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Biochemical and physicochemical characterization of traditional rice wine of Nyishi tribe of Arunachal Pradesh, India

Sony Kumari, Tarh Ama, Pranab Jyoti Koch & Rahel Debbarma*
Department of Applied Biology, University of Science and Technology Meghalaya, Ri-Bhoi 793 101, India
*E-mail: raheldebbarma30@gmail.com

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Rice wine is a traditional alcoholic beverage of the Nyishi tribe of Arunachal Pradesh, prepared using 'Opo,' a traditional starter culture passed down through generations. This study investigates the biochemical significance of different rice wine varieties, focusing on their mineral content, bioactive components, and physicochemical properties. Mineral analysis revealed the presence of essential elements such as iron (Fe), calcium (Ca), zinc (Zn), and potassium (K). Among the studied varieties, Tisar wine exhibited the highest acceptability score, pH, alcohol content, and carbohydrate concentration, while Kecha wine was notable for its high protein, phenolic, and flavonoid contents. Bioactive compound analysis identified α-D-glucopyranoside in Adak wine and hexadecanoic acid in other varieties as the predominant components, contributing to the wines' potential health benefits. These findings provide valuable insights into the physicochemical and biochemical properties of traditional rice wine, highlighting its significance in indigenous practices and potential applications in functional foods.

Keywords: Arunachal Pradesh, GCMS, Nyishi tribe, Opo, Traditional beverage

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The biggest group of tribal communities ever recorded to reside in one place is in the Land of the Rising Sun, also known as Arunachal Pradesh, and practically all of them prepare their own alcoholic beverages[1]. Since their earliest days, the traditional inhabitants of the state have undoubtedly created their unique ways of preparing food and beverages. People have their own traditional practices for making and consuming alcoholic beverages. Although there are many more such groups, the Government of India has only recognized 26 tribes and 110 subtribes from Arunachal Pradesh thus far[2]. Among the significant tribes that prepare and consume rice wine according to their own unique customs are the Nyishi, Adi, Singpho, Galo, Tagin, and Apatani. According to the Indian Constitution, the Nyishi tribe, also known as Nishi or Nyshing, is one of the indigenous groups that inhabits the north-eastern part of the country and is the largest in the state[3]. Rice wine, also known as "Opo" in Nyishi, is a traditional alcoholic beverage made from fermenting rice. It is considered sacred by the Nyishi people and is a significant part of their culture. The art of producing rice wine has been passed down through generations, preserving its unique flavor and brewing techniques. Rice beer is a unique alcoholic beverage that has a variety of flavors and scents due to its starting culture preparation, indigenous plant species, and rice variations[4]. It is a result of climate factors and the usage of natural resources[5]. It has also been used as a medication[6]. Rice beer, a light alcoholic beverage, helps stressed-out folks unwind without having an adverse impact on their health. Due to the taste and alcohol content of glutinous rice, it is popular[7]. Additional research may uncover additional advantageous traits. Improved immunity and digestion are just two of the many health advantages of fermented foods, which are high in probiotics. In a study on Vietnamese rice-based alcoholic beverages, it was discovered that while ethanol level steadily increases after three days, glucose concentration increases during those same days[8]. It has been anticipated that the end product of such indigenous medicinal rich raw material could be a source of health-beneficial and immuno-rich food due to the presence of medicinal plants in the starter culture itself. According to Das *et al.*[9], rice beer has a strong antioxidant activity, a significant alcohol level[4,9] and a high phenolic content[10]. Given their high

*Corresponding author

antioxidant activity, traditional beverages may offer health benefits. This study, rooted in Arunachal tribal culture, aims to raise scientific awareness by quantifying bioactive compounds in traditionally made rice wine.

Materials and Methodology

Sample collection and preparation

The local "Aamtey" rice and three different starter cultures were collected from Naharlagun, Arunachal Pradesh, India. It was cleaned with fresh water and cooked; to prevent burning, a ladle was used to stir the food continuously while cooking. After cooking, the rice was spread evenly on a mat to cool for 30 to 40 min. After cooling, the starter culture (about 4-5 Apaap) was crushed and mixed. It was allowed to ferment in an airtight container for around 21 days. The procedure for making the wine is outlined in (Fig. 1).

Sensory analysis

The freshly prepared beer was served in 20 mL glass for sensory analysis. The entire tests were performed in testing room and were evaluated by 10 volunteers[11]. The consumer preference and all over acceptability was determined with the help of hedonic nine-point scale given by Friedman (1997)[11].

Physiochemical analysis

Measurement of pH

A pH meter (Labtronics, Panchkula, India) was used to monitor the pH of the sample at intervals of 7 days until 21 days.

Total acidity

10 mL of rice beer was mixed with 10 mL distilled water and 2-3 drops of phenolphthalein. The mixture was titrated with 0.1 N NaOH until a persistent pink color appeared. The volume of NaOH used was recorded. The test was repeated on samples aged 14 and 21 days. Total acidity (mg/mL) was calculated as citric acid using Formula[12]:

$$\text{Acidity (g/100 mL)} = \text{Normality of the sample x Equivalent weight of citric acid}$$

Alcohol level

The alcohol level was determined after distillation by using alcohol meter at 7-day interval for 21 days[13].

Moisture content

Hydroscopic water of wine was determined by using the standard method of analysis, by heating in an oven at 105°C. Then weighed 1 g of wine along with the lid and dried in the oven at 105°C with the fitted lid over-night and removed from oven, fit lid, cooled desiccator for at least 30 min and reweighed[14].

Fig. 1 — Preparation of rice wine. A - Rice (cooked for 30-40 min); B - Cooked rice cooled down; C- Mixing of starter culture; D - Stored in airtight container

Using the following formula, the moisture content was calculated:

$$Moisture\ \% = \frac{Initial\ Weight - Final\ Weight}{Initial\ Weight - Container\ Weight} + 100$$

Biochemical study of rice beer

Total carbohydrate content

The estimation of total carbohydrates was carried out by using an Anthrone’s reagent with some modifications[15]. 1 mL of sample was digested with 2N HCl for half an hour and neutralized. It was diluted and treated with 6 mL of Anthrone’s reagent. The mixture was subjected to mild heating. The resultant colour intensity was measured at 630 nm.

Protein estimation

The protein determination was done by Lowry *et al.* 1951. 5 mL of alkaline cupper regent was added to 100 µL of the sample and allowed to stand for 10-15 minutes at room temperature. 0.5 mL of Folin–Ciocalteu (FC) reagent was added and incubated at room temperature in the dark for 30 min. The absorbance was read at 660 nm[16].

Antioxidant activity

Total phenolic content

By using the Folin Ciocalteu method, the total phenolic content of the beer was calculated[17]. For this 2.6 mL of distilled water was added to 400 µL of beer followed by addition of 0.5 mL Folin-Ciocalteu. After adding 0.5 mL 20% sodium carbonate (Na_2CO_3), tubes were placed in boiling water for one minute. At 650 nm, optical density was noted.

Total flavonoid content

A mixture containing 0.5 mL each of beer and distilled water was prepared, to which 0.3 mL of 5% sodium nitrite solution was added. The mixture was incubated at 25°C for 5 min. Subsequently, 0.3 mL of 10% aluminum chloride solution was introduced and thoroughly mixed. Finally, 2 mL of 1 M sodium hydroxide solution was added. The absorbance of the solution was then measured at 510 nm after a 6 min interval[18].

Determination of total antioxidant activity

Beer's ability to scavenge free radicals was calculated using the approach described in Kekuda *et al.*[19]. Beer in various concentrations was added to the reaction mixture, which had been made in methanol, and it was let to sit at room temperature for 30 min. At 517 nm, the absorbance was investigated[19]. Using the following formula, the beer's scavenging capacity was determined:

$$\text{Scavenging activity (\%)} = \frac{A - B}{A} + 100$$

Where, A is the absorbance of DPPH and B is the absorbance of beer combination.

Gas-Chromatography Mass Spectrophotometer analysis

Bioactive compound analysis was conducted using a GC-MS system (Thermo Fisher Scientific, America), consisting of a split/splitless injection port with a split ratio of 1/100, and coupled to an ISQ7000 mass spectrometer. The system was equipped with a TG-5MS fused silica capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness). The temperature program began with an isothermal phase at 60°C for 3 min, followed by a gradual increase to 230°C at a rate of 5°C/min. The injector temperature was maintained at 290°C, while the transfer line temperature was optimized. Component identification was carried out by comparing experimental retention indices and mass spectra to reference standards available in the NIST library, integrated within Chromeleon™ Software[20].

Determination of minerals by AAS

The concentration of the mineral (Mg, Ca, Zn, Fe, and K) was determined by using Atomic Absorption Spectrometer (Spectra AA22PFS model). To measure the mineral content, the ash content was dissolved in HNO_3 with 50 g/L of $LaCI_3$ and the mineral analyzed separately, using an atomic absorption spectrophotometer. The mineral content in the sample were then calculated and results expressed in mg/100 L[21].

Statistical analysis

All experiments were conducted in triplicate, and results are presented as mean ± SD. One-way ANOVA was used to compare physicochemical and antioxidant properties of Tisar, Adak, and Kecha wines. Tukey’s HSD test identified significant pairwise differences ($p < 0.05$), indicated by different superscript letters. Analysis was performed using IBM SPSS.

Results and Discussion

Sensory results

Acceptability of a product is determined by sensory evaluation[22]. Trials by a panel consisting of 10 faculty members from different departments of the University and scoring for sensory analysis was conducted based on nine-point hedonic scale. All values are presented in Table 1 as mean ± standard deviation (SD) of three independent replicates. The sensory evaluation of traditional rice wines; Tisar wine (TW), Adak wine (AW), and Kecha wine (KW) revealed distinct differences in consumer-perceived attributes, suggesting that traditional processing techniques may significantly influence organoleptic properties. In terms of appearance, both TW and KW scored equally high (7.4±0.80 and 7.4±1.20, respectively), while AW scored slightly lower (6.7±1.22). The higher appearance scores for TW and KW may be attributed to clarity, uniformity in texture, and color consistency, which are important parameters in consumer acceptance, as previously noted by Cuamatzin-García *et al.*[23] who emphasized visual appeal as a major driver in traditional fermented beverages.

Taste ratings were highest for TW (7.68±0.80), suggesting a more favorable flavor profile, potentially due to better balance between sweetness and acidity. AW, on the other hand, received the lowest taste score (6.5±1.16). These differences align with the findings of Lee *et al.*[24] who reported that 43% of consumers preferred rice wines exhibiting higher levels of sweetness and herbal or fruity notes. TW may fall within this category, indicating a profile more aligned with consumer preferences. Upon aroma evaluation, TW again received the highest rating (7.6±1.28) indicating a more appealing aromatic profile. In contrast, KW received the lowest aroma score (7.1±0.80) suggesting a relatively subdued olfactory impact. This is particularly relevant given that Lee *et al.*[24] categorized consumer preferences into aroma-based clusters, where approximately 29% of participants favored strong herbal aromas with less bitterness traits that may correspond with TW's sensory profile. Regarding color, TW scored the highest (7.4±0.60), followed closely by AW (7.3 ± 0.66), while KW was slightly lower (7.0 ± 1.06). Color perception often correlates with ingredient composition and fermentation conditions, as noted in the work of Becerra *et al.*[25], who highlighted those differences in raw materials and fermentation length influence pigmentation and visual acceptability.

In terms of texture, KW had the highest score (7.1±1.30), followed by TW (7.0±0.89), and AW (6.6±0.66). The variation may reflect differences in fermentation depth, starch degradation, and protein interaction during the brewing process. A smoother, more consistent mouth feel generally enhances perceived quality[26]. Cloudiness, a traditional trait often associated with authenticity in indigenous rice wines, was highest in TW (8.8±0.40), suggesting higher suspended solid content, which may be desirable in certain cultural contexts. While AW and KW followed closely (8.4±0.66 and 8.0 ± 0.89, respectively) for cloudiness, differences in sedimentation and filtration during brewing could contribute to the variation. According to Zhang *et al.*[27] cloudiness in traditional rice wine is positively correlated with both yeast content and the presence of residual rice solids, both of which influence flavor complexity. The overall acceptability rating was highest for TW (6.9±0.94), followed by AW (5.7±1.26), and KW (4.7±0.90), indicating a clear consumer preference for TW. This correlates strongly with TW's superior scores across most sensory dimensions, particularly taste and aroma. The overall liking patterns are consistent with Lee *et al.*[24] who noted that the most preferred rice wines among consumers were those with rich aroma profiles and sweet flavor tones. Overall, TW showed superior sensory qualities, aligning with consumer preferences. Its unique fermentation practices and ingredients may enhance organoleptic quality, offering insights for standardizing traditional rice wine production in tribal communities.

Physicochemical analysis

pH and total Acidity

The pH of all three rice wines exhibited a gradual increase over the 21-day fermentation period, with TW showing the highest final pH value (4.25±0.38), followed by KW (4.22±0.018), and AW (4.16±0.04). This trend aligns with earlier findings by Deka[29], who reported pH values ranging from 3.75±0.25 to 4.32±0.03 in "Jou," a traditional rice beer consumed by the Bodo tribe in Northeast India[28,29]. Similarly, Teramoto *et al.*[31] reported a pH of 3.6 in Zutho,

Table 1 — Sensory attributes of rice wine (TW: Tisar wine; AW: Adak wine; KW: Kecha wine)

Wine	Appearance	Taste	Aroma	Color	Texture	Cloudiness	Overall Acceptability
TW	7.4+0.80^{a}	7.68+08^{a}	7.6+1.28^{a}	7.4+0.6^{a}	7+0.89^{a}	8.8+0.4^{a}	6.9+0.94^{a}
AW	6.7+1.22^{b}	6.5+1.16^{b}	7.2+0.87^{ab}	7.3+0.66^{a}	6.6+0.66^{ab}	8.4+0.66^{ab}	5.7+1.26^{b}
KW	7.4+1.20^{a}	7.1+0.70^{ab}	7.1+0.8^{ab}	7+1.06^{a}	7.1+1.3^{a}	8+0.89^{b}	4.7+0.90^{c}

another indigenous rice beer, which is comparable to the initial pH values observed in AW and KW.

The rise in pH with time is likely due to the progressive reduction of organic acids during fermentation. According to Guyot-Declerck *et al.*[32], rice beers with pH above 4.4 develop a smooth, biscuit-like mouth feel, while those with lower pH values (<3.7) tend to have a sour or bitter profile. The pH values of the rice wines in this study fall between these two extremes, suggesting a balanced sensory perception.

Total acidity levels observed were highest in TW (1.18±0.08), followed by AW (1.1±0.08), and KW (1.08±0.18). These are lower than the 5.1 value reported by Teramoto *et al.*[31] for Zutho, indicating a milder acidic profile in the Nyishi rice wines. The observed values are consistent with findings from Chai and Rohi beers of Northeast India, which recorded total acidity between 1.08 and 1.23[32].

Alcohol and moisture content

Among the three wine types, TW had the highest alcohol concentration (4.59±0.005%), followed by KW (4.33±0.07%) and AW (4.13±0.09%). This aligns with the general principle that prolonged fermentation increases alcohol content, as observed by Ghosh (2015)[33]. The increased ethanol levels may be attributed to more active or prolonged yeast metabolism in TW[33].

Moisture content varied across the wine types, with KW exhibiting the highest (23.28%), followed by TW (22.22%) and AW (21.01%). These values are slightly lower than the broader range of 26% to 35% (v/v) moisture reported by Ghosh (2015) for similar traditional fermented beverages[33]. Moisture levels influence the microbial dynamics and storage stability of rice wines, which can impact both flavor and safety[34].

Carbohydrate and protein content

Carbohydrate concentration was highest in TW (2.416±0.012 µg/mL), indicating a potentially sweeter profile or residual sugars remaining post-fermentation. This is important because carbohydrate levels influence mouth feel and flavor development. AW and KW followed with slightly lower concentrations (2.278±0.047 and 2.317±0.081 µg/mL, respectively). Interestingly, protein levels were nearly identical between TW and AW (1.847 µg/mL), while KW had marginally higher protein content (1.848 µg/mL). Higher protein content in rice wines can affect turbidity and foaming properties, which are significant for consumer appeal and physical stability[34].

Antioxidant activity

The antioxidant properties of the rice wines, as determined by phenolic and flavonoid contents as well as DPPH radical scavenging activity, varied significantly as shown in Table 2. KW showed the highest total phenolic (2.433±0.015 mg GAE/L) and flavonoid (1.696±0.081 mg catechin/L) contents, followed closely by TW and AW. These results are consistent with observations by Das *et al.*[9], where rice beers from Northeast India showed increased phenolic content during fermentation due to microbial biotransformation of plant compounds.

However, despite KW's higher phenolic and flavonoid content, TW exhibited the highest DPPH-based antioxidant activity (0.365±0.151 mg/mL), suggesting that other bioactive compounds or synergistic effects may enhance its free radical scavenging ability. This is supported by Habschied *et al.*[34], who emphasized that a multitude of bioactive constituents in fermented beverages contribute to antioxidant effects.

All data are presented as mean ± SD from triplicate tests. One-way ANOVA followed by Tukey's HSD test ($p < 0.05$) was used to identify significant differences among rice wines (TW, AW, KW). Different superscripts (a, b, c) in the same column indicate statistically distinct values. Comparatively, Deori-made rice wines have been shown to possess the strongest DPPH activity among several ethnic groups, followed by Mising and Ahom preparations[29]. This highlights the influence of indigenous fermentation techniques and microbial consortia on antioxidant potential. The results of this study further support earlier claims by Lee *et al.*[24] that fermentation enhances the functional quality of rice-based beverages, possibly through microbial enzymatic activity and the release of bound phenolic compounds. As such, traditionally fermented rice wines may serve not only as cultural

Table 2 — Antioxidant activity of different rice wines

Rice wine	Total phenolic content (mg GAE/L)	Total flavonoid content (mg catechin/L)	DPPH radical scavenging activity (mg/mL)
Kecha Wine	2.433 ± 0.015^{a}	1.696 ± 0.081^{a}	0.284 ± 0.098^{b}
Tisar Wine	2.176 ± 0.027^{b}	1.482 ± 0.067^{b}	0.365 ± 0.151^{a}
Adak Wine	2.104 ± 0.019^{c}	1.365 ± 0.054^{c}	0.241 ± 0.104^{c}

artifacts but also as functional beverages with health-promoting properties[10].

Bioactive compounds composition

Figure 2 illustrates the presence of bioactive compounds in rice beer. Each sample has been analyzed and their main components, along with their relative area percentages (RA%), are listed in respective tables. In the Adak (Table 3), the most abundant compound detected is α-D-Glucopyranoside, methyl, making up approximately 22.73% relative area. Following that, Glycerin is the second most prevalent compound, constituting around 17.03% of relative area. Several other organic acids have also been identified in this sample, including Hexadecanoic acid, methyl ester, Methyl stearate, cis-Vaccenic acid, 9,12-Octadecadienoic acid, ethyl ester, Hexadecanoic acid, ethyl ester, 1-Deoxy-d-mannitol, 4-Hydroxybenzoic acid, O-ethoxycarbonyl, ethyl ester, and d-Altronic acid. Gas chromatography and mass spectroscopy analysis of traditional Assam, India rice beer revealed 18 saccharides, 18 organic acids, 11 sugar alcohols, 8 amino acids, and 1 vitamin and nutraceutical compound[9]. In another study, nine samples of rice beer from northeast India were analyzed [20] and the results showed a range of organic acid contents, with lactic acid being the most prevalent. Most of the carbohydrates were monosaccharides and glucose. There were plenty of essential amino acids, with aspartic acid being the most prevalent. Phenylethyl alcohol was the most prevalent volatile or semi-volatile aromatic chemical found in all samples.

In the Kecha (Table 4), the dominant organic acid is Hexadecanoic acid, methyl ester, with RA% of 19.21%. Propanal, 2,3-dihydroxy-, (S), is the second most abundant compound, accounting for around 13.74% (RA). Additionally, other organic acids detected in this sample include Methyl glucopyranoside, Methyl stearate, Diglycerol, 1-Deoxy-d-mannitol, 4-Hydroxybenzoic acid, O-ethoxycarbonyl-, ethyl ester, *cis*-Vaccenic acid, 9,12-Octadecadienoic acid, ethyl ester, and Octadecanoic acid, 2,3-dihydroxypropyl ester.

In the Tisar sample (Table 5), the major compound identified via GC-MS is Propanal, 2,3-dihydroxy-, (S)-, which comprises approximately 15.01% of the total relative area. The second most abundant compound is Hexadecanoic acid, methyl ester, also known as methyl palmitate, contributing around 14.78% of the relative area. This compound is notable for its reported anti-

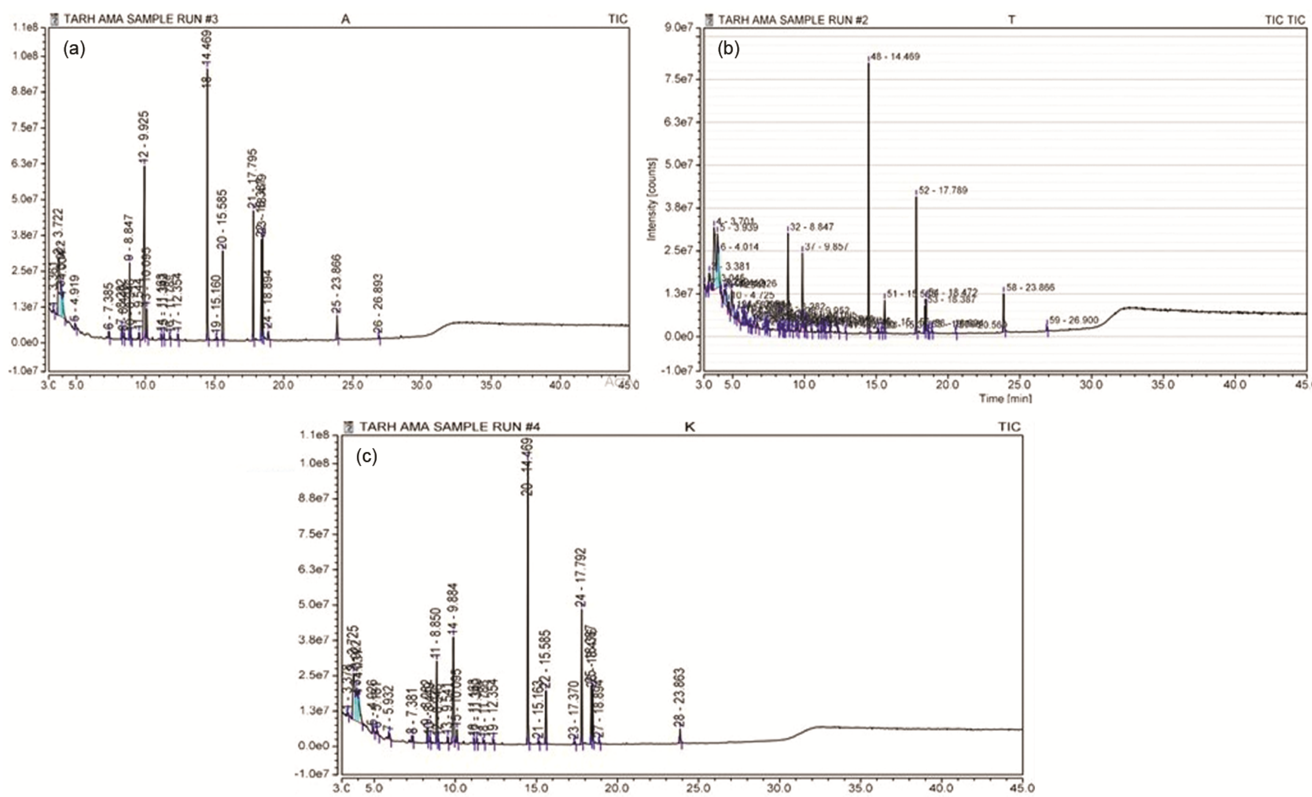
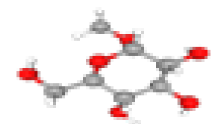
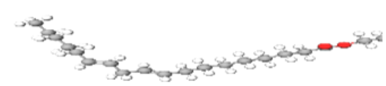
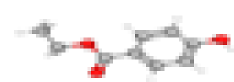
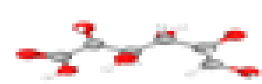


Fig. 2 — GC-MS chromatograms of rice beers (A: AW; B: TW; C: KW)

inflammatory, antioxidant, and anticancer properties; however, excessive accumulation in tissues may result in lipotoxic effects, warranting moderation in its consumption[35].

Among the other organic compounds detected, 1,2,3-Butanetriol has demonstrated antimicrobial activity and may play a role in modulating gut microbiota, contributing to potential health benefits[36]. Methyl stearate, a saturated fatty acid ester, is associated with anti-inflammatory properties but may lead to lipid accumulation when consumed in high amounts[37]. α-D-Glucopyranoside, a sugar derivative, is implicated in antioxidant pathways, offering potential health-promoting effects[35]. Imidazole, 2-amino-5-[(2-carboxy)vinyl]-, belonging to the imidazole group, is recognized for its antimicrobial and anti-inflammatory properties[38]. Another compound, 4-Hydroxybenzoic acid, O-ethoxycarbonyl-, ethyl ester, exhibits antioxidant and preservative characteristics, though concerns

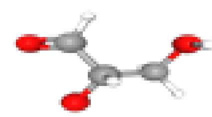
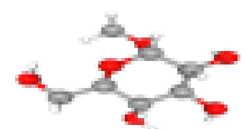
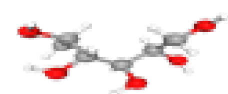
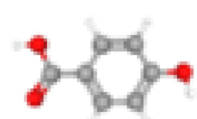
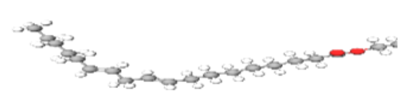
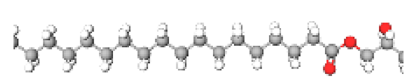
Table 3 — Chemical composition of Adak wine

Sl. No.	Compounds	Retention Time	Relative Area %	Structure
1	α-D-Glucopyranoside, methyl	9.925	22.73	
2	Glycerin	3.722	17.03	
3	Hexadecanoic acid, methyl ester	14.469	14.82	
4	Methyl stearate	17.795	7.90	
5	cis-Vaccenic acid	18.479	6.71	
6	9,12-Octadecadienoic acid, ethyl ester	18.387	5.86	
7	Hexadecanoic acid, ethyl ester	15.585	5.21	
8	1-Deoxy-d-mannitol	3.922	4.24	
9	4-Hydroxybenzoic acid, O-ethoxycarbonyl-, ethyl ester	8.847	3.85	
10	d-Altronic acid	4.004	2.61	

regarding its endocrine-disrupting potential persist in scientific discussions[39].

p-Dioxane-2,5-dimethanol, while structurally relevant to other compounds, requires further research to determine its safety and biodegradability profile[40]. Similarly, 3,7-Diacetamido-7H-s-triazolo[5,1-c]-s-triazole, a nitrogen-rich heterocyclic compound, contributes bioactive potential, but additional studies are needed to evaluate its pharmacological safety[41]. Octadecanoic acid, 2,3-dihydroxypropyl ester, commonly referred to as glyceryl stearate, is generally regarded as safe and is widely used as an emollient and stabilizing agent in various applications[42]. These compounds collectively contribute to the unique flavor, aroma, and potential bioactivity of Tisar. Some compounds like esters and alcohols enhance sensory qualities, while others may provide health benefits, suggesting the nutraceutical potential of Tisar and similar rice wines and the need for further safety and efficacy studies.

Table 4 — Chemical composition of Kecha wine

Sl. No.	Compounds	Retention Time	Relative Area %	Structure
1	Hexadecanoic acid, methyl ester	14.469	19.21	
2	Propanal, 2,3-dihydroxy-, (S)	3.725	13.74	
3	α -D-Glucopyranoside, methyl	9.884	13.67	
4	Methyl stearate	17.792	9.92	
5	Diglycerol	3.922	9.59	
6	1-Deoxy-d-mannitol	4.031	6.20	
7	4-Hydroxybenzoic acid, O-ethoxycarbonyl-, ethyl ester	8.850	4.99	
8	cis-Vaccenic acid	18.476	4.38	
9	9,12-Octadecadienoic acid, ethyl ester	18.387	4.30	
10	Octadecanoic acid, 2,3-dihydroxypropyl ester	23.863	1.39	

Volatile profiling and chemometric techniques have proven effective in classifying rice wines by origin and age. Studies, including Wang *et al.* (2020), identified key compounds-alcohols and phenols in young wines, and furans and aldehydes in aged ones-highlighting how volatiles shape flavor, quality, and consumer perception[26].

Mineral content of the rice wine

The content of minerals (mg/100L) such iron, sodium, calcium, magnesium, zinc, and potassium

Table 5 — Chemical composition of Tisar wine

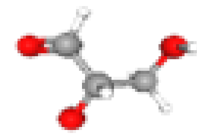
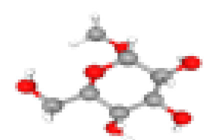
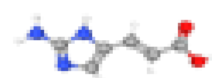
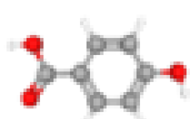
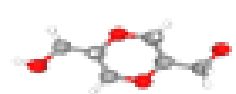
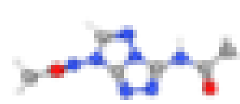
Sl. No.	Compound	Retention Time	Relative Area %	Structure
1	Propanal, 2,3-dihydroxy-, (S)	3.701	15.01	
2	Hexadecanoic acid, methyl ester	14.469	14.78	
3	1,2,3-Butanetriol	3.939	8.68	
4	Methyl stearate	17.789	7.92	
5	α-D-Glucopyranoside, methyl	9.857	7.50	
6	Imidazole, 2-amino-5-[(2-carboxy) vinyl]	4.926	4.40	
7	4-Hydroxybenzoic acid, O-ethoxycarbonyl-, ethyl ester	8.847	4.21	
8	p-Dioxane-2,5-dimethanol	4.014	3.09	
9	3,7-Diacetamido-7H-s-triazolo[5,1-c]-s-triazole	4.412	2.99	
10	Octadecanoic acid, 2,3-dihydroxypropyl ester	23.866	2.73	

Table 6 — Mineral analysis of the rice wine (mg/100 L)

Beer	Iron	Sodium	Calcium	Magnesium	Zinc	Potassium
TW	0.019	-	0.400	-	0.020	0.100
AW	0.030	0.100	0.800	0.300	0.010	0.200
KW	0.081	0.800	1.200	0.900	0.002	2.200

were examined in the rice wine using an Atomic Absorbance Spectrometer (Table 6). The results demonstrate the beneficial effects of rice beer consumption on human health.

The analysis of mineral content in the three rice wines (TW, AW, and KW) revealed notable variation across different mineral types. Potassium was found to be the most abundant mineral, especially in KW (2.2 mg/100 L), followed by AW (0.2 mg/100 L) and TW (0.1 mg/100 L). The high potassium content in KW is consistent with findings by Kumari *et al.*[43], who reported potassium as one of the dominant minerals in Assamese rice beer varieties. Potassium plays a vital physiological role in maintaining electrolyte balance and regulating muscle and nerve functions[7]. Calcium levels were also highest in KW (1.2 mg/100 L), indicating its potential nutritional value. Similar calcium concentrations were reported in traditional fermented beverages across Northeast India, as observed by Bhuyan *et al.*[44], who highlighted that fermentation may enhance mineral bioavailability due to microbial enzymatic activity that breaks down complex matrices, thereby releasing bound minerals. Iron content was highest in KW (0.081 mg/100 L), followed by AW (0.03 mg/100 L), and lowest in TW (0.019 mg/100 L). These values, though relatively low, support the presence of trace essential minerals that contribute to dietary iron intake. Previous studies, including those by Kumari *et al.*[43], reported significantly higher values in some ethnic rice beers, with iron levels varying depending on the type of raw rice, fermentation containers, and microbial diversity involved. Magnesium and sodium were notably absent in TW but present in both AW and KW. The absence of these minerals in TW could be attributed to either their low initial concentrations in the raw material or differences in the fermentation process that may limit their solubilization. In contrast, KW showed higher magnesium (0.9 mg/100 L) and sodium (0.8 mg/100 L) contents, possibly due to extended fermentation and higher mineral solubility under microbial activity. Zinc was found in all samples, with the highest concentration in TW (0.020 mg/100 L), followed by AW (0.010 mg/100 L) and KW (0.002 mg/100 L). Although these levels are low, zinc is essential for enzymatic activities and immune function. Earlier investigations by Kumari *et al.*[43] reported significantly higher zinc concentrations (ranging from 0.0628 ppm to 4.7196 ppm) in traditional rice beers, suggesting that the current samples may have undergone shorter fermentation or used rice varieties with naturally lower zinc content. To ensure safety, further toxicological analysis is needed. Given their cultural importance among tribes like the Nyishi, community awareness and scientific validation are crucial. Standardizing fermentation and hygiene can improve quality while preserving tradition.

Conclusion

One of the traditional, indigenous, and scientific practices in Northeast India is the production of Opo, a rice wine by the Nyishi people. These traditions have been passed down through the generations. More than 150 notable tribes in the area developed unique methods for producing rice beer, which varies in flavour, aroma, colour, and nutritional value. Even if the practice is waning and encountering challenges, there is scope for scientific advancement and commercialization of these traditional drinks. Future research can focus on the standardization of fermentation techniques, enhancement of probiotic potential, and evaluation of the health benefits of Opo. Additionally, exploring ways to commercialize and promote these traditional beverages while preserving indigenous knowledge can open new economic opportunities for local communities.

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Conflict of Interest

The authors have no competing interests.

Author Contributions

SK: Conceptualization, Supervision and Designing, TA: Research work, Validation, PJK: Writing, methodology, RD: Methodology and Editing.

Prior Informed Consent

The PIC has been obtained from the knowledge holders.

Data Availability

The data used to support the findings of this study are included within the article, and will be made available by the authors upon reasonable request.

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