi Society of Agricultural Science, 10*, 1–6. https://doi.org/10.1016/J.JSSAS.2010.06.001

Patel, P., Butani, K., Kumar, A., Singh, S., & Prajapati, B. G. (2023). Effects of fermented food consumption on non-communicable diseases. *Foods, 12*, 68. https://doi.org/10.3390/foods12040687

Pattison, T., Geornaras, I., & von Holy, A. (1998). Microbial populations associated with commercially produced South African sorghum beer as determined by conventional and Petrifilm plating. *International Journal of Food Microbiology, 43*, 115–122.

Pranoto, Y., Anggrahini, S., & Efendi, Z. (2013). Effect of natural and Lactobacillus plantarum fermentation on in-vitro protein and starch digestibilities of sorghum flour. *Food Bioscience, 2*, 46–52. https://doi.org/10.1016/J.FBIO.2013.04.001

Sharma, R., Garg, P., Kumar, P., Bhatia, S. K., & Kulshrestha, S. (2020). "Microbial fermentation and its role in quality improvement of fermented foods". *Fermentation, 6*(4), 106. https://doi.org/10.3390/fermentation6040106

Stone, H., Bleibaum, R., & Thomas, H. (2021). *Sensory Evaluation Practices* (5th ed.).). Academic Press.

Suarti, B., Sukarno, S., Ardiansyah, A., & Budijanto, S. (2021). Bio-active compounds, their antioxidant activities, and the physicochemical and pasting properties of both pigmented and non-pigmented fermented de-husked rice flour. *AIMS Agricultural Food, 6*, 49–64. https://doi.org/10.3934/agrfood.2021004

Tamang, J. P., Watanabe, K., & Holzapfel, W. H. (2016). Diversity of microorganisms in global fermented foods and beverages. *Frontiers in Microbiology, 7*, 377. https://doi.org/10.3389/fmicb.2016.00377

Tamang, J. P., & Thapa, S. (2006). Fermentation dynamics during production of Bhaatijaanr, a traditional fermented rice beverage of the Eastern Himalayas. *Food Biotechnology, 20*, 251–261. https://doi.org/10.1080/08905430600904476

Uddin, A. H., Khalid, R. S., Alaama, M., Abdual kader, A. M., Kasmuri, A., & Abbas, S. A. (2006). Comparative study of three digestion methods for elemental analysis in traditional medicine products using atomic absorption spectrometry. *Journal of Analytical Science and Technology*, 7. https://doi.org/10.1186/s40543-016-0085-6

Vivek, K., Mishra, S., & Pradhan, R. C. (2018). Physicochemical characterization and mass modelling of Sohiong (Prunus nepalensis L.) fruit. *Journal of Food Measurement and Characterization, 12*(2), 923–936. https://doi.org/10.1007/s11694-017-9708-x

Wang, C., Wu, S., & Shyu, Y. T. (2014). Antioxidant properties of certain cereals as affected by food-grade bacteria fermentation. *Journal of Bioscience and Bioengineering, 117*, 449–456. https://doi.org/10.1016/j.jbiosc.2013.10.002

Yonzan, H., & Tamang, J. P. (2010). Microbiology and nutritional value of Selroti, an ethnic fermented cereal food of the Himalayas. *Food Biotechnology, 24*, 227–247. https://doi.org/10.1080/08905436.2010.507133