Journal of Applied Pharmaceutical Research
Volume 14 Issue 3, Year of Publication 2026, Page 403 – 416
DOI: https://doi.org/10.69857/joapr.v14i3.2015

Research Article

JOURNAL OF APPLIED PHARMACEUTICAL RESEARCH | JOAPR
www.japtronline.com *ISSN: 2348 – 0335*

BIOCHEMICAL CHARACTERIZATION, GC-MS METABOLITE PROFILING, AND HEAVY METALS ASSESSMENT OF ROKSHI: SAFETY ASSESSMENT AND NUTRITIONAL POTENTIAL OF A TRADITIONAL FERMENTED RICE BEVERAGE OF MONPA TRIBE

Rosamund Jyrwa[1, 2], Ananta Choudhury[1]*, Andian Ari Anggraeni[3]

Article Information
Received: 22nd January 2026
Revised: 3rd April 2026
Accepted: 29th April 2026
Published: 15th May 2026

Keywords
Traditionally fermented beverage, Rokshi, Monpa tribe, Antioxidant activity, GC-MS analysis, Heavy metals.

ABSTRACT
Background: *Rokshi* is a traditional fermented rice beverage widely consumed by the Monpa tribe of Arunachal Pradesh, a Northeastern State in India, with cultural, nutritional, and biochemical significance. **Methodology:** Standard analytical methods were employed to determine pH, alcohol content, total acidity, viscosity, and antioxidant properties. Microbial count was assessed using the plate count method. Metabolite profiling and heavy metals analysis were conducted using GC-MS and ICP-OES, respectively. **Results and Discussion:** *Rokshi* were reported to be acidic and rich in metabolically important compounds. One-month-old *Rokshi* beverage showed increased alcohol content (7.54% v/v) as compared to fresh *Rokshi* beverage (2.59% v/v), with a pH of 4.10 (fresh), which decreased to 3.09 during storage. Total acidity increased from 0.58% to 0.78% after one month of storage. Total flavonoid and phenolic content were 0.205 mg QE/mL and 0.928 mg GAE/mL, respectively, with moderate antioxidant activity. Microbial load decreased in the starter culture (2.15 log CFU/mL) to 0.55 log CFU/mL in the final product. GC-MS analysis identified 28 metabolites, predominantly amino acids, sugars, organic acids, and alcohol. Heavy metals (Cd, Pb, Cr, Mn, Fe, Zn, Ni) were also detected, within the maximum permissible limit, and TTHQ values (<1) indicated no significant non-carcinogenic risk at 250 mL/day consumption. **Conclusion:** The current study showed that *Rokshi* holds promise for its nutritional and biochemical properties and has moderate antioxidant potential. The presence of a few biogenic amines and trace contaminants emphasizes the need for purification and further toxicological validation to ensure consumer safety and promote nutraceutical applications for commercial use as a functional fermented beverage.

INTRODUCTION

Fermentation is one of the oldest biotechnological processes developed by humankind, serving as a vital method for food preservation and enhancement of nutritional value [1]. Among fermented products, traditional rice-based alcoholic beverages

[1]*Faculty of Pharmaceutical Science, Assam down Town University, Panikhaiti, Guwahati-781026, Assam, India.*
[2]*Pratiksha Institute of Pharmaceutical Sciences, Guwahati, Assam, India- 7810266*
[3]*Universitas Negeri Yogyakarta, Yogyakarta, Indonesia*
***For Correspondence:** **ananta.choudhury@adtu.in**

hold significant cultural, nutritional, and therapeutic importance [2, 3, 4]. In Northeast India, tribal communities in Arunachal Pradesh prepare and consume rice beverages as part of their daily lives, rituals, and festivals, and these beverages are valued not only for their taste but also for their perceived health benefits [5, 6]. Locally known varieties such as *Rokshi* in Arunachal Pradesh are closely associated with indigenous medicinal practices. *Rokshi*, often brewed with medicinal herbs, is traditionally believed to alleviate diarrhea, urinary tract infections, inflammation, insomnia, and body aches, and to strengthen immunity and regulate cholesterol levels. Ethnomedicinal studies have proved the nutritional and potential therapeutic properties of traditional rice beverages consumed and prepared in tribal societies. Mishra *et al*. reported that fermentation enhances the level of amino acids, minerals, prebiotics, probiotics, and vitamins in rice while reducing the non-nutritional factors such as tannins and phytic acid [7, 8]. The fermentation of rice beverage generally involved three stages: (i) saccharification process by amylolytic Molds, (ii) alcohol fermentation by yeast such as *Saccharomyces cerevisiae* and (iii) metabolite and acid production by lactic acid bacteria such as *Lactobacillus, Bifidobacterium* [8, 9] Latif et al. reported that enrichment of rice beverage with probiotics and biochemical metabolites due to microbial succession may improve gastrointestinal health [10]. Antioxidants play a vital role in inhibiting oxidation, which generates free radicals that damage cellular structures. Therefore, an imbalance between antioxidant molecules and free radical production could lead to various health problems, such as cancer, heart disease, and diabetes.

Although many consume antioxidants as dietary supplements for their important role in maintaining optimal health, excessive consumption can lead to adverse health effects. Common antioxidant examples include vitamin C and E, as well as compounds such as flavonoids, carotenoids, and glutathione [11]. In daily food and beverage consumption, essential minerals and trace heavy metals may be present. Essential elements such as zinc (Zn), iron (Fe), calcium (Ca), copper (Cu), magnesium (Mg), and manganese (Mn) are naturally occurring and play important roles in biological function. Toxic elements such as cadmium (Cd), lead (Pb), arsenic (As), and mercury (Hg) pose health risks. During rice beverage fermentation, heavy metals may be introduced from various sources, including raw materials, water, utensils, and environmental contamination. The formation of heavy metal contamination in rice beverages can pose a critical public health concern [12]. Bhattacharya and Chandra Deka reported the presence of heavy metals, including Pb, Cd, As, and Hg, in traditional rice beverages from Northeast India [13]. Prolonged consumption of contaminated rice beverages may lead to serious health issues, including kidney damage, neurological disorders, and increased cancer risk [14]. This study provides a comprehensive biochemical and microbial characterization of *Rokshi*, including alcohol content, acidity, phenolic composition, antioxidant profiling, GC-MS analysis, identification of potentially health-beneficial compounds, and determination of heavy metal concentrations to assess compliance with WHO/FAO maximum permissible limits. The objective of this research was to scientifically evaluate the nutritional value, biochemical composition, biological properties, and heavy metal content of traditional rice beverage from Arunachal Pradesh, with a focus on safety and risk aspects. As limited scientific data are available on *Rokshi,* the current work focuses on this rice beverage by collecting samples directly from the local community without altering the traditional fermentation process, thereby ensuring in situ analysis of authentic samples. The study also aims to validate traditional knowledge, highlight the potential of *Rokshi* as a food, and provide a basis for future research on purification, preservation, and clinical validation.

MATERIALS AND METHODS

Selection of Tribes for Study, Data, and Sample Collection

For the study, starter culture and rice beverage samples were collected from the Monpa tribe residing in the Tawang district of Arunachal Pradesh, India, given their demographic dominance and rich cultural heritage in the region. This tribe was specifically selected because they represent a prominent indigenous group in Northeast India, where traditional fermentation practices are widely preserved across generations. A total of 1L of rice beverage sample and 1 kg of starter cake were collected from Khardung village in the Tawang district during the period from April to August, 2023. Samples were obtained directly from the household to maintain the traditionally prepared product, and proper documentation was completed. All samples were stored in the laboratory under refrigerated conditions and in a humidity chamber at 27℃ for further analysis.

Ethnic Preparation of starter cake (*phap*)

Phap is prepared by mixing three medicinal plants (*P. zeylanica, V. cinerea, B. asiatica*) with two dried chilies and 0.5g of ginger. All ingredients were dried and ground into powder form.

Approximately 2g of this powder was mixed with 3 kg of gelatinous rice flour, which was then inoculated with microorganisms. The mixture was formed into a small cake (approximately 4 cm wide and 2 cm long). These cakes are then placed on a bed of fern leaves, sealed with a jute bag and dry fern, and left over the fireplace smoke for 4 days, depending on seasonal conditions. The cakes were sun-dried for another 4 days. The final product (*phap*) weighed approximately 4-4.5g per piece and was ready for use.

Ethnic preparation of rice beverage (*Rokshi*)

Rokshi was prepared using glutinous rice. 3 kg of rice was cleaned and cooked with 300 ml of water until softened. The cooked rice was spread on a bamboo mat and allowed to cool. After cooling, two pieces of the prepared starter cake (4 g each) were crushed and mixed with the rice. The mixture was transferred into a clay pot and allowed to ferment for 6 days in summer and 12 days in winter. The pot was sealed with *Alocasia* leaves for complete fermentation. After fermentation is complete, the mixture is diluted by filtration through a cloth sieve, and the milky white beverage, known as Rokshi, is ready for consumption.

Sample Handling and Documentation

Ten grams of starter culture, locally referred to as '*phah*' in Arunachal Pradesh, and 5L of rice beverage (*Rokshi*) were collected and stored under controlled laboratory conditions, and stored in a refrigerator at 25°C temperatures and a humidity chamber (27°C temperatures) for subsequent biochemical and microbial analyses. All samples were sourced from different households in the Tawang district of Arunachal Pradesh, which are predominantly inhabited by these communities. Traditional methods for preparing the rice beverage (Rokshi) were carefully observed & recorded in stepwise detail, as illustrated in Figure 1.

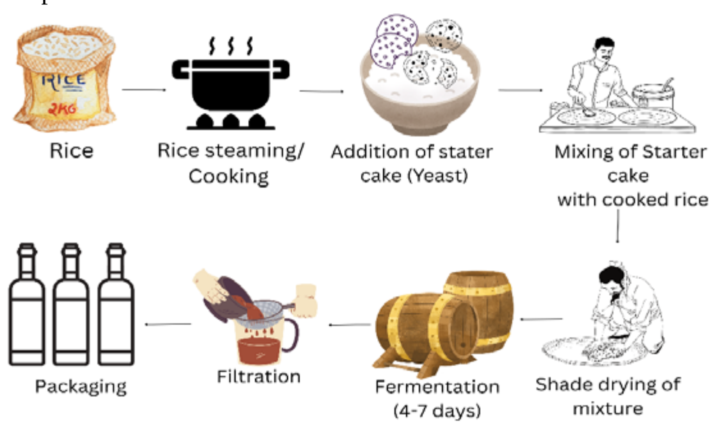

Figure 1: Schematic representation of the traditional preparation of Rokshi, depicting the various stages of the production process from raw rice to the final packaging of beverages

BIOCHEMICAL COMPOSITION OF RICE VARIETIES AND STARTER CAKES

Proximate composition analysis

The proximate composition, including moisture content, crude fiber, ash content, and crude fat, was determined for the collected rice varieties (*sugapankhi*) and starter culture (*phap*).

Moisture Content

Five grams of the sample (starter culture called '*phap*') was ground and placed in a pre-weighed petri dish. The dish containing the sample was dried in a hot-air oven at 130°C for 30 minutes, and its weight was recorded. This process was continued until the constant weight was obtained [15, 1]. Moisture content (%) was calculated using the formula:

$$\textit{Moisture content}\ (\%) = \frac{\textit{Weight of Water}}{\textit{Weight of Dry Solid}} \times 100$$

Ash Content

In a platinum crucible, two grams of the *phap* sample were weighed and heated at 100°C. A few drops of olive oil were added, and the sample was slowly heated over a flame. The crucible was then placed in a muffle furnace at 525°C until white ashes were obtained (all organic compounds had been removed). The collected sample was cooled in a desiccator until white ash was obtained, then weighed [14, 15].

$$\textit{Ash content}\ (\%) = \frac{\textit{Weight of Ash}}{\textit{Weight of Original Sample}} \times 100$$

Crude Fat

Two grams of the rice variety and starter culture sample were ground and subjected to ether extraction for 18 hours. The ether was removed by distillation, and the residue was dried in an oven at 110°C until the mass loss between two successive weighings was observed. Shake the residue with 3 mL of ether at room temperature, allow it to settle, then decant the ether layer. Repeat the extraction process until no residues remain dissolved. The extract was dried, and the final mass difference was recorded in accordance with AOAC Official Method 920.39 [16].

$$\textbf{Crude fat } (\%) \textbf{ by mass } = \frac{(M1 - M2)\ x\ 100}{M}$$

Where M1 is the mass in grams of the extraction flask with extracted fat, M2 is the mass in grams of the empty flask, and M is the mass in grams of the sample taken for analysis.

Sugar Analysis

The total soluble sugars were measured using the Anthrone method. Reducing sugar was estimated using the DNS method (3,5-dinitrosalicylic acid method, obtained from HIMEDIA) in rice variety (sugapankhi) and starter culture (phap) samples. Non-reducing sugars were calculated by subtracting reducing sugars from the total soluble sugars [15, 16].

Preparation of the *Rokshi* rice beverage samples for analysis. Before analysis, 2L of *Rokshi* were made CO_2-free. To do this, the sample *Rokshi* was transferred to a large flask and shaken, first slowly & then violently, while the temperature. maintained at 25°C by following the method described by Singh et al. [14, 15, 16].

Biochemical analysis of rice beverage

The color, taste, and odor of each sample were observed physically.

Measurement of pH

10 ml of rice beverage sample was diluted with 200 ml of CO_2-free distilled water. The pH was determined using a calibrated pH meter with a standard buffer solution.

Total acidity

The indicator titration method was used to determine the total acidity of the rice beverage samples in accordance with AOAC Official Method 950.07, Total Acidity (Total) of Beer, first edition, 1950 guidelines [15, 16]. 250 mL of boiling water was added to the 25mL rice beverage sample, and the mixture was boiled for another 60 seconds. This was then cooled rapidly, and a few drops of 0.5% phenolphthalein (obtained from Fisher Scientific) were added. This was titrated against 0.1M NaOH (Fisher Scientific) until the appearance of a faint pink color occurred as the endpoint. Repeat the process. Results were expressed as % of lactic acid (1ml of 0.1M alkali=0.0090g lactic acid).

Alcohol percentage

The alcohol content of the non-distilled rice beverage sample was evaluated using the dichromate oxidation method and expressed as a volume percentage. 100 ml of rice beer was distilled, and the resulting distillate was collected. The titration method was employed to determine the alcohol %.

Viscosity

The viscosity of the rice beverage sample was determined using a viscometer (at 20℃), EBC method no. 9.38 following the method performed by [17]. Changes in the contents and percentages of the fermented rice beverage produced were analyzed over 30 days.

ANTIOXIDANT ACTIVITY

Total Flavonoid Content (TFC)

The total flavonoid content of the rice beverage sample (*Rokshi*) was determined using the aluminum chloride method. 10mL of rice beverage sample was mixed with 50 mL of alcohol. Then 0.5mL of the extract sample was pipetted out, and 0.1 mL of 10% aluminum chloride solution was added, followed by 3 mL of 5% sodium nitrite. After 5 minutes, 2 mL of 1 M sodium hydroxide was added, and the volume was brought to 10 mL with ethanol. The absorbance was then measured at 415 nm against the blank using a UV-Vis spectrophotometer. For comparison, quercetin was used as a standard. The milligram of quercetin equivalent per 100 mL of sample was used to represent the overall flavonoid concentration (mg/mL) [18, 19]. The determination was carried out in triplicate for the test.

Total Phenolic Content (TPC)

TPC of the *Rokshi* sample was measured using the Folin-Ciocalteu method. 10 mL of rice beverage was taken and dissolved in 50 mL with alcohol; 0.1 mL of the solution was mixed with 0.5mL of 10% Folin-Ciocalteu reagent. The rice beverage sample was then left for 5 min, and 4 mL of distilled water and 1mL of Na_2CO_3 (7%, w/v) were added.

Stabilize the mixture for 30 min until a blue color is observed. Gallic acid was used as a standard for the calibration curve (50-1000 µg/ml). Absorbance was measured at a wavelength of 765 nm against a blank sample on a UV-Vis spectrophotometer. Gallic acid was taken as a standard for the calibration curve. TPC is expressed as milligram Gallic acid equivalents per 1 L of beverage sample (mg/mL) [18, 19].

MICROBIAL ANALYSIS

Microbial plate count method of starter cakes and rice beverages

Microbial analysis of *phap* and *Rokshi* samples was studied after the samples were brought to the laboratory under sterile conditions. The study involves the total viable count (standard plate count (SPC). Plate count agar (PCA) medium was used to quantify the total number of aerobic microorganisms. 10 grams of starter cake and 10 mL of *Rokshi* beverage were transferred into 90 mL of sterile diluent and were homogenized. A serial dilution was prepared by adding 1mL of the homogenate to sterile diluent tubes. 1 g of starter cake and 1mL of *Rokshi* beverage were pipetted into a sterile Petri dish, followed by the addition of 10 mL of molten plate count agar. Allow for solidification. Inoculated plates were incubated at 35± 2°C for 48 hrs. Colonies were counted using a digital colony counter; results are taken in triplicate and expressed as log CFU/g and CFU/mL for starter cake and rice beverage samples [20, 21, 22].

NON-TARGETED METABOLITE PROFILING OF RICE BEVERAGE BY GC-MS ANALYSIS

Sample preparation and analysis

A 100 mL sample was filtered through Whatman filter paper, run centrifuge for the aromatic compounds present in the rice beverage sample were extracted with a slight modification of the method of Patakova-Juzlova et al. 100 mL of the sample was first filtered through Whatman no. 4 filter paper and then distilled in a rotary evaporator (8763.RV0.000 Roteva, Equitron, India). Collect the distillate of 20% of the total volume. 10% (v/v) dichloromethane (DCM) was added to the distillate in a separating funnel, and the mixture was shaken for about 10 minutes. The DCM extract was filtered through a 0.2 µm pore-size filter using a syringe and stored at 4°C until analysis [23, 24].

GC-MS analysis

The analysis was performed using a Gas Chromatography-Mass Spectrometry (GC-MS) system (Agilent 7890 GC coupled with 5977 MSD) with HP-5MS capillary column (30 m × 250 µm × 0.25 µm film thickness). Helium was used as a carrier gas at a flow rate of 1.0 mL/min. Injection volume was 1 µL, and the sample was introduced in split mode with a split ratio of 10:1. The injector temperature ranged from -60℃ to 325℃, the pressure rate was 7.0699 psi, and the average velocity was 36.262 cm/sec. For MS, it was operated in electron ionization (EI) mode at 70 eV, with a run time of 6:50 minutes and a mass scan range of m/z 40-500. 34,593 emissions with 70 eV, 0.503 Repeller, 89- Ion focus, and 1888.848 EM Volts were used. Compounds have been identified by comparing the obtained mass spectra with those available in the NIST (National Institute of Standard and Technology) mass spectra library. Only compounds with a match quality score greater than 90% were considered for identification, a criterion further supported by comparison of retention times with literature-reported values.

Determination of Heavy Metals Concentration

Samples preparation and analysis

A 500 ml *Rokshi* sample was undergoing digestion for metal content analysis in accordance with standard protocols. The sample was first made CO_2-free by using an ultrasonic bath for 30 minutes. 25 mL of the sample was mixed with 10 mL of the conc. HNO_3, 1 mL conc. H_2SO_4 and 1 mL H_2O_2 were added to a beaker and digested on a hot plate for 40 min, until a straw-yellow color appeared. Allow to cool to room temperature, then filter and dilute to a final volume of 25 mL with distilled water [9, 12, 25]. The digested sample was ready for analysis, which was performed in triplicate using ICP-OES (Inductive Coupled Plasma-Optical Emission Spectroscopy). The assessment of 12 elements (cadmium, chromium, nickel, zinc, manganese, iron, copper, lead, sodium, potassium, calcium, and magnesium) was performed using the Avio 220 Max inductively coupled plasma optical emission spectrometer (PerkinElmer). The integration time is 0.1 sec; axial view; peak area read; echelle optical system; power used is 1450 W; plasma gas flow is 151/min; nebulizer gas flow rate is 0.8/min; nitrogen is the pure gas; argon is the plasma gas; and the sheath gas is air. All studies were conducted in triplicate (n=3) respectively.

Method for determination of Estimated daily intake (EDI)

Based on metal concentration found in the rice beverage sample, the EDI (µg/kg bw)/day) The amount of metals for a 60 kg adult was estimated from the daily consumption of these samples, in milliliters. The EDI value is calculated by using the following formula [26, 27].

$$EDI = \frac{C \times V}{W}$$

Where C (µg/mL) is the concentration of metals found in the rice beverage sample, V (mL) is the volume consumed (250 mL), and W (kg) is the body weight for a 60 kg adult.

Methods for the determination of health risk associated with rice beverage

The health risk assessment of beverages measures the adverse health effects on the human body from continuous exposure to chemicals such as metals. Average daily dose (ADD) and target hazard quotient (THQ) are a few parameters to evaluate the health risk. The average daily dose of heavy metals was calculated by using the following formula [28].

$$ADD = \frac{Ci \times MDI \times EF \times ED}{ABW \times AT}$$

where Ci is the metal concentration in rice beverages, MDI is the ingestion rate, EF is the exposure frequency, ABW is the body weight of the consumer, and AT is the average time.

THQ is the estimation of the non-carcinogenic risk level to human health with heavy metals. Consumption of rice beverages 250 mL per day, with a THQ less than 1, indicates a safe level, where a THQ greater than or equal to 1 indicates a level of concern for human health. THQ can be expressed by the following equation [29].

$$THQ = \frac{Ci \times MDI \times EF \times ED}{RfD \times BW \times AT} \times 10^{-3}$$

Where, EF is for exposure frequency (days/year), ED is the exposure duration (year), MDI is the ingestion rate (250 mL/day), Ci is the concentration of metals found in the samples, RfD is the oral reference dose (mg/kg/day), BW is the average adult body weight (assuming 60 kg), AT is the average time for non-carcinogenic (day) and 10-3 is the unit conversion factor. The reference dose (mg/kg/day) was calculated using assumptions from the Integrated United States EPA risk analysis [30].

Statistical analysis

All statistical analyses were performed using the Microsoft Excel software (MS Office 2021). All experiments were performed in triplicate (n=3), and results are expressed as mean ± standard deviation (SD). In addition, one-way ANOVA was performed to study the significance of the data. The minimum value of $^*p<0.05$ is considered to be significant, $^{**}p<0.01$ is considered to be more significant, and $^{***}p<0.001$ is considered to be extremely significant as compared with the control group.

RESULT AND DISCUSSION

A sample for the study was collected from a specific locality in Arunachal Pradesh, and all sample details are presented in Table 1. These included a starter culture (*phah*) and its corresponding rice beverages *(Rokshi)*. The samples were collected from the main tribes of Arunachal Pradesh, a Monpa tribe. The starter cake was prepared by adding various medicinal plants, which are the main ingredients for preparing beverages, and these ingredients are low-cost. Most of the medicinal uses of *Rokshi* have been investigated by elderly members of the Monpa community.

This community uses *Rokshi* throughout the year, and most of the preparations for *Rokshi* are made by the women of the community. Table 1 shows the sample codes, local names, cultural links, and source locations. The phytochemical can play an important role in maintaining human health when consumed properly. These phytochemical constituents present in *Rokshi* can act through complementary and overlapping mechanisms to stimulate the immune system, modulate oxidative stress, regulate gene expression, and induce apoptosis.

Table 1: Details of starter cakes and rice beverages collected from Arunachal Pradesh, a Northeast state, India.

Sample	Local name	Tribe/ Community	Place of collection
Starter Cake	Phap	Monpa	Tawang
Rice beverage	Rokshi	Monpa	Tawang

Note: All the places and locations are from Arunachal Pradesh of northeast India.

Biochemical characterization of starter cake

A starter cake ‘phah’ was prepared from glutinous rice. Table 2 presents the proximate composition of the starter cake samples (SC1, SC2, and SC3), including moisture, crude fat, crude fiber, and ash content; these data agree with other reports on different rice beverages [15, 16, 17]. Moisture content ranged from 14.85% to 15.70%, with the highest value observed in SC3, attributable to differing preparation conditions and practices. Crude fiber content ranged from 7.90% - 8.60%, with the highest in the SC2 sample, due to differences in the plant-based mixture of herbal ingredients during preparation. Similarly, crude fat ranged from 1.02% to 1.15%. Starch content ranged from 50.70 to 51.90 g/100g, with SC2 showing a higher value. Ash content ranged from 0.25% to 0.34%, while amylopectin content remained consistent.

Biochemical composition of rice beverages

The rice beverage samples (RB1, RB2, RB3) underwent physical and biochemical analyses under fresh and 1-month storage conditions, with triplicate measurements (n=3), and are presented in Table 3. The collected traditional rice beverage was stored in the laboratory, and changes in various parameters were observed and analyzed over a period of one month for three samples from three different households in the same village of Khardung. The Fresh *Rokshi* samples exhibited a slightly sweet taste, a cloudy-to-milky-white appearance, and a sour-sweet odor. In contrast, the one-month-stored samples showed a sour taste, a yellow color, and an aromatic odor. The pH deteriorated after one month of storage, with a decrease from 4.10-4.15 in fresh samples to 3.09–3.12 in one-month storage samples, indicating increased acidity due to the fermentation process.

Table 2: Biochemical composition of starter cakes that are used in the preparation of rice beverages.

Sample Name	Moisture %	Crude fiber%	Crude fat %	Ash content	Amylo pectin%	Starch	Total soluble sugar	Reducing sugar	Non-reducing sugar
SC1	15.26 ± 0.053^{a}	8.23 ± 0.035^{b}	1.08 ± 0.004^{a}	0.28 ± 0.005^{b}	67.13 ± 0.002^{a}	51.2± 0.010^{b} (gm/100gm)	2.03 ± 0.002^{b} (gm/100gm)	0.21 ± 0.015^{b} (gm/100gm)	1.33 ± 0.003^{b} (gm/100gm)
SC2	14.86 ± 0.060^{a}	8.60 ± 0.045^{a}	1.15 ± 0.006^{a}	0.34 ± 0.008^{a}	66.80 ±0.003^{a}	50.7± 0.015^{c} (gm/100gm)	2.25 ± 0.018^{a} (gm/100gm)	0.27 ± 0.018^{a} (gm/100gm)	1.43 ± 0.005^{a} (gm/100gm)
SC3	15.70 ± 0.054^{a}	7.90 ± 0.051^{c}	1.02 ± 0.004^{a}	0.25 ± 0.006^{c}	67.45 ± 0.003^{a}	51. 8± 0.013^{a} (gm/100gm)	1.84 ± 0.003^{c} (gm/100gm)	0.16 ± 0.012^{c} (gm/100gm)	1.27 ± 0.004^{c} (gm/100gm)

Note: SCI, SC2, and SC3 represent independent starter cake samples collected from different households of the Monpa tribe, Arunachal Pradesh; results are expressed as mean ± standard deviation (n=3). Moisture content, crude fiber, crude fat, ash content, and amylopectin are expressed in %; starch, total soluble sugar, reducing sugar, and non-reducing sugar are expressed in gm/100 g. Different superscript letters (a,b,c) within the same column indicate statistically significant differences.

As observed from the study of indigenous fermented beverages, the microorganism species, such as yeast sp., could participate in the initial stage of fermentation and subsequently the acid formation, which results in decreasing the value of *p*H until a large amount of acid is produced, which leads to the production of LAB and yeast by-products [3,8,10]. The fresh *Rokshi* samples had a total acidity of 0.49%-0.58% (% lactic acid), whereas the one-month-old samples showed 0.76 ± 0.02% (% lactic acid). This indicates that total acidity increases as the fermentation process progresses, in agreement with previous studies [26,37]. Additionally, the alcohol by volume (% ABV) increased significantly during storage, reaching 7.54 ± 0.02% after one month. This change was directly correlated with the fermentation process and the availability of fermentable sugars in the medium. Furthermore, Cottet et al. [31] and Salanta et al. [17] observed an increase in the beverage's alcohol content during storage, attributing it to active yeast metabolism of available sugars at warm temperatures. Since *Rokshi* is a traditional rice beverage that is also unpasteurized, a drastic change in alcohol content was observed in a one-month-old rice beverage compared with a freshly prepared one. The reason is that the fermentation was still ongoing during storage, and it contained active yeast and a microbial population derived from the starter culture and fermentation environment. The presence of this microorganism can sustain the metabolism of residual fermentable sugar, resulting in gradual ethanol accumulation over time. *Rokshi* has been prepared through traditional fermentation practices, which often lack strict control or a standardized environment for fermentation duration, microbial composition, temperature, and storage conditions. As a result, microbial metabolism depends on the availability of residual sugars, allowing fermentation to continue during storage periods. Therefore, a lower alcohol content was observed in the freshly *Rokshi,* indicating an early stage of fermentation rather than a fully stabilized product. Further studies may consider monitoring the kinetic and stabilization products in these rice beverages. The sensory characteristics changed from slightly sweet and cloudy to sour, and yellow-tinted over time, which agrees with others' report [19]. The observation from the viscosity study shows that viscosity decreased notably after one month, ranging from 18.50 – 20.10 cPs, whereas fresh samples ranged from 58.90 – 61.10 cPs, reflecting the accumulation of alcohol and acidification, unlike the commercial beer that undergoes all the chemical preservation, sterile packaging, and stringent pasteurization methods to inhibit microbial growth and stabilize over a longer period of time, while *Rokshi* beverage did not undergo any of these methods, which led to initial thickness due to microbial degradation of long-chain molecules and affected the viscosity properties. The alcohol content showed a significant increase across all samples, from 2.59% - 2.72% in fresh to 7.30% - 7.80% in aged.

Total flavonoid and total phenolic content of *Rokshi*

Flavonoids are also known as scavengers of free radicals, owing to the presence of aromatic rings and redox-active sites in phenolic compounds, which enable them to absorb and inhibit free radicals. The total flavonoids content of *Rokshi* from three difference household showed RB1 to be at 0.205 ± 0.003 mg QE/mL, RB2 (0.198 ± 0.004 mg QE/mL) and RB3 (0.212 ± 0.003 mg QE/mL) in fresh samples, whereas one-month stored samples observed to be decreased in TPC in which RB1 found to be at 0.178 0.004 mg QE/mL, RB2 (0.174 0.005 mg QE/mL) and RB3 (0.182 0.004 mg QE/mL) respectively. Total phenolic content (TPC) in fresh beverage samples of RB1, RB2, and RB3 was found to be 0.928 ± 0.013 mg GAE/mL, 0.907 ± 0.015 mg GAE/mL, and 0.861 ± 0.016 mg GAE/mL, respectively. As storage time increased, the beverage samples showed a decline in TPC. Therefore, the RB1, RB2, and RB3 samples of one-month-old stored showed TPC at 0.851 ± 0.016 mg GAE/mL, 0.841 ± 0.018 mg GAE/mL, and 0.866 ± 0.016 mg GAE/mL, respectively. The presence of phenolic and flavonoid content in the sample could indicate free radical inhibition. At the same time, its moderate value could explain the high ethanol concentration in the sample, which can inhibit enzymes. Compared with data from previous studies, the TPC content ranged from 0.59 to 2.81 mg GAE/100 mL, indicating that *Rokshi* rice beverage exhibits antioxidant activity similar to that of other rice beverages [6, 14, 15].

Table 3: Comparative study of fresh and aged rice beverage samples of the Monpa tribe from Arunachal Pradesh.

Sample Code	Condition	Taste	Colour	Odour	pH	Alcohol %	Total acidity	Viscosity (CPs)
RB1	Fresh Sample	Slightly sweet	Cloudy white	Sour-sweet	4.10 ± 0.01^{a}	2.59 ± 0.02^{c}	0.58 ± 0.01^{c}	60.34 ± 0.01^{a}
	After 1 month	Sour	Yellow	Aromatic	3.09 ± 0.01^{b}	7.54 ± 0.02^{a}	0.76 ± 0.02^{a}	19.04 ± 0.03^{c}
RB2	Fresh Sample	Mild sweet	Milky white	Slight sour	4.06 ± 0.02^{a}	2.77 ± 0.03^{c}	0.61 ± 0.02^{c}	57.91 ± 0.02^{a}
	After 1 month	Sour	Pale yellow	Strong aroma	3.04 ± 0.02^{b}	7.79 ± 0.03^{a}	0.81 ± 0.03^{a}	18.51 ± 0.02^{c}
RB3	Fresh Sample	Slightly sweet	Cloudy white	Mild sour	4.12 ± 0.01^{a}	2.44 ± 0.02^{c}	0.49 ± 0.01^{c}	60.10 ± 0.02^{a}
	After 1 month	Sour	yellowish	aromatic	3.11 ± 0.01^{b}	7.30 ± 0.02^{b}	0.74 ± 0.01^{b}	20.12 ± 0.03^{b}

CPs- (centipoise) unit for Viscosity. Result is expressed as mean ± standard deviation (n=3). Different superscript letters (a, b, c) within the same column revealed statistically significant differences ($p < 0.05$) according to one-way ANOVA.

Microbial count in *Rokshi* rice beverage

Microbial enumeration showed the starter culture of *phap* levels is 2.15 ± 0.024 log_{10} CFU/mL. These levels gradually decreased in the finished beverages of *Rokshi* to 0.55 ± 0.024 log_{10} CFU/mL, as shown in Table 4. The *phah* starter cake showed a higher microbial count than the finished product, *Rokshi*. The microbial numbers from the starter cake to the finished product indicate partial microbial die-off during the alcoholic fermentation phase. The patterns of bacterial and fungal growth matched traditional back-slopping fermentation practices.

GC-MS analysis for metabolites profile

The use of various medicinal plants in the traditional preparation of starter cake, which is mixed with cooked rice for fermentation, yields the final rice beverage. Therefore, the rice beverage product contains various bioactive compounds that contribute to human health benefits when used in appropriate, limited amounts. In this study, GC-MS is used to determine and identify these biochemical compounds produced in the *Rokshi*. From Table 5, the GC-MS analysis reported about 28 compounds across the sample. Shared metabolites included 2,3-butanediol, lactic acid, and isopropyl alcohol. 2,3-butanediol is formed by yeast and has a characteristic odor during carbohydrate fermentation. Cis-vaccenic acid is one of the fatty acid compounds that can be used against cardiovascular diseases and is also a precursor to conjugated linoleic acid. DL-xylose has been reported to support gut function and was produced in the *Rokshi* beverage [18, 23]. Ethyl acetate is a major compound that can be metabolized into ethanol by esterase in the body. Tyramine, N-formyl and N-acetyl tyramine, which are biogenic amine derivatives, and valeramine, N-propyl, are also in the biogenic amine derivative family and can pose health concerns at high concentrations. These products primarily originate from yeast, lactic acid bacteria, and environmental flora. Compounds were grouped into six main classes: alcohols, acids, sugars, amino acids, fatty acids, and minor classes, including aldehydes, lactones, esters, and ethers. Notably, some compounds, if present at higher concentrations in rice beverages, might have harmful effects on consumers. Compounds like tyramine, dibutyl phthalate, hydrazine derivatives, and N-formyl-tyramine were also identified in a rice beverage. All chemical compounds produced by fermented rice beverages, such as Rokshi, have been categorized as shown in Table 5. After the investigation, it was observed that alcohol compounds accounted for the largest proportion, followed by acid compounds, amino acids, sugars, fatty acids, aldehydes, and other compounds in a lower range (hydrocarbons, ketones, lactones, esters, and ethers), as shown in Figure 2.

Table 4: Microbial colony counts of starter culture and rice beverage samples.

Sample Name	Dilution factor	Mean log CFU/mL (x 10^7)	Mean log CFU/g (x 10^7)	Inoculum Volume (ml)	SD	1 SD range (x 10^7)
Phap	10^{-4}	-	2.15	0.1	0.024	2.17-2.12
Rokshi	10^{-4}	0.55	-	0.1	0.024	0.58-0.53

Note: Mean CFU/mL and CFU/g - calculate average microbial count for each sample, SD-standard deviation, 1 SD Range- range of values within 1 SD from the mean. The value is the mean of triplicates (n=3) for 3 different dilutions, and the most reliable and statistically significant dilution (10^{-4}) is selected.

Table 5: List of the biochemical metabolites obtained in *Rokshi* rice beverage and their relative percentage detected through GC-MS analysis.

Compound Name	Molecular formula	Molecular weight (g/mol)	Ret. Time (min)	Relative %
2,3-butanediol	$C_4H_{10}O_2$	90.12	3.657	6.57
2-Deoxy-O-galactose	$C_6H_{12}O_5$	164.16	5.121	4.69
2-hydroxy-gamma-butyrolactone	$C_4H_6O_3$	102.09	4.913	1.92
2-methyl, hexanoic acid	$C_7H_{14}O_2$	130.18	6.021	3.15
3-hydroxy, Butanoic acid	$C_4H_8O_3$	104.1	6.487	4.91
3-methyl,4-Heptanol	$C_8H_{18}O$	130.229	15.25	2.17
Acetic acid	$C_2H_4O_2$	60.05	23.41	3.52
Acetic acid,trifluoro-dodecyl ester	$C_{14}H_{23}F_3O_2$	282.34	21.564	2.57
Acetoin	$C_4H_8O_2$	88.11	5.544	2.16
Butyrolactone	$C_4H_6O_2$	86.09	4.864	20.60
cis-vaccenic acid	$C_{18}H_{34}O_2$	282.47	27.859	1.92
D-Aspartic acid	$C_4H_7NO_4$	133.1	18.154	2.37
D-Fucose	$C_6H_{12}O_5$	164.16	5.27	4.69
dl-beta-phenyllactic acid	$C_9H_{10}O_3$	166.17	16.149	2.22
DL-Xylose	$C_5H_{10}O_5$	150.13	11.1	3.31
Ethyl acetate	$C_4H_8O_2$	88.11	5.405	2.42
Glycerol,1-palmitate	$C_{19}H_{38}O_4$	330.5	31.488	2.41
isopropyl alcohol	C_3H_8O	60.1	3.66	7.43
L-Arabinose	$C_5H_{10}O_5$	150.13	7.81	1.39
L-lactic acid	$C_3H_6O_3$	90.08	5.565	3.88
N-acetyl tyramine	$C_{10}H_{13}NO_2$	179.22	8.11	1.80
O-Acetyl-L-Serine	$C_5H_9NO_4$	147.13	20.35	1.41
Phenylethyl Alcohol	$C_8H_{10}O$	122.16	7.82	2.30
propanoic acid,2-hydroxy-ethyl ester	$C_5H_{10}O_3$	118.13	3.48	1.62
Propylene glycol	$C_3H_8O_2$	76.09	4.225	1.99
Tryptophol	$C_{10}H_{11}NO$	161.2	24.322	1.69
Tyramine, N-formyl	$C_9H_{11}NO_2$	165.18	17.217	1.98
Valeramine, N-Propyl	$C_8H_{19}NO$	143.23	9.935	2.92

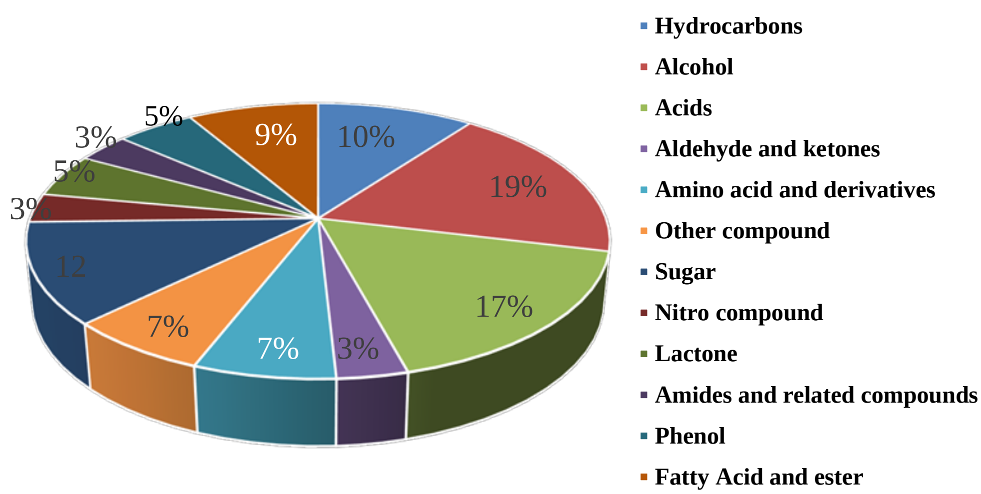

Figure 2: Chemical compound distribution according to their functional groups in the samples.

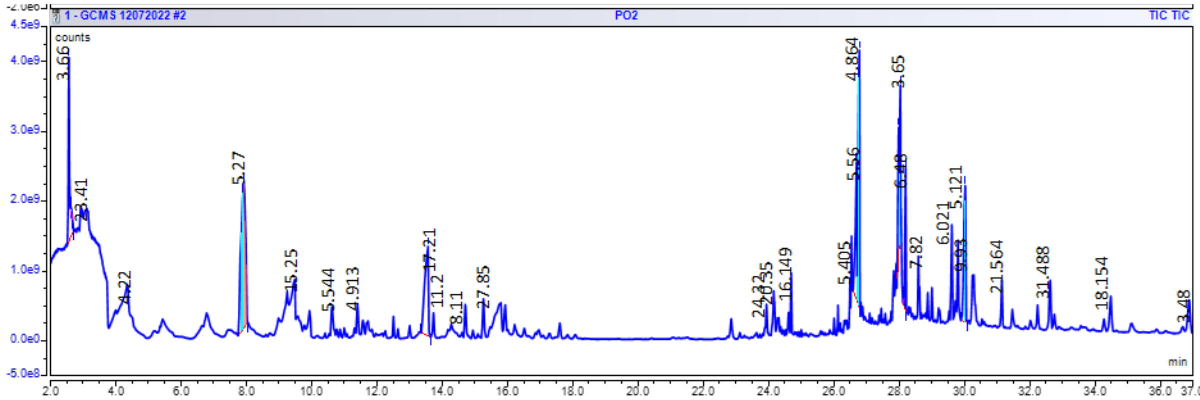

Figure 3: GC-MS total ion chromatogram of *Rokshi* beverage sample showing peaks separation of metabolites based on retention time (RT) with compound identification using NIST library matching.

Heavy Metals Determination

In the *Rokshi* rice beverage sample, the metal concentration usually depends on factors such as the raw materials used, the preparation procedure, storage, and the utensils used. Table 7 presents the essential element concentrations and standard deviations for Cr, Mn, Cu, Zn, Fe, Ni, Cd, Pb, Na, Mg, K, and Ca in the *Rokshi* rice beverage sample from Arunachal Pradesh in Northeast India. All metal levels have been compared with the maximum permissible limit (MPL) or the recommended daily allowance (RDA) as specified by the International Organization for Grapes and Wines, as shown in Table 6. All the heavy metals in *Rokshi* are found to be within the set permissible limits. Lead concentration in the sample is high but remains within safe limits. Since all the metal concentrations present in this rice beverage are below the permissible limit or within the safe limit, it is safe to consume it. The estimated daily intake (EDI) is determined in µg/kg bw/day of various metals present in rice beverages. The EDI of Cr from consumption of rice beverage in *Rokshi* is 0.132 µg/kg bw/day, which is comparable to the reported values for beers [29, 30]. It is found to be below the EDI tolerance value of 1 µg/kg bw/day. From consumption of *Rokshi* rice beverage, for metals like manganese (Mn), copper (Cu), iron (Fe), and nickel (Ni), the EDI concentration of these heavy metals was reported to be 0.345 µg /kg bw/day,0.095 µg /kg bw/day, 1.317 µg /kg bw/day, and 0.151 µg /kg bw/day, respectively. The reported intake values were below the maximum permissible limits for the recommended dietary allowance (Table 7) and were consistent with those reported in previous studies [28]. According to the JECFA (Joint (FAO/WHO) Export Committee on Food Additives), the tolerable daily intake for metals such as zinc is 1000 µg/kg bw/day [33]. Based on *Rokshi* consumption, the EDI for Zn was 3.75 µg/kg bw/day, which is within the maximum permissible limit as per WHO and FAO guidelines, as shown in Table 7. The value was compared with the previously available data [27, 28].

Table 6: Oral reference doses (RfD) for metals (assigned by USEPA, WHO, FAO)

Metal	RfD (mg/kg bw/day) for heavy metals
Cd	1 × 10 -3
Co	3 × 10 -4
Cr	1.5
Cu	4 × 10 -2
Fe	7 × 10 -1
Mn	1.4 × 10 -1
Ni	2.0 × 10 -3
Pb	4 × 10 -3
Zn	3 × 10 -1
Na	-
Mg	-
K	-
Ca	-

Cd; cadmium, Co; cobalt, Cr; chromium, Cu; copper, Fe; iron, Mn; manganese, Ni; nickel, Pb; lead, Zn; zinc, Na; sodium, Mg; magnesium, K; potassium, and Ca; calcium.

Harmful elements that pose severe health risks, such as lead and cadmium, were also found in the *Rokshi* rice beverage sample. Their estimated daily intake values were 0.146 µg/kg bw/day and 0.013 µg/kg bw/day for Pb and Cd, respectively (Table 7). When the EDI for metals was compared with the WHO-recommended dietary intake, it was found to be below the 1 µg/kg bw/day limit [33]. In this rice beverage sample, some essential minerals that are important for the biological functioning of the body were also present. Such minerals are calcium, potassium, sodium, and magnesium [34]. The values are expressed in µg /kg bw/day for these minerals. The EDI value of these essential minerals, such as Na, K, Ca, and Mg, is represented in Table 7. Their values were under the maximum permissible limit [35, 36].

Table 7: Heavy metals concentrations in *Rokshi* rice beverage, Estimated Daily Intake (EDI), Average Daily Dose (ADD), and non-carcinogenic risk (THQ) for consumption of *Rokshi* rice beverage.

Elements	Metal Conc. (mg/L)		EDI (mg/Kg Bw/day)	ADD (mg/Kg Bw/day)	THQ (mg/Kg Bw/day)	Recommended Daily Allowance (RDA) values
	Conc. (mg/L) ± SD	RSD %				
Cr	0.031 ± 0.09	0.31	0.132	0.00013	0.0001	2.2μg/kg bw/day [13, 26]
Mn	0.08 ± 0.64	0.53	0.345	0.00034	0.0024	2- 5mg/day/person [28]
Cu	0.022 ± 0.12	0.53	0.095	0.00009	0.0023	15-500μg/kg bw/day [27,28]
Zn	0.9 ± 1.29	0.14	3.750	0.00375	0.0120	1000μg/kg bw/day [28, 29
Fe	0.31 ± 7.31	0.45	1.317	0.00132	0.0018	10-18mg/day/person [28, 29]
Ni	0.03 ± 0.32	0.89	0.151	0.00015	0.0628	5μg/kg bw/day [5, 12]
Cd	0.003 ± 0.05	4.81	0.013	0.00001	0.0129	1μg/kg bw/day [13, 26]
Pb	0.035 ± 0.29	0.83	0.146	0.00015	0.0351	7.14μg/kg bw/day [13, 26]
Na	5.206 ± 0.1	1.3	14.35	0.021693402	-	2000 mg/day/ adult person [5, 12, 13]
Mg	9.773 ± 0.02	0.37	42.70	0.040724091	-	400 mg/day/person 12, [13]
K	29.7 ± 0.31	0.35	108.7	0.1237599	-	3510 mg/day/ adult person [5, 12]
Ca	9.935 ± 0.11	0.46	30.56	0.041399145	-	1200 mg/day/person [12,13]

RSD: Relative standard deviation, EDI: Estimate daily intake, ADD: Allowance daily dose, THQ: Target hazard quotient, RDA: Recommended Daily Allowance. All metal concentration values are expressed in mean ± SD (n=3)

In the ingestion of *Rokshi* rice beverage samples, there will be exposure to these contaminated metals, and the average daily dose of these metals needs to be determined. Therefore, the ADD values for all metals, both essential minerals and harmful metals, are calculated and presented in Table 7. They equally had low values of ADD. Target hazard quotient (THQ) is defined as an estimate of the health risk from heavy metals in rice beverages consumed daily. To assess the dietary intake of these heavy metals, Rokshi, a traditional rice beverage from Arunachal Pradesh, was used. The ingestion rate was assumed to be 250mL/day of rice beverages. In this study, the average weight was taken as 60kg for adults. The estimated THQ values for individual metals in a sample, as shown in Table 7, from consuming 250 mL of glass per day, were all less than 1. The THQ values 1 indicate levels of concern. For THQ value =20 is larger but not 10-fold bigger than a THQ=2, since THQ values are additive, not multiplicative. From the current data, it was observed that each metal such as Cd, Cu, Cr, Pb, Fe, Mn, Zn present in *Rokshi* rice beverage, the total target hazard quotients (TTHQ) value is TTHQ< 1, indicated that it is in safe level for consumption of these beverages and no health risk according to previous studies and international guidelines such as USEPA and WHO [27, 28, 36].

DISCUSSION

The present study reported that the preparation of the traditional starter cake (phap) and the fermented rice beverages (Rokshi) were drawn from the indigenous knowledge system passed down from one generation to another. The composition of ingredients may vary depending on the communities, the type of rice variety (glutinous or non-glutinous) used, and the specific herbs or medicinal plants used. These ingredients influence the flavor, aroma, taste, color, and biochemical composition of the rice beverages. Additionally, the utilization of medicinal herbs can contribute to the production of nutritional and bioactive compounds in the rice beverages of the Monpa tribe from Arunachal Pradesh. The findings in this study are consistent with previous reported data on traditional fermented rice beverages. Microbial activity also plays an important role in the fermentation process (*Rokshi* beverages), which could be beneficial for gut health, by converting fermentable sugar into various by-products such as organic acid, lactic acid, amino acid, alcohol, and various secondary metabolites through different pathways, such as lipid metabolism, resulting in the production of fatty acid, transamination, deamination, decarboxylation, and glycometabolism. As a result, a one-month-old sample of *Rokshi* was found to have higher acidity and a lower pH, which, if not addressed, could lead to sourness and damage to the drink.

The GC-MS results also showed support that, in the presence of key fermentation metabolites such as organic acid, 2,3-butanediol, sugar, fatty acid, volatile aromatic compounds (such as aldehyde, ketone, and ester), and alcohol, contribute to flavor, odor, and aroma during the LAB fermentation process [10, 20, 22]. Similarly, the high abundance of a compound such as butyrolactone could reflect secondary metabolic activity. Decreased viscosity in the *Rokshi* beverage after one month of storage, relative to a freshly prepared sample, suggests structural changes in the sample matrix during aging due to enzymatic degradation of complex macromolecules by microorganisms present in the fermented beverage. Since the traditional fermented beverage contains diverse microbial communities, including lactic acid bacteria and some yeast, these microbes can produce hydrolytic enzymes, such as amylase and glucoamylase, thereby reducing the viscosity of the rice beverage stored for over one month.

The flavonoid compound can increase blood antioxidant capacity and the activity of antioxidant enzymes, thereby reducing oxidative stress. The presence of flavonoid and phenolic compounds in the rice beverage was attributable to the ingredients used in preparing the starter cake. These ingredients are no less than medicinal plant materials, which is why the identification and evaluation of antioxidant compounds in rice beverages were undertaken, contributing to human health benefits [6, 15]. However, excessive consumption of this beverage for a longer period of time can be a serious cause of liver disease because there is the metabolism of ethanol, which generates the reactive oxygen species (ROS). Therefore, the presence of antioxidant phytochemicals, such as polyphenols, can contribute to health at higher concentrations. Still, their activity may be reduced at lower concentrations due to ethanol's indirect inhibition of antioxidant enzymes in the human body. Thus, the increase or decrease in antioxidant activity in rice beverage can depend on the alcohol concentration and the fermentation temperature [37].

According to the literature, organic acids play a vital role in regulating the acid-base balance during fermentation, which may affect the color, flavor, and biological stability of the rice beverage. Many nutritional and bioactive components have been reported in this study; the detection of a few components, such as dibutyl phthalate, hydrazine derivatives, and bioactive amines like tyramine, raises important health concerns about food safety. Previous studies on biogenic amine production in beverages indicate that biogenic amines, such as tyramine, putrescine, and cadaverine, have inflammatory effects that can cause adverse health effects. These BAs are by-product compounds formed by microbial activity in beverages; if present at high concentrations, they can cause toxic or inflammatory effects on human health [36, 36]. Biochemical transformations in rice beverages during storage or fermentation can lead to the formation of many bioactive compounds, as explained earlier, which may contribute to nutritional benefits. Still, if the traditional method is not conducted under standardized protocols and environmental conditions, uncontrolled fermentation can lead to undesirable microbial growth and the production of toxic compounds. Therefore, it is very important to understand both microbial growth and biochemical production under fermentation and storage conditions to ensure human health. The study also reported the presence of essential minerals and heavy metals in contaminated samples. Although all detected metals were within maximum permissible limits, the THQ value was low; however, continuous consumption of these beverages may still pose long-term health risks, including kidney damage, cancer, and neurological disorders. The findings highlight both nutritional and functional benefits of consuming these *Rokshi* beverages, while also needing evaluation for potential safety risks.

CONCLUSION

The study reveals that the rice beverage, namely Rokshi, produced in Arunachal Pradesh, has significant antioxidant properties, nutritional value, and beneficial microbial activity, making it a potential functional fermented beverage. This study provides novel insights into *Rokshi* rice beverages from the Monpa tribe of Arunachal Pradesh, India. The findings provide that fermentation could contribute to the production of alcohol, organic compounds, and other metabolites that could enhance the nutritional properties of the *Rokshi* beverage. However, some components, such as tyramine, hydrazine-related derivatives, and dibutyl phthalate, are also identified as produced in this beverage, suggesting a health concern that warrants evaluation. It could be due to the use of raw materials that are not properly cleaned and washed before fermentation, such as water and plant materials, or to the use of utensils that release harmful components during fermentation. Such components include biogenic amines and heavy metals such as lead and cadmium, since the beverage was made under household conditions. Therefore, their storage conditions or preparation may not be standardized, as they should be for health reasons. Even though consuming *Rokshi* beverage may not be harmful to consumers whose consumption is under limit, it can lead to some serious health risks (such as neurological disorders, kidney damage, and cancer) if consumed on an irregular basis. The presence of such compounds highlights the need for further quantitative toxicological studies, quality control, safety checks, and improvement. This study also emphasizes preserving the traditional fermentation process; therefore, collecting the rice beverages directly from the local without preparing them in the laboratory is important. It also ensures consumer safety through scientific methods or traditional methods, if available. Future work should focus on purification and pasteurization, and on conducting a clinical trial to establish health benefits and consumer safety.

FINANCIAL ASSISTANCE
NIL

CONFLICT OF INTEREST
The authors declare no conflict of interest.

AUTHOR CONTRIBUTION
Rosamund Jyrwa and Ananta Choudhury conducted the research design, data collection, and manuscript writing and editing. The manuscript was critically revised by Ananta Choudhury and finally approved by all the authors for further publication.

REFERENCES

[1] Taveira IC, Nogueira KMV, Olivera DLG, Silva RN. Fermentation: humanity's oldest biotechnological tool. *Front Young Minds*, **9** (2021) https://doi.org/10.3389/frym.2021.568656

[2] Koay M, Fan H, Wong C. An overview of fermentation in rice winemaking. *Canrea J Food Technol Nutr Culinary J*, **5(1)**, 12–37 (2022) https://doi.org/10.20956/canrea.v5i1.629

[3] Salanță LC, Coldea TE, Ignat MV, Pop CR, Tofană M, Mudura E, Borșa A, Pasqualone A, Zhao H. Non-alcoholic and craft beer production and challenges. *Processes*, **8(11)**, 1382 (2020) https://doi.org/10.3390/pr8111382

[4] Rios-Corripio G, Hernández-Carranza P, Ruiz-López I, Guerrero-Beltrán JÁ, Ochoa-Velasco CE. Health benefits of fermented alcoholic beverages. In: *Bioactive Compounds in Fermented Foods*, 265–299 (2021) https://doi.org/10.1201/9780429027413-14

[5] Deka AK, Handique P, Deka DC. Ethnic food beverages with heavy metal contents: parameters for associated risk to human health, North-East India. *Toxicol Rep*, **8**, 1220–1225 (2021) https://doi.org/10.1016/j.toxrep.2021.06.013

[6] Handique P, Deka AK, Deka DC. Antioxidant properties and phenolic contents of traditional rice-based alcoholic beverages of Assam, India. *Natl Acad Sci Lett*, **43(6)**, 501–503 (2020) https://doi.org/10.1007/s40009-020-00903-5

[7] Deka DC. Traditional rice-based alcoholic beverages in India. *Ref Ser Phytochem*, 889–921 (2025) https://doi.org/10.1007/978-3-031-38663-3_140

[8] Devika HR, Harsha KM, Kalpana R, Shet KG, Rashmi D. Development of a fermented probiotic beverage inoculated with kefir grain. *Asian J Dairy Food Res* (2024) https://doi.org/10.18805/ajdfr.dr-2155

[9] Lange KW, Nakamura Y. Amazake (Japanese fermented rice beverage) and its potential health benefits. *Food Sci Hum Wellness*, **14(1)**, 9250076 (2025) https://doi.org/10.26599/FSHW.2024.9250076

[10] Latif A, Shehzad A, Niazi S, Zahid A, Ashraf W, Iqbal MW, Rehman A, Riaz T, Aadil RM, Khan IM, Özogul F, Rocha JM, Esatbeyoglu T, Korma SA. Probiotics: mechanism of action, health benefits and their application in food industries. *Front Microbiol*, **14**, 1216674 (2023) https://doi.org/10.3389/fmicb.2023.1216674

[11] Peng B, Huang H, Xu J, Xin Y, Hu L, Wen L, Li L, Chen J, Han Y, Li C. Rice wine fermentation: unveiling key factors shaping quality, flavor, and technological evolution. *Foods*, **14(14)**, 2544 (2025) https://doi.org/10.3390/foods14142544

[12] Dobrowolska-Iwanek J, Zagrodzki P, Galanty A, Fołta M, Kryczyk-Kozioł J, Szlósarczyk M, Rubio PS, de Carvalho IS, Paśko P. Determination of essential minerals and trace elements in edible sprouts from different botanical families—application of chemometric analysis. *Foods*, **11(3)**, 371 (2022) https://doi.org/10.3390/foods11030371

[13] Balali-Mood M, Naseri K, Tahergorabi Z, Khazdair MR, Sadeghi M. Toxic mechanisms of five heavy metals: mercury, lead, chromium, cadmium, and arsenic. *Front Pharmacol*, **12** (2021) https://doi.org/10.3389/fphar.2021.643972

[14] Rai AK, Appaiah KAA. Bioactive compounds in fermented foods. *Bioactive Compounds in Fermented Foods* (2021) https://doi.org/10.1201/9780429027413

[15] Najman K, Ponikowska P, Sadowska A, Hallmann E, Wasiak-Zys G, Świderski F, Buczak K. Physicochemical properties and nutritional relevance of rice beverages available on the market. *Appl Sci*, **14(19)** (2024) https://doi.org/10.3390/app14199150

[16] Muzaifa M, Abubakar Y, Safrida N, Nilda C, Sapitri M. Alcohol content and chemical characteristics of fermented beverages in Aceh Province, Indonesia. *IOP Conf Ser Earth Environ Sci*, **1241(1)**, 012095 (2023) https://doi.org/10.1088/1755-1315/1241/1/012095

[17] Cuamatzin-García L, Rodríguez-Rugarcía P, El-Kassis EG, Galicia G, Meza-Jiménez ML, Baños-Lara MDR, Zaragoza-Maldonado DS, Pérez-Armendáriz B. Traditional fermented foods and beverages from around the world and their health benefits. *Microorganisms*, **10**, 1151 (2022) https://doi.org/10.3390/microorganisms10061151

[18] Ofoedu CE, You L, Osuji CM, Iwouno JO, Kabuo NO, Ojukwu M, Agunwah IM, Chacha JS, Muobike OP, Agunbiade AO, Sardo G, Bono G, Okpala COR, Korzeniowska M. Hydrogen peroxide effects on natural-sourced polysaccharides: free radical formation/production, degradation process, and reaction mechanism—A critical synopsis. *Foods*, **10(4)**, 699 (2021) https://doi.org/10.3390/foods10040699

[19] Lorencová E, Salek RN, Černošková I, Buňka F. Evaluation of force-carbonated Czech-type lager beer quality during storage in relation to the applied type of packaging. *Food Control*, **106**, 106706 (2019) https://doi.org/10.1016/j.foodcont.2019.106706

[20] Loying R, Kalita J, Manna P. Rice-based alcoholic fermented beverages of North-East India: insight into ethnic preparation, microbial intervention, ethnobotany, and health benefits. *J Food Biochem*, e7769743 (2024) https://doi.org/10.1155/2024/7769743

[21] Canaviri-Paz P, Oscarsson E, Kjellström A, Olsson H, Jois C, Håkansson Å. Effects on microbiota composition after consumption of quinoa beverage fermented by a novel xylose-metabolizing *Lactiplantibacillus plantarum* strain. *Nutrients*, **13(10)**, 3318 (2021) https://doi.org/10.3390/nu13103318

[22] da Silva Vale A, Venturim BC, da Silva Rocha ARF, Martin JGP, Maske BL, Balla G, De Dea Lindner J, Soccol CR, de Melo Pereira GV. Exploring microbial diversity of non-dairy fermented beverages with a focus on functional probiotic microorganisms. *Fermentation*, **9(6)**, 496 (2023) https://doi.org/10.3390/fermentation9060496

[23] Bian M, Fang Y, Yang S, Yuan T, Zhou X, Xu Q, Jiang D, Han B. Dynamic changes in physicochemical properties, amino acid content, and flavour-related substances during fortified fermentation of rice wine with *Saccharomycopsis fibuligera*. *J Food Process Preserv*, 5581068 (2024) https://doi.org/10.1155/2024/5581068

[24] Coelho E, Lemos M, Genisheva Z, Domingues L, Vilanova M, Oliveira JM. Validation of a LLME/GC-MS methodology for quantification of volatile compounds in fermented beverages. *Molecules*, **25(3)**, 621 (2020) https://doi.org/10.3390/molecules25030621

[25] Bhattacharya B, Deka DC. Metal contents in traditional alcoholic rice beers prepared by Rabha and Sonowal Kachari tribes of Assam, India. *Asian J Chem*, **36(1)**, 201–205 (2024) https://doi.org/10.14233/AJCHEM.2024.30782

[26] Ariaya Hymete TE. Health risk assessment of heavy metals in locally produced beer to the population in Ethiopia. *OMICS J Radiol*, **6(6)** (2014) https://doi.org/10.4172/1948-593X.1000114

[27] Debebe A, Chandravanshi BS, Redi-Abshiro M. Assessment of essential and non-essential metals in Ethiopian traditional fermented alcoholic beverages. *Bull Chem Soc Ethiop*, **31(1)**, 17–30 (2017) https://doi.org/10.4314/bcse.v31i1.2

[28] Ramić E, Žero S, Memić M. Determination, estimation of dietary intake and target hazard quotients of heavy metals in wines from the Bosnia and Herzegovina market. *Kem Ind*, **73**, 457–466 (2024) https://doi.org/10.15255/KUI.2024.006

[29] Scutarasu EC, Trincă LC. Heavy metals in foods and beverages: global situation, health risks and reduction methods. *Foods*, **12(18)**, 3340 (2023) https://doi.org/10.3390/foods12183340

[30] Cottet C, Ramirez-Tapias YA, Delgado JF, de la Osa O, Salvay AG, Peltzer MA. Biobased materials from microbial biomass and its derivatives. *Materials*, **13(6)**, 1263 (2020) https://doi.org/10.3390/ma13061263

[31] Singh A, Tiwari A, Singh N. Physico-chemical analysis of developed rice beer from *Oryza sativa*. *Asian Food Sci J*, **22(5)**, 28–34 (2023) https://doi.org/10.9734/afsj/2023/v22i5634

[32] Koyama M, Kitamura Y. Development of a new rice beverage by improving the physical stability of rice slurry. *J Food Eng*, **131**, 89–95 (2014) https://doi.org/10.1016/j.jfoodeng.2014.01.030

[33] Cabrera-Vique C, Teissedre PL, Cabanis MT, Cabanis JC. Determination and levels of chromium in French wine and grapes by graphite furnace atomic absorption spectrometry. *J Agric Food Chem*, **45(5)**, 1808–1811 (1997) https://doi.org/10.1021/jf960691b

[34] Mishra S, Mithul Aravind S, Charpe P, Ajlouni S, Ranadheera CS, Chakkaravarthi S. Traditional rice-based fermented products: insight into their probiotic diversity and probable health benefits. *Food Biosci*, **50**, 102082 (2022) https://doi.org/10.1016/j.fbio.2022.102082

[35] Sarma Bora M, Devi U, Bharadwaj N, Sharma P, Kalita S, Baruah S, Das AK, Sarma KP. Pollution and health risk assessment of toxic metals in solid waste dumping site soil and its impact on groundwater: a case study. *Int J Environ Anal Chem*, **103(3)**, 612–632 (2023) https://doi.org/10.1080/03067319.2020.1862813

[36] Tang Z, Wang Q, Zhao Z, Shen N, Qin Y, Lin W, Xiao Y, Yuan M, Chen H, Chen H, Bu T, Li Q, Huang L. Evaluation of fermentation properties, antioxidant capacity in vitro and in vivo, and metabolic profile of a fermented beverage made from apple and cantaloupe. *LWT*, **179**, 114661 (2023) https://doi.org/10.1016/j.lwt.2023.114661

[37] Lekjing S, Venkatachalam K. Quality changes of HomChaiya rice beer during storage at two alternative temperatures. *J Biosci Bioeng*, **133(4)**, 369–374 (2022) https://doi.org/10.1016/j.jbiosc.2021.12.011