Indian Journal of Traditional Knowledge
Vol 25(4), April 2026, pp 384-396
DOI: 10.56042/ijtk.v25i4.20314

Bio-functional and anti-inflammatory properties of *Chubitchi*: A traditional fermented rice beverage of Meghalaya, India

Subrota Hati[a], Bethsheba Basaiawmoit[b], Krupali Ramanuj[a], Sreeja V[a], Arka Bhattacharya[c], Mahendra Bishnoi[c], Kanthi Kiran Kondepudi[c] & B K Mishra[b,*]

[a]Dairy Microbiology Department, SMC College of Dairy Science, Kamdhenu University, Anand 388 110, Gujarat, India
[b]Department of Rural Development and Agricultural Production, North-Eastern Hill University, Chasingre 794 002, Meghalaya, India
[c]Healthy Gut Research Group, Food & Nutritional Biotechnology Division, National Agri-Food Biotechnology Institute, Knowledge City, Punjab 140 306, India

[*]E-mail: birendramishra14@gmail.com

Received 04 June 2025; revision on 23 February 2026; accepted 17 March 2026

Chubitchi, a traditional brewed alcoholic beverage from Meghalaya, was prepared under controlled laboratory conditions. A comprehensive storage study was conducted over four weeks under refrigerated conditions to examine its physicochemical properties (pH, acidity, alcohol content) and bio-functional attributes (antimicrobial, antioxidative, ACE inhibitory, anti-diabetic activities, and phenolic content). Over the four weeks, the pH decreased to 3.31, while acidity increased to 2.03%. Ethanol content peaked at 5.62% during week 2. Lactic acid bacteria and yeast counts declined to 5.21 log CFU/mL and 4.86±0.19 log CFU/mL, respectively, by week 4. *Chubitchi* demonstrated antimicrobial activity against *K. pneumoniae*, *E. coli*, and *S. aureus*. Antioxidative activity peaked in weeks 3 (86.19±0.00%) and 4 (86.04±0.06%). ACE inhibition increased non-significantly from 17.03±0.28% to 23.10±0.92%. Anti-diabetic potential peaked at 3.53% (α-amylase inhibition), 20.42% (α-glucosidase inhibition), and 39.50% (lipase inhibition). Total phenolic content rose from 0.22 mg/mL to 0.27 mg/mL. Organoleptic evaluation revealed declining sensory scores through day 40. Furthermore, a 1:8 dilution of *Chubitchi* was found to be non-cytotoxic in a murine macrophage cell line, exhibiting significant anti-inflammatory activity and reduced reactive oxygen species (ROS) production. This study provides critical insights into physicochemical and biofunctional dynamics of *Chubitchi* during storage, supporting its potential as a functional beverage.

Keywords: Anti-inflammatory activity, Biofunctional properties, *Chubitchi*, Meghalaya, Traditional fermented rice beverage

IPC Code: Int Cl.[26]: A232 2/38

The Northeastern region of India, particularly Meghalaya, boasts a vibrant cultural mosaic characterized by diverse tribal communities, including the Garo, Khasi, and Jaintia. An integral part of this cultural heritage is the traditional crafting and consumption of fermented rice beverages; each community infuses unique variations while upholding fundamental methods passed down through generations[1]. Among these groups, the Garo tribes of Meghalaya maintain a profound connection with their traditional fermented rice beverage, locally known as '*Chubitchi*.' This blend of rice and indigenous knowledge plays a pivotal role in the economic, cultural, and spiritual practices of the Garo people[2].

Chubitchi, a traditional fermented rice beverage, is central to the tribal culture of Garo Hills. Its authentic preparation, wisdom, and cultural significance are primarily preserved within rural communities. Unregulated fermentation, influenced by local microorganisms and environment, causes variable *Chubitchi* quality and yield. Efforts aim to enhance safety and quality via hygienic brewing[3].

Fermented rice beverages like *Chubitchi* are recognized as 'probiotic' due to their diverse yeast and lactic acid bacteria (LAB) strains. These microorganisms contribute to unique sensory attributes while offering potential antioxidant and antibacterial benefits[2]. Alcoholic rice beverages exhibit health-promoting antioxidant and antibacterial properties. *Chubitchi*, akin to other global fermented rice beverages, shows bio-functional promise, attracting research interest[4,5].

This study formulates a laboratory-scale *Chubitchi*, based on traditional Garo Hills fermentation. It comprehensively analyzes physicochemical properties (pH, acidity, alcohol content), 4-week refrigerated

[*]Corresponding author

storage stability, and diverse bio-functional attributes. These include antimicrobial, antioxidative, ACE-inhibitory, anti-diabetic, total phenolic content, and anti-inflammatory activities on RAW 264.7 macrophage cell lines. This research aims to preserve traditional knowledge, enhance quality, and explore indigenous *Lactobacillus* isolates for novel probiotic foods, promoting broader application and health advantages.

Materials and Methods

Preparation of a defined starter culture (*Wanti*) for *Chubitchi* production

A defined starter culture was developed, adapted from Dung's methodology[6]. Washed and dried (50°C) sticky red rice (*Minil*, 500 g) was ground; 100 g of this autoclaved powder served as inoculum. Utensils were meticulously washed, rinsed, and UV-sterilized (20 min)[7], while glassware was autoclaved to ensure sterility.

Scoparia dulcis stems and leaves were surface sterilized with 1% sodium hypochlorite for 15 min[8,9], then rinsed. The sample was crushed into a paste using a Philips (India) mixer and filtered through Whatman No. 1 filter paper. A 2 mg/mL extract was prepared. The extract was sterile-filtered (0.45 µm Millex-HP, Merck Pvt. Ltd.) for sterility and particulate removal.

Lactiplantibacillus plantarum KGL3A (MG722814), *Limosilactobacillus fermentum* KGL4 (MF951099), and *Saccharomyces cerevisiae* WTS1A (MG183699) were cultured in their respective growth media: MRS broth for the *Lactobacillus* species and YPD broth for the yeast. Cell pellets, collected during exponential growth, were rinsed twice in PBS and reconstituted in 4 mL of sterile distilled water.

For biomass production, autoclaved whey (from channa/paneer) was cooled to 37°C. The whey was then inoculated separately with 4% (v/v) cell pellets of *L. plantarum* and *L. fermentum*, followed by a 24-h incubation at 37°C. For *S. cerevisiae* production, 2% dextrose was added to the whey before inoculation with a 4% cell suspension.

A defined starter culture was formulated by combining 100 g of autoclaved red rice powder, 5 mL of filter-sterilized *Scoparia dulcis* extract (from stems and leaves), 4 mL each of KGL3A and KGL4 suspension, and WTS1A in 100 mL of whey medium, along with sterile-filtered water. These components were aseptically kneaded into the dough in a laminar air flow (LAF) chamber. The fresh starter culture was then oven-dried at 40°C for four days in a sterile desiccator, bottled, and stored at 4°C for future use (Fig. 1 & Fig. 2).

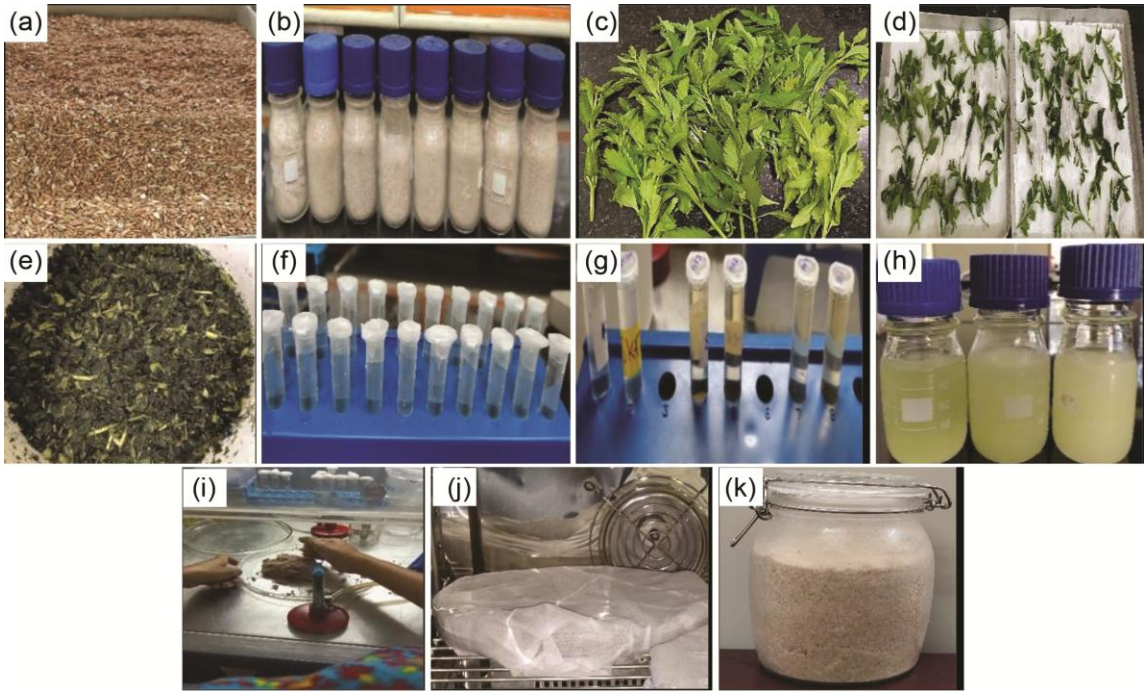

Fig. 1 — Preparation of a defined starter culture (*Wanti*) for *Chubitchi* production; (a) Washed and dried red sticky rice (*Minil*), (b) Powdered and autoclaved *Minil* rice powder, (c) *Scoparia dulcis*, (d) Washed and dried plant, (e) Plant extract, (f) Filter-sterilized extract of *Scoparia* sp., (g) KGL3A, KGL4, and WTS1A cell suspensions, (h) Whey medium for growth of KGL4, KGL3A, & WTS1A, (i)- (b), (f), & (h) combined with sterilized distilled water, (j) Dried for 4 days in a 40°C oven, (k) Stored in an aseptic vessel at 4°C

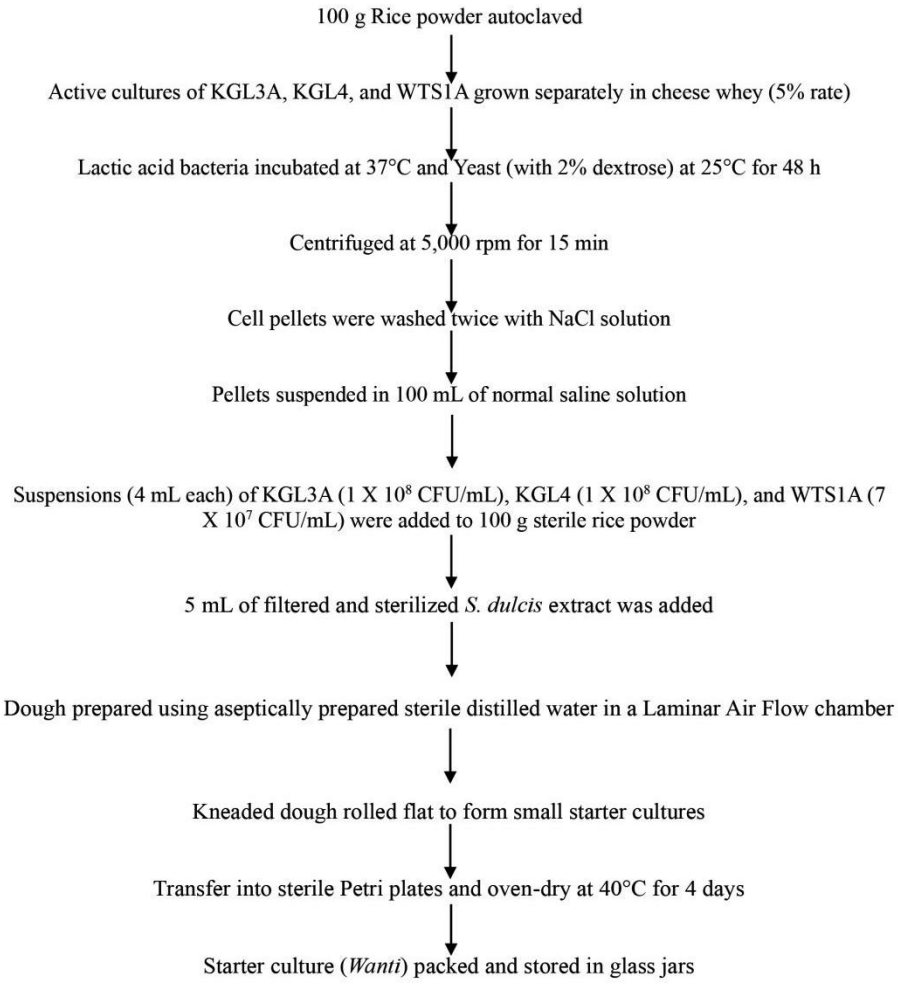

Fig. 2 — Flow diagram for starter culture (*Wanti*) preparation

Preparation of *Chubitchi*

Local red sticky rice (*Minil*, 100 g) was washed, combined with filtered water (1:3 w/v) in glass jars, and autoclaved. Following cooling to 37°C, the mixture was supplemented with 1% (w/w) α-amylase and held at 50°C for 12 h for saccharification. Subsequently, 2 g of starter culture (*Wanti*) was inoculated, and fermentation proceeded at 32°C for 15 days. The resulting liquid was extracted via centrifugation (15,000 rpm, 4°C for 30 min), filtered three times through muslin cloth, and stored at 8°C (Fig. 3 & Fig. 4).

Physicochemical properties: pH, acidity, alcohol content

The pH and titratable acidity of *Chubitchi* were measured according to the methods described by Mishra *et al*.[9]. Ethanol content was rapidly determined from 40 mL samples in sterile Falcon tubes using an Anton Paar Alex 500 computerized alcohol extractor.

Microbiological properties- LAB count, yeast count

Chubitchi total *Lactobacillus*, yeast, and coliform counts were quantified weekly for four weeks at 7°C±2°C. MRS, YM, and EMB agar were used, respectively, with results expressed as log CFU/mL.

Analysis of bio-functional properties

Antioxidative activity

Chubitchi's antioxidative activity was assessed via its ABTS radical-scavenging capacity[10]. An ABTS working solution was prepared by reacting 7 mM ABTS in deionized water with 2.45 mM potassium persulfate for 16 h in the dark, then diluted with deionized water to 0.7 OD at 734 nm. *Chubitchi* supernatant (200 µL) was added to 2300 µL ABTS solution, and absorbance at 734 nm was measured for 10 min at 30-second intervals. Antioxidative activity was quantified by the decrease in absorbance using a specific formula.

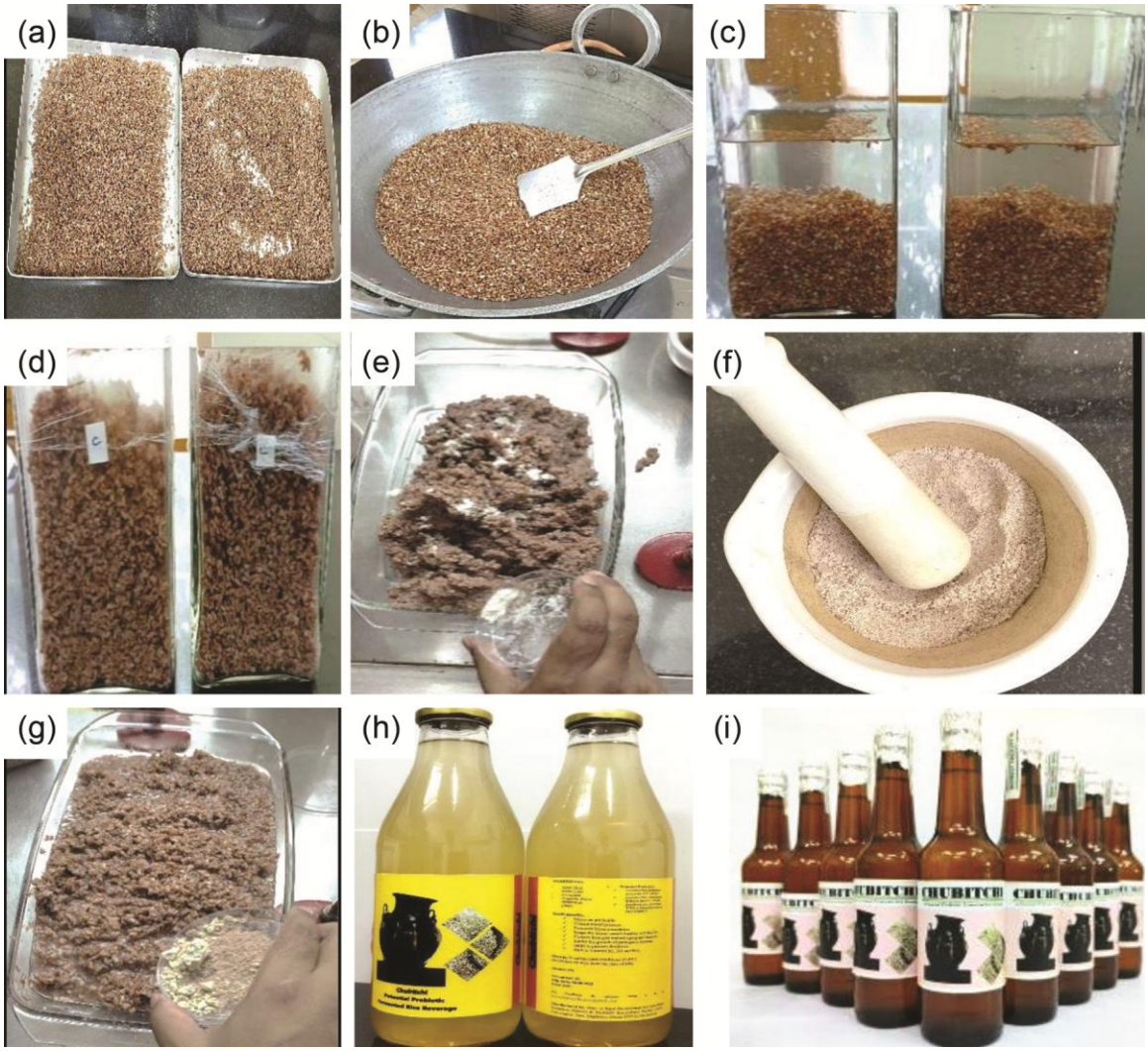

Fig. 3 — Development of *Chubitchi* (fermented rice beverage). (a) Red sticky rice (*Minil*), (b) Rice roasted for 20 min; (c) Rice soaked in distilled water at a 1:3 ratio and autoclaved; (d) Autoclaved rice; (e) Inoculated with 1% α-amylase and incubated for12 h at 50°C; (f) Starter culture crushed uniformly using a mortar and pestle; (g) Inoculated with starter culture and incubated for15 days at 32°C; (h) Development of rice beverage: centrifugated for 30 min at 15,000 rpm, filtered thrice with muslin cloth, and stored at 8°C until use; (i) Bottled rice beverage

ABTS radical scavenging activity (%) =

$$[(A_{control}-A_{sample})/ (A_{control})] \times 100$$

Were,

A_{Sample} = absorbance of the sample

$A_{Control}$ = absorbance of the control sample

ACE inhibitory activity

ACE inhibitory activity in *Chubitchi* was determined using hippuryl-histidyl-leucine (HHL) as a substrate, following the method described by Shukla *et al*.[11]. The reaction mixture- comprising HHL, sample supernatant, deionized water, and ACE enzyme-was incubated at 37°C. After the reaction was terminated with HCl, the resulting hippuric acid was extracted with ethyl acetate, evaporated to dryness, and reconstituted. Absorbance was then measured spectrophotometrically at 228 nm.

Anti-diabetic activity

α-Amylase inhibition activity

α-Amylase inhibitory activity was evaluated according to the method described by Khakhariya *et al*.[12]. A reaction mixture containing phosphate buffer, α-amylase, and *Chubitchi* supernatant was pre-incubated at 37°C for 15 min. Following the addition of soluble starch and a subsequent 20-min incubation, 3,5-dinitrosalicylic acid (DNS) reagent was added. The mixture was then heated in a boiling water bath, and the absorbance was measured at 540 nm.

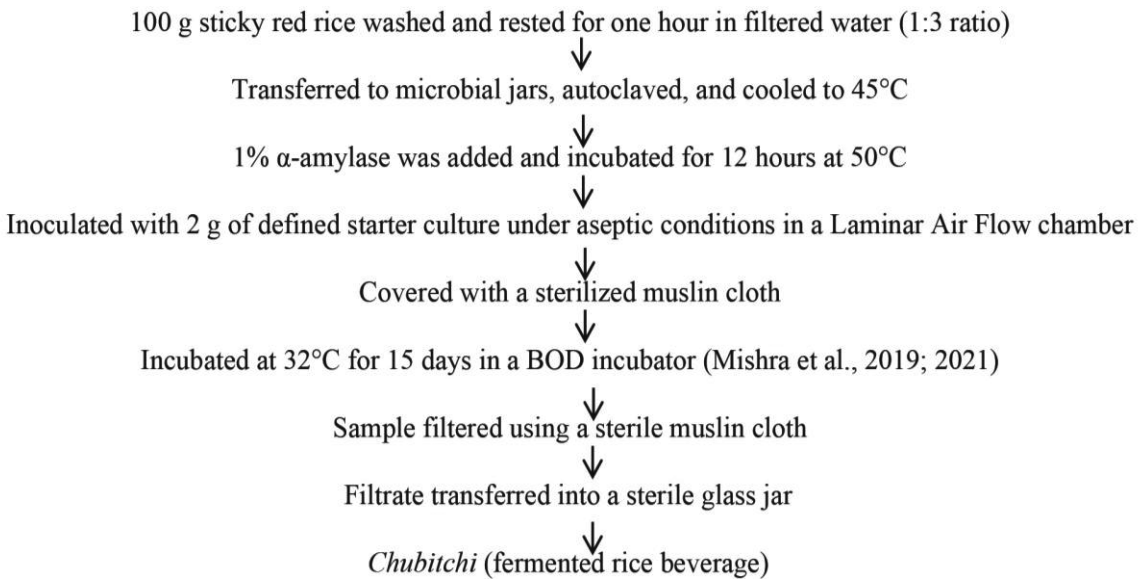

Fig. 4 — Flow diagram for *Chubitchi* (fermented rice beverage) preparation

α- Glucosidase inhibition activity

α-glucosidase inhibition by *Chubitchi* supernatant was assessed following Kinariwala *et al*.[13]. The reaction, containing phosphate buffer, enzyme, and PNPG, was incubated at 37°C, stopped with Na_2CO_3, and measured spectrophotometrically at 405 nm.

Lipase inhibition activity

Lipase inhibitory activity was assessed according to the method described by Shukla *et al*.[11]. A reaction mixture containing phosphate buffer, *Chubitchi* supernatant, 4-methylumbelliferyl oleate (4MUO), and pancreatic lipase were incubated at 37°C for 30 min. The reaction was terminated by the addition of sodium citrate, and the resulting 4 MU fluorescence was measured using a spectrophotometer at 260 nm.

Phenol content

Total phenolic content was determined using a modified method based on Subrota *et al*.[14]. To prepare the sample, the fermented Chubitchi was centrifuged at 10,000 rpm for 15 min. The resulting supernatant was then diluted 50-fold and filtered. Subsequently, 1 mL of the filtrate was mixed with 0.5 mL of Folin-Ciocalteau reagent and 10 mL of 7.5% sodium carbonate. After incubation for 60 min in the dark, the absorbance was measured at 750 nm. The results are expressed as mg gallic acid equivalents GAE/100 mL.

Organoleptic evaluation

Sensory evaluation was conducted by nine panelists accustomed to *Chubitchi* from NEHU, Tura Campus. Refrigerated samples (6-8°C) were evaluated for color, clarity, aroma, taste, mouth feel, flavor, and overall acceptability using a 9-point hedonic scale.

Anti-inflammatory and ROS activities

RAW264.7 cell culture

RAW264.7 cells (obtained from NCCS, Pune, India) were cultured in DMEM (high-glucose) supplemented with 10% (v/v) FBS and 1% penicillin-streptomycin. The cells were maintained at 37°C in a humid atmosphere with 5% CO_2 and passed every 1-2 days.

Cytotoxicity assay

Confluent RAW264.7 cells were seeded in 96-well plates at a density of 1×10^5 cells/well and incubated overnight. The cells were then treated with various *Chubitchi* dilutions (1:1 to 1:8) for 24 h. Following treatment, 100 µL of MTT reagent (0.5 mg/mL) was added to each well and incubated for an additional 4 h. The resulting formazan crystals were dissolved with 100 µL of DMSO, and the absorbance was read at 570 nm.

IL-6, IL-1β, and TNF-α cytokine measurements

TNF-α, IL-6, and IL-1β levels were measured in culture supernatants using a commercial ELISA kit, according to the manufacturer's instructions.

Reactive oxygen species detection

Total ROS production in RAW264.7 cells was evaluated following exposure to LPS (1 µg/mL) and *Chubitchi* (1:8). After 24 h of treatment, the cells were harvested by scraping and resuspended in 20 µM

DCFDA(2′,7′-dichlorodihydrofluorescein diacetate). Following a 30-min incubation in the dark, ROS levels were analyzed using a BD FACS Aria III flow cytometer via the FITC-A channel.

Statistical analysis

Data is presented as the mean ± SEM from three independent experiments. Statistical significance was determined using one-way analysis of variance ANOVA. Mean differences were analyzed using the Duncan Multiple Range Test for sample comparisons and the Tukey post-hoc test for the cell culture study groups. A p-value of $p<0.05$ was considered statistically significant. All cell culture assay data were analyzed using GraphPad Prism 8.0.

Results and Discussion

Physicochemical properties- pH, acidity& alcohol content

During four weeks of storage, the pH of *Chubitchi* decreased from 3.93 to 3.31, while the titratable acidity increased from 0.60% to 2.03% (tartaric acid equivalent; Table 1). The observed decline in pH is likely attributable to the production of organic acids by lactic acid bacteria (LAB), which consequently increases acidity. Similar pH trends were reported by Zhao *et al*.[15] in black glutinous rice wine, where the pH initially decreased before stabilization. This increase in acidity aligns with findings by Khan *et al*.[16] and Mangang *et al*.[17] for other cereal-based fermented beverages.

The ethanol concentration of *Chubitchi* initially increased during the first two weeks, reaching 5.62±0.20%. Although a minor, non-significant decrease was noted after the third week, this decline signifies the completion of fermentation, consistent with the findings of Palaniveloo & Vairappan findings[18] (Table 1). The gradual reduction in ethanol content from the third week (5.58±0.18%) onwards can be attributed to yeast producing glycerol to maintain redox balance during later fermentation stages (Scanes *et al*.)[19]. Additionally, ethanol evaporation and chemical changes in ethanol-water molecules contribute to this decrease during storage - a phenomenon also reported by Pomar and Gonzalez-Mendoza[20] and Mishra *et al*.[9].

Nutritional composition of *Chubitchi*

The composition of *Chubitchi* was analyzed according to FSSAI guidelines[21]. It contained less than 0.1% total fat and protein, 12.61% carbohydrates, and an energy value of 50 Kcal/100 g. The moisture content was 87.17%, while the total ash and total soluble solids were 0.22% and 12.83%, respectively.

Microbiological properties- LAB count, yeast count

Microbial counts in *Chubitchi* were monitored over a four-week period. The Lactic Acid Bacteria (LAB) count ranged from 5.21 to 8.67 log CFU/mL. The peak LAB count was observed at the outset (8.67±0.33 log CFU/mL), followed by a steady decrease to 5.21±0.07 log CFU/mL by the fourth week (Table 1). Similarly, the yeast count was highest at the start (7.86±0.38 log CFU/mL) before declining to 6.48±0.22 log CFU/mL by the end of the first week.

The observed decline in microbial counts is likely attributable to the inhibitory effects of alcohol and organic acid production during fermentation. These findings corroborate studies on other traditional fermented rice wines. For instance, Song *et al*.[22] noted a decline in both LAB and yeast counts in *Gayangju* fermentation following an initial increase. Similarly, Ghosh *et al*.[23] reported a decrease in yeast counts and a fluctuating LAB count in an Indian rice beverage. In contrast, Cichonska *et al*.[24] observed no significantreductionin the viable cell populations in rice-based drinks fermented with specific starter cultures.

Table 1 — Physicochemical, microbial, and antioxidant profile of *Chubitchi* during refrigerated storage (7±2°C)

Time (Weeks)	pH	Acidity (% Tartaric Acid)	Ethanol (%)	LAB Counts (log CFU/mL)	Yeast Counts (log CFU/mL)	Antioxidant Activity (%)	Phenol Content (mg/mL)
0	3.93±0.009^{a}	0.60±0.009^{c}	5.16±0.35^{a}	8.67±0.33^{a}	7.86±0.38^{a}	85.89±0.06^{d}	0.22±0.001^{d}
1	3.78±0.003^{a}	0.70±0.004^{c}	5.44±0.22^{a}	6.27±0.13^{b}	6.48±0.22^{b}	85.94±0.04^{cd}	0.25±0.004^{abc}
2	3.55±0.006^{a}	1.75±0.132^{bc}	5.62±0.20^{a}	6.06±0.12^{bc}	5.64±0.18^{c}	85.94±0.04^{cd}	0.24±0.002^{bcd}
3	3.39±0.009^{a}	1.85±0.065^{ab}	5.58±0.18^{a}	5.58±0.19^{cd}	5.10±0.21^{cd}	86.19±0.00^{a}	0.26±0.005^{ab}
4	3.31±0.009^{a}	2.03±0.043^{a}	5.04±0.06^{a}	5.21±0.07^{d}	4.86±0.19^{d}	86.04±0.06^{b}	0.27±0.015^{a}
CD (0.05)	1.95	0.22	N/A	0.57	0.75	0.13	0.022
CV %	8.96	10	N/A	5.99	8.27	0.10	6.01

*Values are expressed as Mean ± SEM (n=4). Values with different superscripts within the same column differ significantly ($p\leq0.05$). LAB: Lactic Acid Bacteria; CD: Critical Difference; CV: Coefficient of Variation.

Bio-functional properties

Antimicrobial activity

Chubitchi demonstrated notable antimicrobial activity against all tested pathogens. Against *K. pneumoniae*, the zone of inhibition was 14 mm in week 1, decreasing to 12 mm by week 4. Activity against *E. coli* remained largely constant at 13 mm, despite a temporary dip to 11 mm in week 2. Notably, *Chubitchi* was most effective against *S. aureus*, exhibiting an initial inhibition zone of 16 mm, which reduced slightly to 15 mm by week 4 (Table 2, Fig. 5).

These findings are comparable to those reported forother fermented beverages. Al-Mohammadi *et al*.[25] observed significant inhibition zones for fermented

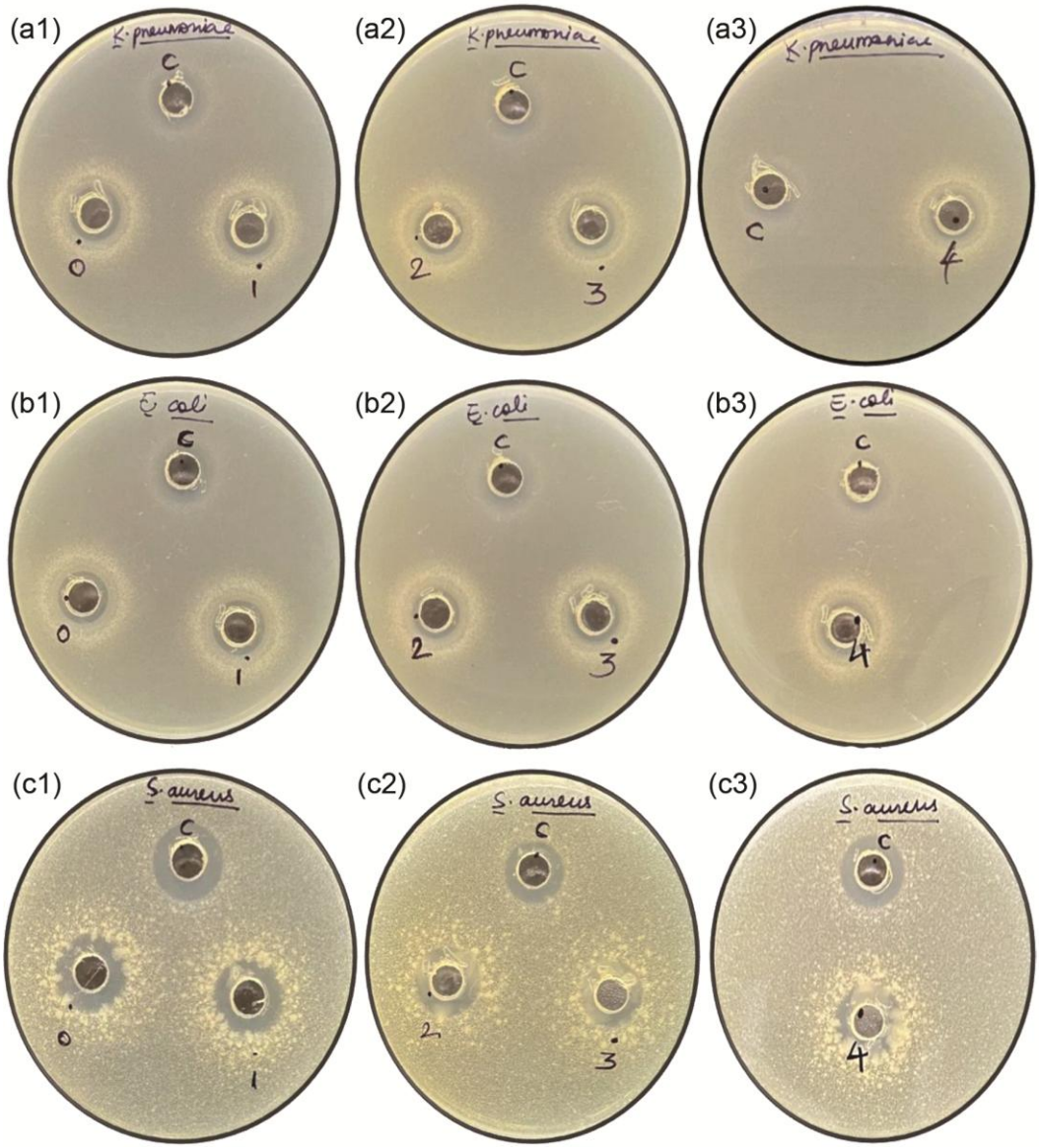

Fig. 5 — Antimicrobial activity (Zone of Inhibition) of *Chubitchi* against selected pathogens over a 4-week storage study: (a1-a3) *K. pneumoniae*, (b1-b3) *E. coli*, and (c1-c3) *S. aureus*

Table 2 — Antimicrobial activity (Zone of Inhibition) of *Chubitchi* against selected pathogenic bacteria during a 4-week storage study

Pathogenic bacteria	0 Week (mm)	1 Week (mm)	2 Week (mm)	3 Week (mm)	4 Week (mm)
K. pneumoniae	13	14	12	12	12
E. coli	13	13	11	13	13
S. aureus	16	15	16	14	15

kombucha and kefir against *S. aureus* (19-21 mm) and *E. coli* (14-18 mm). Similarly, Tano *et al*.[26] demonstrated that *L. fermentum*-fermented sorghum beer inhibited *E. coli* (15 mm) and *S. aureus* (11-13 mm). Furthermore, rice vinegar has been shown to exertantimicrobial effects against *S. aureus* (11 mm), *E. coli* (9 mm), and *S. typhimurium* (9 mm)[27].

Antioxidative activity

The antioxidative activity of *Chubitchi*, assessed by its ABTS radical-scavenging capacity, remained high and stable throughout the four-week refrigerated storage (Table 1). Peak activity was observed in the third week (86.19±0.00%), followed closely by the fourth week (86.04±0.06%). This sustained high potential suggests that during fermentation, lactic acid bacteria (LAB) and yeast may act on bound phenolic compounds, releasing more free antioxidants. Notably, the antioxidative activity in *Chubitchi* was higher than that reported for other fermented rice beverages by Cheirsilp *et al*.[28] and Hernandez-Garcia *et al*.[29].

ACE inhibitory activity

ACE catalyzes the conversion of angiotensin I to the hypertensive angiotensin II and the breakdown of the vasodilator bradykinin; therefore, ACE inhibition is critical for hypertension management. *Chubitchi* demonstrated ACE inhibitory activity ranging from 17.03% to 23.10% throughout the study, with a consistent increase from an initial 17.03±0.28% to 23.10±0.92% by week four (Fig. 6). This *in vitro* ACE inhibition is likely attributable to bioactive compounds derived during the fermentation process.

Compared to the current findings, probiotic yogurt enriched with rice bran exhibited peak ACE inhibition on day 7, followed by a decline[30]. Fermented wheat-based *dosa* displayed significantly higher ACE inhibitory activity (38.4%)[31]. Conversely, some Korean rice wines like *Makgeolli* showed no ACE inhibitory activity whatsoever[32].

Anti-diabetic activities

α- Amylase inhibition activity

Pancreatic α-amylase catalyzes the breakdown of starch, thereby increasing postprandial glucose levels; consequently, inhibiting this enzyme is a key strategy for diabetes management. *Chubitchi* exhibited α-amylase inhibitory activity ranging from 0.37% to 3.53%, peaking in the third week (3.53±0.18%). While these values are lower than those reported for other fermented products -such aslupin (26.4-72.4%)[33], *L. plantarum*-fermented camel milk (55.64-80.94%)[34], and fermented buffalo/camel milk (57.09-81.66%)[12]- *Chubitchi* nonetheless demonstrated detectable anti-diabetic potential (Fig. 6).

α- Glucosidase inhibition activity

α-Glucosidase inhibitors prevent the breakdown of complex carbohydrates, thereby delaying glucose absorption and modulating postprandial blood

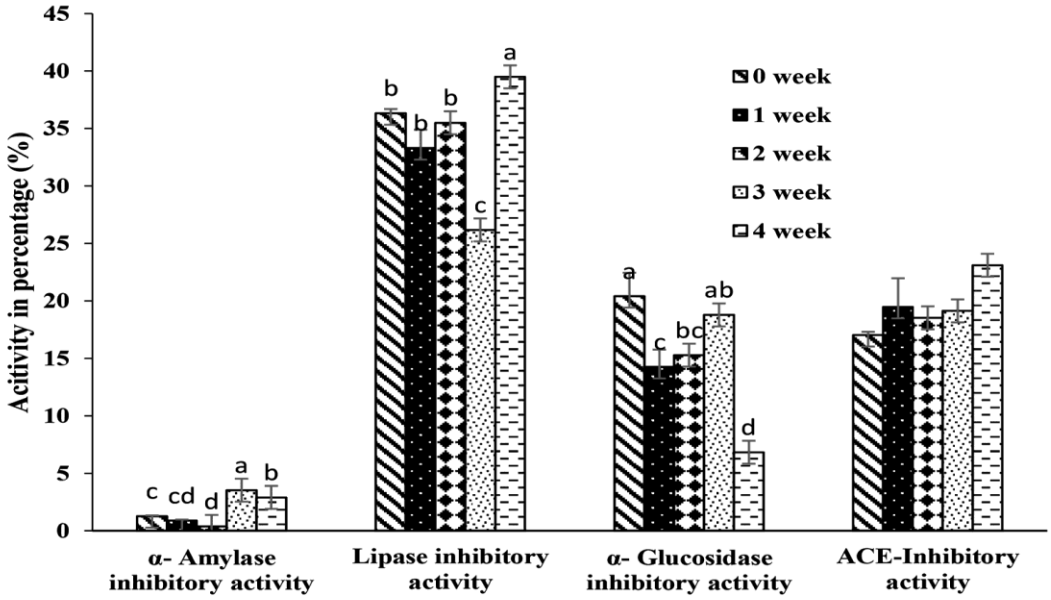

Fig. 6 — α- Amylase inhibitory activity, Lipase inhibitory activity, α- Glucosidase inhibitory activity, ACE-inhibitory activity of *Chubitchi* during storage under refrigeration conditions (7±2°C)

glucose elevations[35]. *Chubitchi* exhibited α-glucosidase inhibitory activity ranging from 14.26% to 20.42% (Fig. 6). The peak activity was observed initially (20.42±2.03%), which was statistically comparable to the activity in the third week (18.78±1.37%). These results align with inhibition levels reported for certain lactic acid bacteria-fermented vinegars (9.65-19.23%)[36] and fermented Roselle wine (up to 24%)[37]. However, this activity was considerably lower than that of specific Peruvian Chicha samples, which demonstrated substantially higher inhibition (44.47-75.73%)[38].

Lipase inhibition activity

Pancreatic lipase is essential for dietary fat digestion; consequently, its inhibition is a key strategy for managing obesity and type 2 diabetes by reducing intestinal fat absorption[39]. *Chubitchi* demonstrated lipase inhibitory activity ranging from 26.18% to 39.50%, with the peak activity (39.50±1.05%) occurring by the fourth week of storage. This upward trend suggests a gradual accumulation of lipase-inhibiting metabolites during the refrigeration period.

These findings are comparable to those reported forother fermented products. For instance, *Lacticaseibacillus casei*-fermented barley beverages demonstrated an increase in lipase inhibition from 32% to 40% during fermentation[40]. Furthermore, while some fermented rice beverages showed limited lipase inhibition[41], probiotic brown rice milk fermented with *Lactiplantibacillus pentosus* exhibited an activity level of 22.62±2.75%[42]. (Fig. 6)

Phenol content of Chubitchi

During fermentation, microorganisms convert complex phenolic compounds into more bioaccessible forms, potentially increasing antioxidative activity through the release of free aglycones[43]. The total phenolic content (TPC) of *Chubitchi* ranged from 0.22 mg/mL to 0.27 mg/mL (Table 1), with the highest concentration observed in the fourth week (0.27±0.015 mg/mL). This peak was statistically comparable to the levels recorded in the first and third weeks.

This increase in phenolic content during storage aligns with observations in other fermented products. Wang *et al.*[44] reported a significant rise in total phenolic content (TPC) in an eight-grain fermented beverage over 13 weeks. Similarly, Dela Rosa and Medina observed increased phenolic concentrations in Philippine rice wines produced from various rice varieties following a 7-day fermentation. Furthermore, Das *et al.*[2], and Mangang *et al.*[17] ocumented a similar increase in total phenol content during the extended storage of fermented rice beers.

Organoleptic evaluation

Organoleptic evaluations of *Chubitchi* over a 40-day storage period (Table 3) revealed significant sensory changes ($p \leq 0.05$). Whilescores for color, clarity, aroma, taste-mouthfeel, flavor, and overall acceptability remained above 7 (indicating good acceptability) between days 0 and 20, they significantly declined to below 6 by day 40. This decline effectively rendered the product unacceptable to the sensory panel by the end of the storage period.

The color of *Chubitchi* transitioned from a pale yellow-amber to a golden yellow-amber, with scores peaking on day 20 (8.10±0.10) before declining to 5.98±0.08 by day 40. This deepening of color is likely attributable to the polymerization of monomeric pigments during storage. Furthermore, clarity decreased from 7.25±0.15 (day 0) to a hazy 5.60±0.16 (day 40), a change potentially caused by suspended microbialcells, protein precipitation, or the solubilisation of specific compounds, as previously suggested by Cheirsilp *et al.*[28].

The aroma scores peaked on day 20 (7.78±0.04), characterized by sweet and fruity notes, but became increasingly acidic and reached their lowest point by day 40 (5.87±0.14). This acidity may stem from

Table 3 — Organoleptic evaluation of *Chubitchi*

Parameters	Storage period (days)				
	0	10	20	30	40
Colour	7.15±0.10^{a}	7.42±0.21^{a}	8.10±0.10^{b}	6.42±0.11^{c}	5.98±0.08^{d}
Clarity	7.25±0.15^{a}	7.50±0.12^{a}	7.33±0.12^{a}	6.15±0.18^{c}	5.60±0.16^{d}
Aroma	7.48±0.20^{a}	7.66±0.10^{a}	7.78±0.04^{a}	6.48±0.21^{c}	5.87±0.14^{d}
Taste &mouth feel	7.32±0.24^{a}	7.77±0.28^{a}	7.82±0.15^{a}	6.20±0.20^{c}	5.74±0.12^{d}
Flavor	7.20±0.15^{a}	7.80±0.11^{a}	8.11±0.17^{b}	6.43±0.10^{c}	5.66±0.15^{d}
Overall acceptability	7.33±0.21^{a}	7.57±0.33^{a}	7.73±0.19^{a}	6.78±0.15^{c}	5.82±0.11^{d}

* Values with different superscripts differ significantly ($p \leq 0.05$), Mean ± SEM, n=9

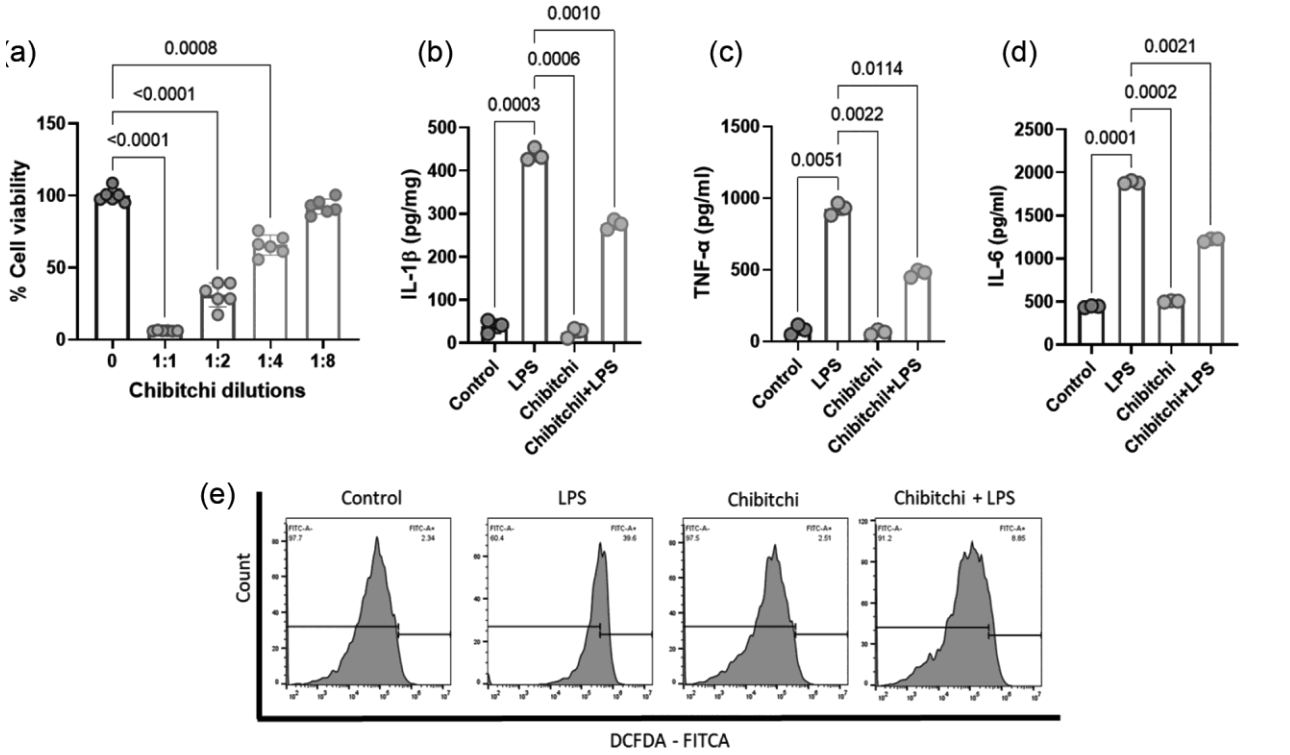

Fig. 7 — Anti-inflammatory activity and ROS of *Chubitchi*: (a) cell viability of *Chubitchi* in RAW264.7 cells, (b) IL-1β, (c) TNF-α, and (d) IL-6 production; (e) ROS production. Data are expressed as mean ± SEM and analyzed by one-way ANOVA followed by Tukey's post hoc test

acetaldehyde oxidation during the later stages of fermentation. Similarly, taste and mouth feel varied significantly; a sweet-bitter savory peak was recorded on day 20 (7.82±0.15), which transitioned to a tangy, low score by day 40 (5.74±0.12). This shift is potentially attributable to LAB-induced sourness and incipient microbial spoilage. Consequently, flavour scores also peaked on day 20 (8.11±0.17) before declining as acidic notes became more prominent.

Overall, the laboratory-prepared *Chubitchi* was rated as "very good" on day 20 (7.73±0.19) but was deemed unacceptable by day 40. Notably, the traditionally prepared *Chubitchi* remained acceptable only until day 20. These findings align with previous research regarding the impact of increasing acidity on the sensory attributes of fermented beverages during the aging process[9].

Anti-inflammatory and ROS in *Chubitchi* (Rice fermented beverage)

Initial evaluations confirmed the non-cytotoxic nature of *Chubitchi* at dilutions up to 1:8, which was subsequently utilized for further studies. *Chubitchi* alone did not induce an elevation in inflammatory cytokines within murine macrophage cells, demonstrating its non-inflammatory character. Crucially, co-treatment with LPS significantly reduced extracellular inflammatory cytokine production and ROS levels (from 36.9% to 8.85%). This highlights the potent anti-inflammatory and antioxidant bio-functionality of *Chubitchi* (Fig. 7), aligning with prior research on fermented grain beverages where similar effects were attributed to compounds such as terpenoids and fatty acid esters. These findings are consistent with studies on cereal-based fermented drinks that report the prevention of ROS regeneration and the inhibition of NF-κB pathway activation.

Conclusion

Chubitchi, a traditional Garo rice beverage, was prepared aseptically using a defined starter culture of lactic acid bacteria (LAB), yeast, and *S. dulcis* extract. The laboratory-prepared version exhibited sensory attributes comparable to those of traditionally made *Chubitchi* while demonstrating significant health benefits, including antioxidant, antimicrobial, ACE inhibitory, anti-inflammatory, and anti-diabetic properties. Throughout refrigerated storage, LAB and

yeast populations remained stable. This research underscores the potential of *Chubitchi* as a functional food, offering a standardized production method to enhance the socioeconomic well-being of tribal communities in Meghalaya and promoting the global recognition of traditional Indian fermented foods.

Acknowledgments

We thank NABI, Mohali, Punjab, India, for assistance with the cell culture study.

Funding

Funding was provided by the Department of Biotechnology, Ministry of Science and Technology [Grant BT/PR41738/ NER/95/1857/2021], Government of India.

Author Contributions

SH, BKM, KKK, KR, BB: conceptualized, designed, and supervised; AB, BB, KR: analyzed data, drafted the manuscript; SH, KKK, MB, BB: edited it. All authors approved the final version.

Conflict of Interest

Authors declare that there is no conflict of interest.

Ethics Approval and Consent to Participation

Approved by the North-Eastern Hill University Ethical Committee (Ref: TC/RDAP/ DBT Twinning/Sens./2023-01, dated: 10.05.2023). All panelists gave informed consent, affirming that they understood no harm would result from product consumption.

Data Availability

Data available from the corresponding author upon reasonable request.

References

1 Jyrwa Y W, Palika R, Boddula S, Boiroju N K, Madhari R, *et al*., Retention, stability, iron bioavailability and sensory evaluation of extruded rice fortified with iron, folic acid and vitamin B12, *Matern Child Nutr*, 16 (2020) e12932. https://doi.org/10.1111/mcn.12932

2 Das A J, Deka S C & Miyaji T, Methodology of rice beer preparation and various plant materials used in starter culture preparation by some tribal communities of North-East India: a survey, *Int Food Res J*, 19 (2012) 101-107.

3 Bala Murugan N, Mishra B K & Paul B, Antibacterial activity of indigenous fermented rice beverage of West Garo Hills, Meghalaya, India, *Int J Ferment Food*, 7 (1) (2018) 39-44. http://dx.doi.org/10.30954/2321-712X.01.2018.5

4 Que F, Mao L, Zhu C & Xie G, Antioxidant properties of Chinese yellow wine, its concentrate and volatiles, *LWT-Food Sci Technol*, 39 (2) (2006) 111-117. https://doi.org/10.1016/j.lwt.2005.01.001

5 Bhuyan D J, Barooah M S, Bora S S & Singaravadivel K, Biochemical and nutritional analysis of rice beer of North East India, *Indian J Tradit Know*, 13 (1) (2014) 142-148.

6 Dung N T P, Defined fungal starter granules for purple glutinous rice wine, Ph.D. thesis, (Wageningen University, Wageningen, The Netherlands), (2004).

7 Kawachi M & Noel M H, Sterilization and sterile technique, In: *Algal Culturing Techniques*, (Elsevier Academic Press, USA) (2005) 65-81.

8 Balamurugan N, Physicochemical sensory and microbial analysis of fermented rice beverage *Chubitchi* in West Garo Hills, Meghalaya. Ph.D. thesis, North-Eastern Hill University, Meghalaya, India, (2018). http://hdl.handle.net/10603/299444

9 Mishra B K, Das S, Prajapati J B & Hati S, Bio-functional properties and storage study of '*Chubitchi*'-a fermented rice beverage of Garo Hills, Meghalaya, *Indian J Tradit Know*, 20 (2) (2021) 498-511. https://doi.org/10.56042/ijtk.v20i2.31946

10 Mishra B K, Hati S, Brahma J & Das S, Evaluation of probiotic potentials of yeast isolates from traditional fermented rice beverages of Meghalaya, India, *Rev Med Microbiol*, 32 (1) (2021) 28-38. https://doi.org/10.1097/MRM.0000000000000230

11 Shukla P, Sakure A, Pipaliya R, Basaiawmoit B, Maurya R, *et al*., Exploring the potential of *Lacticaseibacillus paracasei* M11 on antidiabetic, anti-inflammatory, and ACE inhibitory effects of fermented dromedary camel milk (*Camelus dromedaries*) and the release of antidiabetic and anti-hypertensive peptides, *J Food Biochem*, 46 (12) (2022) e14449. https://doi.org/10.1111/jfbc.14449

12 Khakhariya R, Sakure A A, Maurya R, Bishnoi M, Kondepudi K K, *et al*., A comparative study of fermented buffalo and camel milk with anti-inflammatory, ACE-inhibitory and anti-diabetic properties and release of bioactive peptides with molecular interactions: *In vitro, in silico* and molecular study, *Food Biosci*, 52 (2023) 102373. https://doi.org/10.1016/j.fbio.2023.102373

13 Kinariwala D, Panchal G, Sakure A & Hati S, Exploring the potentiality of *Lactobacillus* cultures on the production of milk-derived bioactive peptides with antidiabetic activity, *Int J Pept Res Ther*, 26 (2020) 1613-1627. https://doi.org/10.1007/s10989-019-09958-5

14 Subrota H, Shilpa V, Brij S, Vandna K & Surajit M, Antioxidative activity and polyphenol content in fermented soy milk supplemented with WPC-70 by probiotic *Lactobacilli*, *Int Food Res J*, 20 (5) (2013) 2125-2131.

15 Zhao C, Su W, Mu Y, Jiang L & Mu Y, Correlations between microbiota with physicochemical properties and volatile flavor components in black glutinous rice wine fermentation, *Food Res Int*, 138 (2020) 109800. https://doi.org/10.1016/j.foodres.2020.109800

16 Khan M, Singh K & Pandey S, Quality aspects of rice-based fermented beverage, *Biomed J Sci Tech Res*, 34 (5) (2021) 27096-27104. https://doi.org/10.26717/BJSTR.2021.34.005609

17 Mangang K C S, Das A J & Deka S C, Comparative shelf life study of two different rice beers prepared using wild-type and established microbial starters, *J Inst Brew*, 123 (4) (2017) 579-586. https://doi.org/10.1002/jib.446

18 Palaniveloo K & Vairappan C S, Biochemical properties ofrice wine produced from three different starter cultures, *J Trop Biol Conserv*, 10 (2013) 31-41.

19 Scanes K T, Hohmann S & Priori B A, Glycerol production by the yeast *Saccharomyces cerevisiae* and its relevance to wine: A review, *S Afr J Enol Vitic*, 19 (1) (1998) 17-24.

20 Pomar M & Gonzalez-Mendoza L A, Changes in composition and sensory quality of red wine aged in American and French oak barrels, *J Int Sci Vigne Vin*, 35 (1) (2001) 41-48.

21 Notification issued by the Ministry of Health and Family Welfare (Food Safety and Standards Authority of India), New Delhi, dated December 23, 2016. Available online: https://www.fssai.gov.in/upload/advisories/2018/02/5a93d37400ba9Directions_Health_Suppliments_06_01_2017.pdf (Accessed February 21, 2026)

22 Song Y-R, Lim B-U & Baik S-H, Microbial diversity and volatile flavor changes during *Gayangju* fermentation, a traditional Korean house rice wine, *Foods*, 11 (17) (2022) 2604. https://doi.org/10.3390/foods11172604

23 Ghosh K, Ray M, Adak A, Dey P, Halder S K, *et al*., Microbial, saccharifying and antioxidant properties of an Indian rice-based fermented beverage, *Food Chem*, 168 (2015) 196-202. https://doi.org/10.1016/j.foodchem.2014.07.042

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

25 Al-Mohammadi A-R, Ismaiel A A, Ibrahim R A, Moustafa A H, Zeid A A, *et al*., Chemical constitution and antimicrobial activity of Kombucha fermented beverage, *Molecules*, 26 (16) (2021) 5026. https://doi.org/10.3390/molecules26165026

26 Tano M B, Aka-Gbezo S, Attchelouwa C K & Koussémon M, Use of lactic acid bacteria as starter cultures in the production of Tchapalo, a traditional sorghum beer from Côte d'Ivoire, *Am J Food Sci Health*, 6 (2020) 23-31.

27 Bakir S, Devecioglu D, Kayacan S, Toydemir G, Karbancioglu-Guler F, *et al*., Investigating the antioxidant and antimicrobial activities of different vinegars, *Eur Food Res Technol*, 243 (2017) 2083-2094. https://doi.org/10.1007/s00217-017-2908-0

28 Cheirsilp B, Satansat J, Wanthong K, Chaiyasain R, Rakmai J, *et al*., Bioprocess improvement for fermentation of pigmented Thai glutinous rice-based functional beverage (Sato) with superior antioxidant properties, *Biocatal Agric Biotechnol*, 50 (2023)102701. https://doi.org/10.1016/j.bcab.2023.102701

29 Hernandez-Garcia Y, Melgar-Lalanne G, Téllez-Medina D I, Ruiz-May E, Salgado-Cruz M D L P, *et al*., Scavenging peptides, antioxidant activity, and hypoglycemic activity of a germinated amaranth (*Amaranthus hypochondriacus* L.) beverage fermented by *Lactiplantibacillus plantarum*, *J Food Biochem*, 46 (7) (2022) e14139. https://doi.org/10.1111/jfbc.14139

30 Hu J, Wang H, Weng N, Wei, Tian X, *et al*., Novel angiotensin-converting enzyme and pancreatic lipase oligopeptide inhibitors from fermented rice bran, *Frontiers in Nutrition*, 9 (2022) 1-9. https://doi.org/10.3389/fnut.2022.1010005

31 Darwish M S, Gomaa M S, Elsherbiny E S & Mostafa M S, Angiotensin-converting enzyme (ACE-1) inhibitory and antioxidant activities of probiotic yogurt enriched with rice bran, *Egypt J Chem*, 66 (12) (2023) 353-365. https://dx.doi.org/10.21608/ejchem.2023.190160.7527

32 Zareian M, Oskoueian E, Forghani B & Ebrahimi M, Production of a wheat-based fermented rice enriched with γ-amino butyric acid using *Lactobacillus plantarum* MNZ and its antihypertensive effects in spontaneously hypertensive rats, *J Funct Foods*, 16 (2015) 194-203. https://doi.org/10.1016/j.jff.2015.04.015

33 Choi J-S, Seo H J, Lee Y-R, Kwon S-J, Moon S H, *et al*., Characteristics and *in vitro* anti-diabetic properties of the Korean rice wine, Makgeolli fermented with *Laminaria japonica*, *Prev Nutr Food Sci*, 19 (2) (2014) 98-107. https://doi.org/10.3746%2Fpnf.2014.19.2.098

34 Ayyash M, Johnson S K, Liu S Q, Mesmari N, Dahmani S, *et al*., *In vitro* investigation of bioactivities of solid-state fermented lupin, quinoa, and wheat using *Lactobacillus* spp, *Food Chem*, 275 (2019) 50-58. https://doi.org/10.1016/j.foodchem.2018.09.031

35 Shukla P, Sakure A, Maurya R, Bishnoi M, Kondepudi K K, *et al*., Antidiabetic, angiotensin-converting enzyme inhibitory and anti-inflammatory activities of fermented camel milk and characterization of novel bioactive peptides from lactic-fermented camel milk with molecular interaction study, *Int J Dairy Technol*, 76 (1) (2023) 149-167. https://doi.org/10.1111/1471-0307.12910

36 Chin Meng Khoo, Diabetes Mellitus Treatment, In: *International Encyclopedia of Public Health*, (Second Edition), Stella R Quah(Ed), (Academic Press), (2017) 288-293, ISBN 9780128037089, https://doi.org/10.1016/B978-0-12-803678-5.00108-9

37 Ifie I, Marshall L J, Ho P & Williamson G, *Hibiscus sabdariffa* (Roselle) extracts and wine: Phytochemical profile, physicochemical properties, and carbohydrase inhibition, *J Agric Food Chem*, 64 (24) (2016) 4921-4931. https://doi.org/10.1021/acs.jafc.6b01246

38 Vargas-Yana D, Aguilar-Morón B, Pezo-Torres N, Shetty K & Ranilla L G, Ancestral Peruvian ethnic fermented beverage "Chicha" based on purple corn (*Zea mays* L.): unraveling the health-relevant functional benefits, *J Ethn Foods*, 7 (2020) 35. https://doi.org/10.1186/s42779-020-00063-3

39 Zhu G, Fang Q, Zhu F, Huang D & Yang C, Structure and function of pancreatic lipase-related protein 2 and its relationship with pathological states, *Front Genet*, 12 (2021) 693538. https://doi.org/10.3389%2Ffgene.2021.693538

40 Guo W, Chen M, Cui S, Tang X, Zhang Q, *et al*., Effects of *Lacticaseibacillus* casei fermentation on the bioactive compounds, volatile and non-volatile compounds, and physiological properties of barley beverage, *Food Biosci*, 53 (2023) 102695. https://doi.org/10.1016/j.fbio.2023.102695

41 Otunola G A, Talabi O T, Oduro-Yeboah C, Nyako J, Ojo O, *et al*., Fermented rice beverages modulate key enzymes related to type 2 diabetes *in vitro*, *Preprints*, 2023 (2023)

2023070617. https://doi.org/10.20944/preprints202307.0617.v1

42 Kittibunchakul S, Yuthaworawit N, Whanmek K, Suttisansanee U & Santivarangkna C, Health beneficial properties of a novel plant-based probiotic drink produced by fermentation of brown rice milk with GABA-producing *Lactobacillus pentosus* isolated from Thai pickled weed, *J Funct Foods*, 86 (2021) 104710. https://doi.org/10.1016/j.jff.2021.104710

43 Melini F & Melini V, Impact of fermentation on phenolic compounds and antioxidant capacity of quinoa, *Fermentation*, 7 (1) (2021) 20. https://doi.org/10.3390/fermentation7010020

44 Wang C-F, Huang C-R & Lu Y-C, Changes in bio-functional compounds, ACE inhibition, and antioxidant capacity after mixed fermentation of eight whole grains, *Fermentation*, 9 (3) (2023) 209. https://doi.org/10.3390/fermentation9030209