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Bio-functional properties and storage study of '*Chubitchi*'- a fermented rice beverage of Garo Hills, Meghalaya

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In this study, an ethnic fermented rice beverage- '*Chubitchi'* from Garo Hills region of Meghalaya was closely monitored and was improvised with well characterised *Lactobacillus* and *Saccharomyces* strains to develop a product similar to it under laboratory conditions. A comparative analysis between the traditionally made and laboratory-made rice beverage was made to assess their storage period and bio-functional properties up to 40 days. During the organoleptic evaluation based on the scores of all sensory attributes, the laboratory-made rice beverage was acceptable till day 30 and the traditionally made rice beverage was acceptable till day 20. A decrease in pH and increase in acidity (%) with increase in fermentation time was observed for both rice beverage types. An initial rise in ethanol percentage was also witnessed with increase in fermentation time till day 20 for both the rice beverage types. The ACE inhibitory property of laboratory-made *Chubitchi* enhanced from 22.64% at day 0 to 86.87% at day 20 followed by a reduction at day 30 (68.04%). The antioxidative activity was highest at day 0 (95.18%) followed by lowering down at day 10 (81.59%). The laboratory prepared *Chubitchi* showed rapid reduction in the polyphenol content and high antimicrobial activity against major test organisms during the storage study. The results show longer storage period and higher ACE-inhibition, antioxidative, total phenol reduction and antimicrobial activity of the laboratory prepared *Chubitchi* against the traditional made. This study could further provide the rural tribes of Garo Hills with well-defined novel starter cultures as well as optimized procedure for rice beverage development with numerous health benefits.

Keywords: *Chubitchi,* Fermented rice beverage, Garo Hills, *Lactobacillus, Saccharomyces*

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The North-eastern part of India is generally characterized with a diverse population of tribal communities with different ethnic backgrounds who bear their very own methods of fermenting rice and employing different starter cultures. As a part of the rich tribal diet and culture in the region of Garo Hills of Meghalaya state, indigenous fermented beverage brewed from rice is considered as an integral part[1]. The rural ethnic tribal population in the Garo Hills of Meghalaya state holds a rich sense of traditional knowledge and conventional culture of which traditionally brewed rice beverage has been an indispensable part economically, culturally, and spiritually and assumes a very eminent role in their socio-cultural activities[2]. The traditional beverage of Garo tribes is locally termed as '*Chubitchi*' (*'Chu'*- rice, *'bitchi'*- beer/beverage), which holds a rich source of heritage handed down from their ancestors and till now remains to be produced at household level. Hence, the authentication of this traditional knowledge is currently limited to only the local tribal communities in rural villages. Moreover, development of the traditional fermentation of rice beverages remains uncontrolled and is dependent on micro-organisms for the fermentation substrate and from the environment that can result in products of low yield and variable quality. However, abiding by proper hygienic brewing techniques would improve the beverages' quality and safety under ambient conditions[3].

Besides, fermented rice beverages have also been considered as 'probiotic' due to various strains of lactic acid bacteria (LAB) and yeast present in it, which are often conceived to bear probiotic attributes[4]. Likewise, several positive health claims have been linked to fermented rice beverages claimed to be obtained from added medicinal plants/herbs that apparently increase the beverage's antioxidative

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potential and assist with the free radical disruption[3]. The lactic acid bacteria and yeasts also contribute to various organoleptic and specific medicinal properties to the beverage that could be dependent on the type of medicinal plants used[1]. Fermented rice beverage of Taiwan has been reported with antibacterial effect against gram-positive bacteria such as such as *Bacillus subtilis* and *Staphylococcus aureus* and gram-negative bacteria, such as *Escherichia coli* and *Pseudomonas aeruginosa*[5]. There have been several claims that alcoholic rice beverages' health-promoting effects may be related to their antioxidant and anti-bacterial activity. Japanese rice wine *Sake* has also been widely known for its antimicrobial and anticancer properties. Like *Sake*, the Korean rice wine has recently been reported to have beneficial health effects, with gastro-protective, antioxidant and antibacterial properties[6].

The study aimed to develop fermented rice beverage product by improvising the methodology of traditionally made rice beverage (*Chubitchi*) of Garo Hills using well characterized indigenous *Lactobacillus* and *Saccharomyces* strains. Furthermore, the storage study and bio-functional attributes of the laboratory-made and traditionally-made rice beverages were assessed.

Materials and Methods

Bacterial and yeast strains

The lactic acid bacteria and yeast cultures employed in the study viz., *Lactobacillus plantarum* KGL3A (MG722814), *Lactobacillus fermentum* KGL4 (MF951099) and *Sachharomyces cerevisiae* WTS1A (MG183699) were isolated previously from various ethnic fermented rice-based beverages of Garo Hills, Meghalaya, India which were deposited in culture collections of Animal Science Laboratory, Department of Rural Development and Agricultural Production, North-Eastern Hill University, Tura Campus, Meghalaya, India. These isolates were previously purified and studied for estimating the morphological and biochemical features using the API Kit (Biomerieux, Germany)[7]. The molecular characterization of isolates was evaluated by 16s rRNA (for bacteria) and 5.8s rRNA (for yeast) gene sequencing and the amplified gene sequences were submitted to NCBI (National Center for Biotechnology Information). These isolates were further considered for this study because of their rich probiotic attributes reported earlier[7].

Indicator strains for performing the antimicrobial activity consisted of *Bacillus cereus* ATCC 14459, *Enterococcus faecalis* NCDC 115, *Staphylococcus aureus* MTCC 114, *Escherichia coli* ATCC 25922, *Listeria monocytogenes* and *Salmonella typhi* NCTC 5017. These organisms were obtained from the culture collection maintained by Dept. of Dairy Microbiology, SMC College of Dairy Science, Anand Agricultural University, Anand, Gujarat, India.

Preparation of Chubitchi (traditional method in Garo tribes of Meghalaya, India)

Development of starter culture (Wanti) by the Garo tribes of Meghalaya

The traditional practice for the processing of *Chubitchi* was observed and monitored in a step by step manner in a village (Chasingre) located in the West Garo Hills district of Meghalaya, India, as depicted in Fig. 1 (A-J) and Fig. 2 (A-J) . Around 500 g sticky rice, *Menil* was taken, followed by the addition of 2-3 red chilies (optional step) and was finely ground to powder in a traditional wooden mortar pestle. Around 40 mL normal tap water was added to the mix of powdered rice and chilies. The mixture was rolled into small round doughs. Charcoal blocks from the fireplace are put into the rice dough for generating a smoky flavour (optional step). The small doughs were covered and packed with the leaves of *Scoparia dulcis* or *Thelypteris clarkei* and stored in bamboo baskets for 5-6 days under room temperature. Dried starter cultures (*Wanti*) are formed after the 6th day [Fig. 1 (A-J)].

Development and brewing of fermented rice beverage (Chubitchi) by the traditional Garo tribal process

The process [Fig. 2 (A-J)] begins after the starter culture gets completely dried up after storing it in the indigenous bamboo baskets. Boiled rice (*Menil*) was cooled, transferred in banana leaves and few grams (approx. 5-6) of starter culture was added to the rice and was mixed uniformly. A bamboo basket perforated with uniform holes throughout its body was placed at the center of an earthen pot. The rice-starter mix was stuffed around the bamboo basket. The entire pot was tied and covered with ficus/banana leaves and was kept above a fireplace for 15-20 days for fermentation to take place. The fermented rice beverage accumulates within the bamboo sieve between 15-20 days of fermentation. Dried bottle gourd shells (*Pong*) are used to remove the beverage from the bamboo basket. The obtained beverage is

further sold in the local market or used for consumption purposes.

Preparation of Chubitchi like product under laboratory conditions

Development of defined starter culture

The formulation and development of defined starter cultures were executed with methods by Dung[8] with requisite modifications as depicted in Fig. 3 (A-J). The locally grown sticky red rice, *Menil* (500 g) was washed with clean filtered water and dried in the oven at 50, after which it was powdered and stored in a sterilized airtight reagent bottle. 100 g of rice powder was autoclaved each time before being used in starter culture preparation. The utensils used during the preparation (mortar, pestle, mixer grinder jars, desiccator, etc.) were washed well, rinsed with distilled water and UV sterilized (20 min). Other items such as petriplates and spoons used were autoclaved. The vernal stem and leaves of *Scoporia dulcis* plant were surface sterilized with 1% sodium hypochlorite by immersing them in the solution for 15 min after which they were rinsed well with sterile water thrice and ground to paste consistency in an

Fig. 1 — Traditional process for Starter culture preparation *(Wanti)* for development of fermented rice beverage by the indigenous Garo tribes of Meghalaya. a) Sticky rice (*Menil*) and red chilli; b) Vernal stems and leaves of *S. dulcis* plant; c) Uniform crushing the mix of rice, red chili and stems/leaves of *S. dulcis* plant in a traditional wooden mortar pestle; d) Addition of water into the mix; e) Rolling the mix into round doughs; f) The dough is placed in the leaves of *Thelypteris clarkei*; g) Charcoal blocks from the fire places are put into the rice dough for generating smoky flavour (optional step); h) Packing of the starter culture (*Wanti*) with the leaves of *Thelypteris clarkei*; i) Storage in bamboo baskets for 6 days under room temperature; j) Formation of the dried starter culture after the 6th day.

Fig. 2 — Brewing methodology of fermented rice beverages by the pre-dominant Garo tribes of West Garo Hills. a) Boiled red sticky rice *(Menil)* uniformly spread in the banana leaves; b) After the rice cools down to room temperature, the starter culture is added; c) Rice-starter mix is packed in the pot around the bamboo sieve; d) The pot was covered by the banana leaves; e) The pot is placed above a fireplace; f) The pot is kept for 15-20 days at room temperature for fermentation to take place; g) Accumulation of fermented rice beverage in the bamboo sieve; h) *Pong*- Dried bottle groud shells; i) Beverage is removed with pong; j) A Garo tribal folk collecting the beverage for either selling in the local market or consumption purpose.

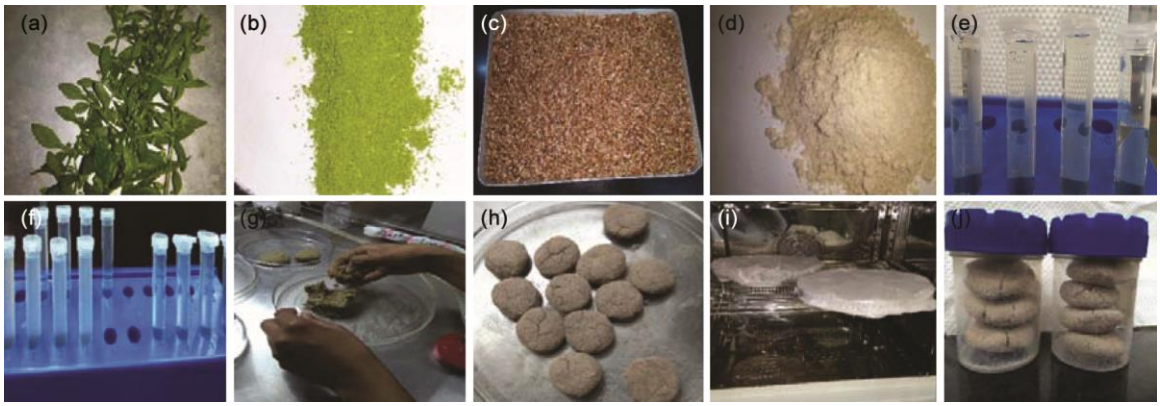

Fig. 3 — Preparation of starter culture under laboratory conditions. a) Medicinal plant to be used- *Scoporia dulcis*; b) Plant extract; c) Red sticky rice (*Menil*); d) Autoclaved rice powder; e) Filter sterilized extract of *S. dulcis*; f) Cell suspensions of KGL3A, KGL4 and WTS1A; g)- D, E, & F were mixed and kneaded into dough with sterilized filter water; h) Dough rolled into small slightly flat cakes-starter culture (*Wanti*); i)- Dried in the oven at 40ºC for 4 days; j) Stored in an aseptic container at 4ºC.

electrical blender (Philips, India) and filtered using Whatman filter paper (110 mm). The plant extracts (2mg/mL) were passed subsequently through Millex-HP syringe filter unit bearing a 0.45 µm pore size hydrophilic polyethersulfone (PES) membrane (Merck Pvt. Ltd.). *L. plantarum* KGL3A (MG722814), *L. fermentum* KGL4 (MF951099), *S. cerevisiae* WTS1A (MG183699) were grown in their respective broth (MRS- deMan, Rogosa Sharpe/YPD- Yeast Peptone Dextrose) and harvested at their exponential growth phase, washed twice in phosphate-buffered saline (PBS) and each was resuspended to 4 mL in sterile distilled water (KGL3A, 1 X 10^8 CFU/ml; KGL4, 1 X 10^8 CFU/ mL; WTS1A, 7 X 10^7 CFU/mL). The different ingredients (autoclaved red rice powder- 100 g, filter-sterilized extract of vernal stem and leaves of *S. dulcis*- 5 mL, 4 mL of each suspension of KGL3A, KGL4 and WTSA) were pooled together and were kneaded to a dough using sterilized filter water (as per requirement) and rolled into small slightly flat balls under aseptic conditions under the laminar airflow (LAF). The freshly prepared defined starter cakes were enclosed in sterilized desiccator and dried in the oven at 40° C for 4 days. They were packed in previously sterilized sample bottles and refrigerated at 4° C.

Brewing of functional fermented rice beverage in the laboratory

The sticky red rice, *Menil* was weighed to 200 g, washed properly with filtered water, transferred into microbial jars with rice to water ratio of 1:3, covered with a sterile muslin cloth and subsequently autoclaved. The microbial jar containing the steamed rice was allowed to cool at room temperature. It was inoculated with 1% α-amylase and incubated at 50° C in a hot air oven for 12 h to crusade the saccharification process. Further, the jar was inoculated with 2 g of defined starter culture and was incubated at 32° C to facilitate fermentation for 15 days. After the 15th day, the jars were taken out, followed by centrifugation (Sorvall™, Legend XTR, ThermoFisher Scientific, India) at 15,000 rpm for 30 min to separate the beverage from the red rice. The beverage obtained was filtered by muslin cloth thrice and was stored at 8 until further use [Fig. 4 (A-J)].

Shelf-life study of the traditional and laboratory-made Chubitchi like product

Organoleptic evaluation

The quality of the laboratory-made and traditional fermented beverages samples was determined by employing indigenous expert panelists (5 members) to measure specific attributes of the samples (color, clarity, aroma, flavour, taste, mouthfeel and overall quality) for 40 days with 10 days interval. The samples were subjected to ortho-nasal and retronasal perception, taste and mouthfeel gustatory modality by five native panelists (who have been known to be a regular patron in devouring the fermented rice beverage) in over three tasting sessions in an attempt to generate terms and identify key product attributes and deduce appropriate intensity ratings on the 9-point hedonic scale (9=like very much to 1=dislike very much). The beverage bottles were opened immediately before tasting, and 30 mL of each sample was served in standard wine glasses. The samples were evaluated according to the methodology described by Japan Sake and Shochu Makers Association[9].

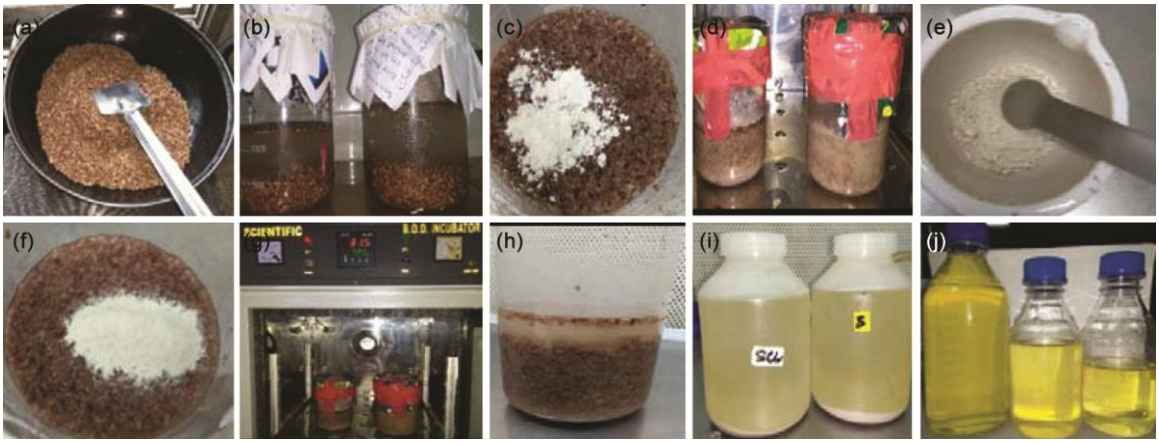

Fig. 4 — Development and brewing of rice beverage under laboratory conditions. a) Roasting of red rice variety for 20 min.; b) Rice soaked and washed in filtered water followed by autoclaving; c) Inoculation with 1% α-amylase; d) Incubation at 50℃ for 12 h; e) Uniform crushing of starter culture by mortar-pestle; f) Inoculation with the starter culture; g) Incubation at 32℃ for 15 days; h) Formation of rice beverage; i) Centrifugation at 15,000 rpm for 30 min; j) The rice beverage obtained was filtered by muslin cloth thrice and was stored at 8℃ until further use.

Physicochemical properties

pH of the laboratory-made and traditional fermented beverage samples was determined using a pH meter (Model EI 112) and the titratable acidity was evaluated following the guidelines of AOAC[10]. Around 40 mL of each sample viz., traditional and laboratory-made rice beverage was collected in fresh sterile falcon tubes and was processed for rapid ethanol estimation by digital Alcohol Extract Meter (Alex 500, Anton Paar).

Microbial analysis

One mL of each laboratory-made and traditionally fermented beverages sample was taken separately and diluted with 9 mL of 0.85% normal saline solution (v/v). The serial dilutions were arranged using the 1:10 dilution technique. Total *Lactobacillus* counts (in MRS agar), coliform counts (in VRBA agar), yeast and mold (in SCA agar) count of traditional and laboratory-made rice beverage samples were analyzed up to 40 days with 10 days interval under refrigeration conditions (6^0C-8^0C) and the viable cell counts were reported in terms of log CFU/mL[11].

Bio-functional properties of the traditional and laboratory-made Chubitchi-like product under different time periods

Determination of ACE inhibitory activity (in vitro)

The ACE inhibition property of traditional and laboratory-made rice beverage samples was analyzed for 40 days, with 10 days intervals following the method of Donkor *et al*.[12]. Around 200 µL from each sample was added with 50 µL of 5 mM HHL solution, followed by further addition of 100 µL 0.1 M sodium borate buffer (pH-8.3). The reaction was induced by adding 20 µL (4mU in 250 µL) of ACE enzyme followed by incubation at 37^0C for 60 min. The reaction was completed by adding 500 µL of chilled 1N HCL, followed by incubation at 37^0C for 30 min. The ACE-released hippuric acid was collected after centrifugation at 14,000 rpm for 10 min. Then the samples were placed in a hot water bath and further 2 mL deionized water was added and passed through a 0.22 µM membrane filter. The inhibition activity of the solution was estimated spectrophotometrically applying Systronic Double beam Spectrophotometer 2202, India at 228 nm using the following Eq. (1) respectively:

$$\text{ACE-inhibition (\%)} = (A_{control} - A_{sample})/A_{control} \text{ X } 100 \ldots (1)$$

Where, $A_{control}$ = the absorbance without ACE inhibitory component, A_{sample} = the absorbance in the presence of ACE and ACE inhibitory component.

Total antioxidative capacity

The total radical scavenging capacity of the traditional rice beverage and the rice beverage developed in the laboratory were measured by the methodology of Hati *et al*.[13]. These samples were analyzed for 40 days with 10 days interval. The antioxidant activity of the tested samples was determined using the following Eq. (2):

$$\text{Antioxidative capacity (\%)} = (A_C - A_T)/A_C \text{ x } 100 \quad \ldots (2)$$

where A_C and A_T are respective absorbance of $ABTS^+$ [2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)] and tested samples, was expressed as inhibition percentages.

Total phenol content

Using Folin-Ciocalteu reagent[13], the total polyphenol content was determined. The samples of rice beverages (0.1 mL of each form of *Chubitchi*) were diluted with 0.9 mL distilled water and collected from a mechanical shaker after extraction for 2 h at room temperature. 1 mL Folin-Ciocalteu (1:2 dilution) reagent and 2 mL 10% Na_2CO_3 were added. The mixture was centrifuged at 12,000 rpm for 20 min and the supernatant was decanted and filtered through Whatman No. 1 filter paper. Absorbance of the supernatant was monitored at 765 nm using UV-Vis double beam spectrophotometer (model no. 220; Systronics, Ahmedabad, India). Gallic acid was taken as a standard. The findings were presented as milligrams of gallic acid equivalents (GAE) per 100 mL (mg/100 mL).

Antimicrobial activity

The method of agar well diffusion[14] was used to investigate the antimicrobial activity of the traditional and laboratory-made rice beverage samples for 40 days storage with 10 days interval. Both the beverage samples were assessed to check the antimicrobial activity against six major test organisms, i.e., *E. faecalis* NCDC 115, *S. aureus* MTCC 114, *E. coli* ATCC 25922, *L. monocytogenes* and *S. typhi* NCTC 5017. The inhibition activities of the rice beverage samples on the indicator bacteria were depicted by the development of a clear inhibition zone around the agar wells.

Statistical analysis

The average of three independent assays is provided here and the results obtained are expressed as mean ± standard deviation (M±SD). One-way analysis of variance (ANOVA) was implemented and correlation was made using the IBM SPSS Statistical program (ver. 20) by Bonferroni's test with the least significant difference of $p \leq 0.05$. Origin (version 8.0) was used for graphical presentation and data analysis.

Results and Discussion

Shelf-life study of the traditional and laboratory-made Chubitchi-like product

Organoleptic evaluation

For profiling the different sensory attributes of the traditional and the laboratory-made fermented rice beverage (*Chubitchi*), the organoleptic evaluation was carried out to 40 days of storage study as presented in Table 1. For laboratory-made rice beverages, the scores (>7) of color, clarity, aroma, taste-mouthfeel, flavour and overall acceptability on days 0, 10, 20 significantly differed ($p \leq 0.05$) from that on day 30 (>6). A significant difference ($p \leq 0.05$) was also observed from the sensory scores (<6) of day 40 against the scores (>6) of the rest of the storage days viz., 0, 10, 20 and 30. For traditionally made rice beverages, except the scores of colour and clarity, those of aroma, taste-mouthfeel, flavour and overall acceptability on days 0 and 10 significantly differed ($p \leq 0.05$) from day 20. There was a significant difference ($p \leq 0.05$) between the sensory scores of days 30 and 40 against the scores of the rest storage days viz., 0, 10 and 20 for each of the sensory attribute.

Colour is considered a primary sensory parameter that plays a crucial role in assessing fermented beverage by yielding information regarding the age, method of preparation and the downstream process treatments. The sensory results recorded for colour witnessed clear distinctions between the beverages brewed under laboratory conditions and traditionally. The laboratory-made rice beverage was observed to change colour from pale yellow amber to golden yellow amber from day 0 to day 40 of the storage period. An increase in the colour trend was recorded, with a score of 8.10±0.10 being the highest at day 20, which decreased to 5.48±0.08 on day 40. The traditionally made rice beverage was observed to change colour from pale to deep amber during the storage study, with the highest colour score of 8.13±0.15 on day 20 and scoring lowest (5.13±0.15) on day 40. Overall, the colour intensity was found to increase or deepen during the analysis period, which was in agreement with previous reports that stated the colour of alcoholic beverages generally deepens during storage or aging due to the transformation of monomeric pigments to more stable polymeric forms[15].

The results inferred from the analysis of clarity of the different beverage samples during the shelf life assessment of 40 days exhibited a decreased score with an increase in storage time. A decrease in trend was observed for rice beverage prepared under laboratory conditions with scores from 7.96±0.06 at day 0 (clear bright) to 5.40±0.16 at day 40 (slightly hazy). Similarly, traditionally made rice beverage also showed a decreasing trend of 7.88 ± 0.28 on day 0 (clear) to 5.87 ± 0.20 on day 40 (partially cloudy). This could be attributed to the presence of suspended

Table 1 — Organoleptic evaluation of the fermented rice beverages (*Chubitchi*) during 40 days of storage

Samples	Sensory attributes	Storage period (Days)				
		0	10	20	30	40
FRB	Colour	7.25±0.21 [a]	7.42±0.21 [a]	8.10±0.10 [a]	6.42±0.11 [b]	5.48±0.08 [c]
	Clarity	7.96±0.06 [a]	7.50±0.12 [a]	7.33±0.12 [a]	6.15±0.18 [b]	5.40±0.16 [c]
	Aroma	7.45±0.10 [a]	7.66±0.10 [a]	7.78±0.04 [a]	6.48±0.21 [b]	5.57±0.14 [c]
	Taste & Mouthfeel	7.65±0.15 [a]	7.77±0.28 [a]	7.82±0.15 [a]	6.20±0.20 [b]	5.54±0.12 [c]
	Flavour	7.44±0.11 [a]	7.80±0.11 [a]	8.11±0.17 [a]	6.43±0.10 [b]	5.66±0.15 [c]
	Overall acceptability	7.35±0.10 [a]	7.57±0.33 [a]	7.73±0.19 [a]	6.78±0.15 [b]	5.22±0.11 [c]
FTB	Colour	7.05±0.10 [a]	7.15±0.10 [a]	7.13±0.15 [a]	6.81±0.10 [b]	5.13±0.15 [c]
	Clarity	7.88±0.28 [a]	7.25±0.15 [a]	7.11±0.09 [a]	6.55±0.24 [b]	5.87±0.20 [c]
	Aroma	7.24±0.20 [a]	7.48±0.20 [a]	6.92±0.12 [b]	5.92±0.25 [c]	5.43±0.12 [c]
	Taste & Mouthfeel	7.15±0.09 [a]	7.32±0.24 [a]	6.15±0.10 [b]	5.85±0.33 [c]	5.20±0.10 [c]
	Flavour	7.05±0.10 [a]	7.20±0.15 [a]	6.12±0.12 [b]	5.77±0.20 [c]	5.12±0.12 [c]
	Overall acceptability	7.18±0.08 [a]	7.33±0.21 [a]	6.35±0.20 [b]	5.85±0.15 [c]	5.73±0.20 [c]

FRB- Fermented rice beverage made under laboratory conditions; FTB- Traditionally made rice beverage by the Garo Tribes of Meghalaya. Values are mean ± SD of three independent determinations (n=3) of each sample. Values bearing different superscripts in each column differ significantly ($p<0.05$).

dead yeast cells, bacteria, the acids that are involved in the precipitation of proteins, the presence of polymerized and precipitated anthocyanins, tannins, tartrate crystals and solubilization of copper and iron[15]. Our present study was found to be quite in agreement with Chay *et al*.[16] who reported that the clarity of laboratory brewed samples (*Medombae*, a rice wine starter culture of Cambodia) with developed starter cultures was found to be superior to the traditionally brewed samples.

Fermented rice beverage brewed under laboratory conditions was recorded with the highest score (7.78±0.04) for aroma (sweet fruity) on day 20 followed by the lowest score (5.57±0.14) on day 40 with an acidic note. Rice beverage brewed traditionally showed the highest score of 7.48±0.20 (sweet fruity) on day 10 with a decreasing trend from day 20 (6.92±0.12) onwards up to day 40 (5.43±0.12) with a slightly acidic and pungent note. The aroma description of 'fruity' used in our study stands in agreement with various previous studies that used similar terminology to distinguish the key aroma attribute in fermented rice beverages[9,17]. However, it has been stated that further oxidation of acetaldehyde might result in the generation of scant amounts of acetic acid during the final stages of fermentation,[18] which could also possibly explain the acidic intensities obtained in the aroma in case of both the beverage samples fermented during the later stages of the storage study.

Fermented rice beverage brewed under laboratory conditions recorded with the highest score (7.82±0.15) for taste and mouthfeel (sweet-bitter-savoury) on day 20, followed by the lowest score (5.54±0.12) on day 40 with a tangy mouthfeel note. Rice beverage brewed traditionally showed the highest score of 7.32±0.24 (sweet-bitter) on day 10 with a decreasing trend from day 20 (6.15±0.10) onwards up to day 40 (5.20±0.10) with sour-bitter-savour mouthfeel. During aging, LAB is believed to produce organic acids that contribute in causing sour taste gradually followed by microbial spoilage[15,19]. Hence, specific appropriate levels of acids could balance as well as enhance the taste and mouthfeel palatability while too much acid would cause distastefulness[19] as noticed in our beverage samples after 40 days storage period.

Flavour is a combination of aroma and taste and it is also a primary parameter with respect to a panelist's perception that determines the quality of the rice beverages. The flavour evaluations showed the highest score of 8.11±0.17 on day 20 for fermented rice beverage brewed under laboratory conditions and on day 10 with a score of 7.20±0.15 for traditionally brewed rice beverage with a fruity-sweet-acidic flavour. The decline in flavour scores from day 30 to day 40 for laboratory-made rice beverage and declining scores from day 20 to day 40 for traditionally made rice beverage might be due to the pronounced acidic notes. Hence a proper amount of acid could balance and enhance the fruity flavours while too much acid would cause an unsatisfied flavour[20] as observed in our beverage samples. Oxidation of alcoholic beverages during the time of

aging has been linked to off-flavours characterized as pungent[15] and also contributing to perceptible sourness during aging[15].

The evaluation of the fermented beverages with respect to their overall quality showed that the beverage prepared under laboratory conditions (hedonic score- 7.73±0.19) on day 20 was preferred the most and were rated as 'very good' by the sensory panelists. The traditionally brewed rice beverage was rated as 'liked a lot' on day 10 (7.33±0.21). After the sensory scores (<6) from day 40, both the beverage samples were considered as 'unacceptable' and thus were not considered for further evaluation. The sensory scores of the laboratory-made *Chubitchi* were almost at par with that of the traditionally made one during the 40 days storage study. But the laboratory-made *Chubitchi* was preferred till day 30, whereas the traditionally made *Chubitchi* was accepted till day 20 with hedonic scores >6.

Physicochemical analysis

During the 40 days of storage study, there was no significant difference ($p \leq 0.05$) in the pH of fermented rice beverage brewed under laboratory conditions that ranged between 4.09±0.07 to 3.61±0.04 and that of traditionally brewed rice beverage ranged between 3.87±0.07 to 3.15±0.06 (Table 2). The decrease in pH with an increase in fermentation time was in complete agreement with various previous studies[17,20,22]. pH has been regarded to be an important factor that affects virtually all of the physical, chemical, and biochemical reactions that take place during the brewing process[8]. The pH of the very popular fermented rice beverage of Japan, *Sake* has been reported to be in the ranges of 3.8 to 4.6[(ref 9)]. The low pH level in beverages produced by fermenting rice might be due to the presence of different organic acids and byproducts released during the anaerobic process[23]. However, the variation in pH among beverages might be related to factors such as different rice varieties, temperature, time period of fermentation, and indigenous microbial pool involved in the fermentation[24].

The total acidity (%) of the fermented rice beverage brewed under laboratory conditions ranged between 0.70±0.07 to 1.38±0.18 and that of traditionally brewed rice beverage ranged between 0.88±0.10 to 1.08±0.07 after 40 days of storage study. The acidity (%) of day 0 and 10 differed significantly from day 30 and 40 for laboratory-made rice beverage. The acidity (%) of traditionally made rice beverage significantly differed between day 0 against day 10, 20, 30, 40 (Table 2). Our results showed a significant increase in total acidity with an increase in fermentation time, which was in similar agreement with the results of several previous studies[21,24]. The findings of pH and acidity values were within the acceptable levels cited in several literature reports[8,17]. The results justify that with a decrease in pH, the total acidity increases as organic acids (citric, malic, succinic, lactic and tartaric acids) may get accumulated due to microbial activity[17]. It is worthwhile noting that such high acidity in fermented rice beverages has been admitted in the elimination of enteropathogens, coliforms, and other spoilage microorganisms[23].

An initial rise in ethanol percentage was witnessed in Table 2 with an increase in fermentation time till 20th day for both the rice beverage samples viz., laboratory-made (7.83±0.15) and traditionally made (11.75±0.22). For laboratory-made rice beverage, although a slight reduction in the ethanol percentage was observed after day 30, no significant differences ($p \leq 0.05$) were noted during the 40-day storage study.

Table 2 — Physicochemical analysis of the fermented rice beverages (*Chubitchi*) during 40 days of storage

Samples	Parameters	Storage period (Days)				
		0	10	20	30	40
FRB	pH	4.09±0.07 ^{a}	3.80±0.05 ^{a}	3.73±0.08 ^{a}	3.64±0.03 ^{a}	3.61±0.04 ^{a}
	Acidity (%)	0.70±0.07 ^{a}	0.85±0.10 ^{a}	1.15±0.15^{b}	1.47±0.05 ^{b}	1.38±0.18 ^{b}
	Ethanol (%)	7.42±0.18 ^{a}	7.65±0.20 ^{a}	7.83±0.15 ^{a}	7.41±0.20 ^{a}	7.26±0.23 ^{a}
FTB	pH	3.87±0.07 ^{a}	3.62±0.06 ^{a}	3.38±0.08 ^{a}	3.25±0.07 ^{a}	3.15±0.06 ^{a}
	Acidity (%)	0.88±0.10 ^{a}	1.12±0.08 ^{b}	1.37±0.05 ^{b}	1.17±0.10 ^{b}	1.08±0.07 ^{b}
	Ethanol (%)	11.20±0.30 ^{a}	11.42±0.12 ^{a}	11.75±0.22 ^{a}	11.15±0.35^{a}	10.84±0.25^{b}

FRB- Fermented rice beverage made under laboratory conditions; FTB- Traditionally made rice beverage by the Garo Tribes of Meghalaya. Values are mean ± SD of three independent determinations (n=3) of each sample. Values bearing different superscripts in each column differ significantly ($p<0.05$).

Traditionally made rice beverages showed no significant differences in the alcohol percentages till day 30, a significant reduction ($p \leq 0.05$) was observed after day 40. The reduction in alcohol-reducing values indicated the end of the fermentation process, which was found to be in agreement with the findings of Palaniveloo and Vairappan[25]. The ethanol content of both the rice beverage types started to decrease slightly after day 30 (laboratory-made- 7.41±0.20, traditionally made- 11.15±0.35) till the end of the storage period. It would also be worth highlighting that for maintaining a redox balance, the yeast cells have been known to produce glycerol as a by-product of the ethanol fermentation in the later stages. This could plausibly explain the gradual decrease in ethanol production observed in both the laboratory-made and traditionally made rice beverage samples towards the end of fermentation[26]. A decline in the alcohol content during the natural aging process of alcoholic beverages has been generally recognized and linked to evaporation loss of ethanol and the changes in the structure of water and ethanol molecules[27].

Microbial analysis

Lactobacillus counts were significantly increasing up to 30th day (7.88±0.09 log CFU/mL) and yeast count till the 20th day (6.21±0.08) of the storage study for laboratory-made rice beverage stored at refrigerated conditions (6-8) as shown in Table 3. For laboratory-made *Chubitchi*, the *Lactobacillus* counts of day 0, 10, 20, 30 significantly differed ($p \leq 0.05$) from day 40 and the yeast counts differed considerably in each of the storage days. The traditionally prepared rice beverage showed increasing *Lactobacillus* counts till the 20th day (5.92±0.05 log CFU/mL) and yeast counts till day 30 (5.04±0.07 log CFU/mL) of the storage study. There were no significant differences observed for *Lactobacillus* and yeast counts in the traditionally made *Chubitchi* during the 40-day storage study. A decreasing growth trend for lactobacilli (6.82±0.07 log CFU/mL) was observed on day 40 for laboratory-made beverage and on day 30 (5.67±0.15 log CFU/mL) for the traditionally made beverage that might be due to exhaustion of nutrients. Similarly, a decrease in yeast count was reported after day 30 (6.11±0.08 log CFU/mL) for laboratory-made rice beverage and after day 40 (4.43±0.05 log CFU/mL) for traditionally prepared rice beverage (Table 3). No coliforms were detected in the samples tested during the shelf life study of laboratory-made rice beverage, claiming it to be safe from faecal contamination. Contrastingly, on the final day of the study, coliforms were detected from the rice beverage brewed under traditional process that might be due to contamination or due to the preparation under unhygienic conditions (Table 3). The decreasing growth trend witnessed for both lactic acid bacteria and yeast might be related to the inhibitory activity of alcohol production via fermentation and the presence of organic acids such as lactic acid and other organic acids. The various organic acids viz., lactic, acetic, propionic and butyric acids, produced by fermented cereal beverages released during fermentation could function in prolonging the shelf life by reducing pH to lower than 3 or 4 that helps to inhibit the growth and colonization of spoilage and pathogenic organisms viz., *Shigella*, *Salmonella* and *E. coli*[28]. Similarly, Kim *et al*.[17] and Shrivastava *et al*.[28] had also reported a decrease in microbial counts in fermented rice beverages during storage correlating with storage time as depicted in our study.

Table 3 — Microbial analysis of the fermented rice beverages (*Chubitchi*) during 40 days of storage

Samples	Microbial load (log CFU/mL)	Storage period (Days) 0	10	20	30	40
FRB	Total *Lactobacillus* count	7.65±0.08 ^{a}	7.70±0.13 ^{a}	7.79±0.11 ^{a}	7.88±0.09 ^{a}	6.82±0.07 ^{b}
	Yeast count	4.62±0.06 ^{a}	5.89±0.07 ^{b}	6.21±0.08 ^{c}	6.11±0.08 ^{c}	5.58±0.07 ^{b}
	Coliform count	Absent in 1 mL	Absent in 1 mL	Absent in 1 mL	Absent in 1 mL	Absent in 1 mL
FTB	Total *Lactobacillus* count	5.35±0.11 ^{a}	5.42±0.12 ^{a}	5.92±0.05 ^{a}	5.67±0.15 ^{a}	5.16±0.06 ^{a}
	Yeast count	4.34±0.07 ^{a}	4.57±0.05 ^{a}	4.88±0.01 ^{a}	5.04±0.07 ^{a}	4.43±0.05 ^{a}
	Coliform count	Absent in 1 mL	Absent in 1 mL	Absent in 1 mL	Absent in 1 mL	2.72±0.12^{a}

FRB- Fermented rice beverage made under laboratory conditions; FTB- Traditionally made rice beverage by the Garo Tribes of Meghalaya. Values are mean ± SD of three independent determinations (n=3) of each sample. Values bearing different superscripts in each column differ significantly ($p<0.05$).

Bio-functional properties of laboratory-made and traditionally made Chubitchi under different storage periods

ACE-inhibition activity

During the storage study, it was found that ACE inhibitory property of *Chubitchi* developed under laboratory conditions with *Lactobacillus* and *Saccharomyces* cultures enhanced from 22.64% at day 0 to 86.87% at day 20, followed by a lowering from day 30 (68.04%) up to day 40 (49.29%).

Traditionally prepared *Chubitchi* resulted in increasing ACE inhibition till day 20, followed by a significant reduction after day 30 to day 40. It was found that the ACE-inhibitory activity of *Chubitchi* prepared under laboratory conditions was quite higher and differed significantly ($p \leq 0.05$) than traditionally prepared *Chubitchi* (Fig. 5A). Therefore, it was found from the above analysis that potent ACE inhibitory peptides, which were significantly essential, were produced at day 20 during storage at 6-8°C. However, during days 30 and 40, the values decreased sharply due to proteolytic activity. Kancabas and Karakaya[29] have previously recorded Boza's ACE-inhibitory activity, with 76.76% being a typical Turkish beverage produced by fermenting barley, maize, wheat, or rice with yeast and lactic acid bacteria. Vermeirssen *et al*.[30] evaluated the ACE-inhibitory property of fermented drinks and whey protein foods using *Lactobacillus helveticus* and *Saccharomyces cerevisiae* and recorded an 18%-30% increase in ACE inhibitory property after fermentation, which stands in agreement to our study as well. Jang and Lee[31] reported a rise in ACE inhibitory activity (67.8%) for Vitis hybrid-*Vitis coignetiae* red wine fermented by *S. cerevisiae* after 10 days of fermentation.

Antioxidative activity

During the storage study of the rice beverage made under laboratory conditions, at day 0, the antioxidative radical scavenging activity of 95.18% was recorded, followed by 81.59% at day 10; 67.31% at day 20; 52.66% on day 30, and 41.79% at day 40. The results were statistically significant ($p \leq 0.05$) during the 40 days period. Similarly, the radical scavenging activity of traditionally made rice beverage of 82.69% at day 0 to 30.13% at day 40 was observed during the storage study. The highest radical scavenging activity of both the fermented beverages right after the 15 day fermentation period (day 0 of the storage study) was thought to be associated with the release of phenolic compounds possessing antioxidant capacity (Fig. 5B). Comparatively, with lactic and yeast cultures, laboratory-made *Chubitchi* exhibited the maximum antioxidant ability, preventing the release of radical cations in a concentration gradient way to capture $ABTS^{+}$. In a similar study by Hong *et al*.[32], the *Takju* wine of Korea reported 32.08-61.99% antioxidant activity using ABTS assay. The *Tapuy* samples of Ibaloi tribe (a traditional alcoholic beverage in the northern region of the Philippines), in a study, ranged from 44.88-69.14%, using ABTS assay[33]. In a study by Yang *et al*.[34], the trends in ABTS radical scavenging activity of two *L. plantarum* strains were recognized by a significant rise of antioxidative activity for the first 8 days and then reduced during the time of fermentation in a vegetable–fruit beverage. Hur *et al*.[35] observed that the accumulation of antioxidant compounds, particularly phenolic compounds, flavonoids, and superoxide dismutase, lead to the enhancement of antioxidant activity in fermented foods. The laboratory-made *Chubitchi* could be served as a kind of functional food to relieve free radical species-induced disease based on the above-stated results.

Total polyphenol content

The total polyphenol content of the laboratory and traditionally made rice beverages was measured using the Folin Ciocalteu method till 40-day shelf-life (Singleton *et al*., 1999) as depicted in Fig. 5C. The total polyphenol levels of the laboratory-made *Chubitchi* were significantly different ($p \leq 0.05$) during each 10-day interval of the 40 days storage study. During the storage study, a rapid reduction in the polyphenol content was observed from day 0 (68.18±1.12 mg/100 mL) up to day 40 (24.72±0.42 mg/100 mL) in the laboratory made *Chubitchi*. The traditionally made *Chubitchi* showed low polyphenol content with no significant difference ($p \leq 0.05$) in reduction from day 20 to day 40 of the storage study. The total phenolic contents in mg/L GAE were reported by various workers as: in beer, 270-600[(ref 36)] and 206-374[(ref 37)]; in wine, 178-284[(ref 37)] and 1648-4495[(ref 38)]. The reduction of phenolic compounds is seen as a significant contributor to improve the antioxidant efficacy of beverages[39]. The decline of the polyphenol content by fermentation may improve the digestibility and also increase the bioavailability of minerals in the fermented product by enhancing the overall nutritional values. Fermentation with different probiotics results in the reduction of polyphenols[40]. The decreasing effect of fermentation

on polyphenols might be the result of the activity of polyphenol oxidase enzyme existing in the food grain or microflora. The lowering in polyphenolic content by fermentation improve the sensory quality[41].

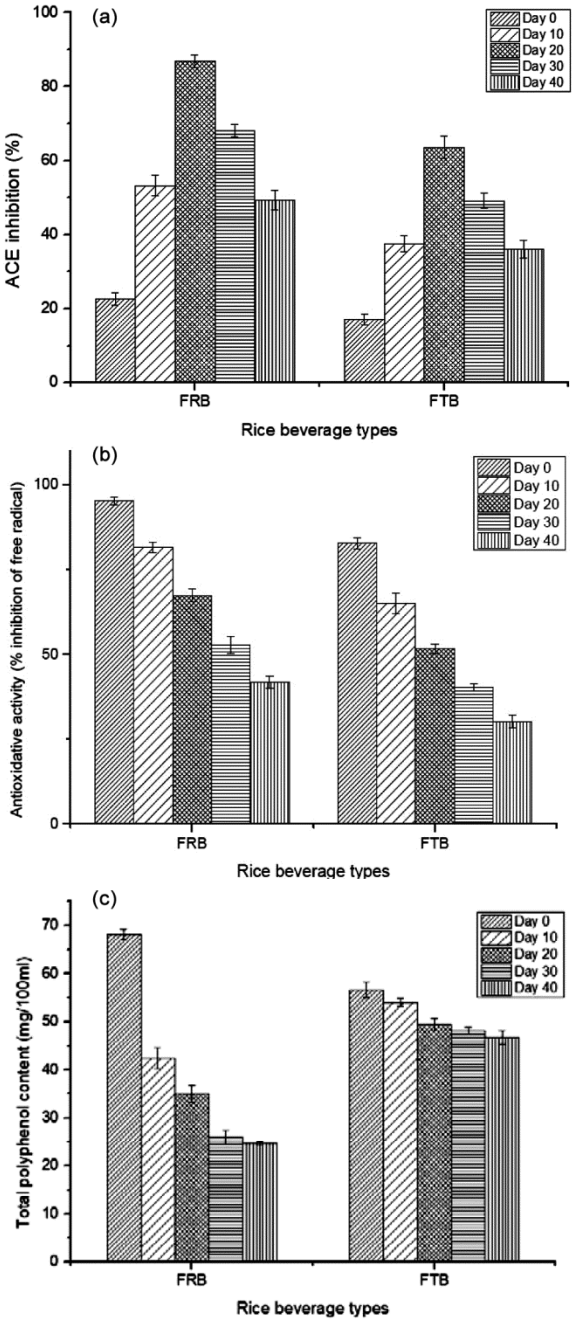

Fig. 5 — (a) ACE inhibitory activity, (b) Antioxidative activity (% inhibition of free radical) and (c) Total polyphenol content of FRB (Fermented rice beverage made under laboratory conditions) and; FTB (Traditionally made rice beverage by the Garo Tribes of Meghalaya) during 40 days of storage. Error bars show standard deviation (n=3); $p<0.05$.

Antimicrobial activity

The antimicrobial activity of both the laboratory (Fig. 6A) and traditionally made (Fig. 6B) *Chubitchi* was evaluated against six crucial test organisms, viz., *B. cereus, E. faecalis, S. aureus, E. coli, L. monocytogenes, S. typhi* in a 40-day storage study with an interval of 10 days. The antimicrobial results of the laboratory-based *Chubitchi* differed significantly ($p\leq0.05$) from the traditional rice beverage. The laboratory-made *Chubitchi* showed an increasing trend of antimicrobial activity against *B. cereus* (26.66 mm), *E. faecalis* (27.33 mm), *S. aureus* (28.33 mm), *E. coli* (23.33 mm), *L. monocytogenes* (26.33 mm) after day 30 and against *S. typhi* (22.66 mm) after day 20. A significant decrease in the antibacterial activity was witnessed after day 20 for *S. typhi* and the rest of the five test organisms after day 40. In the traditionally

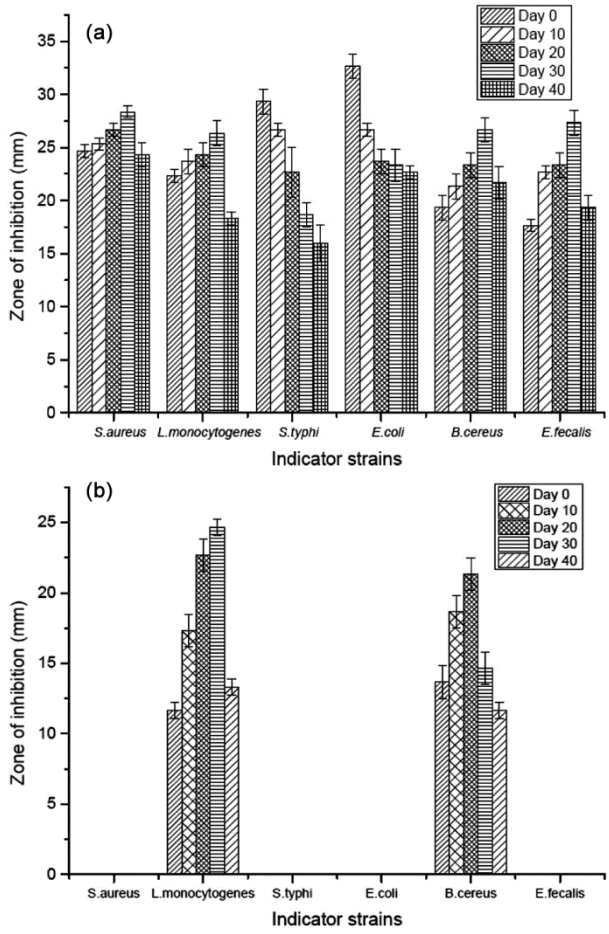

Fig. 6 — Antimicrobial activity of a) FRB (Fermented rice beverage made under laboratory conditions) and; b) FTB (Traditionally made rice beverage by the Garo Tribes of Meghalaya) during 40 days of storage. Error bars show standard deviation (n=3); $p<0.05$.

made *Chubitchi, E. faecalis, S. aureus, E. coli, S. typhi* showed complete resistance with no inhibition zone till 40 days of storage study. The traditional *Chubitchi* showed the highest antimicrobial activity against *L. monocytogenes* (24.66 mm) after 30 days and against *B. cereus* (21.33 mm) after 20 days, which decreased significantly after the final day of the storage study.

Very little literature is available on antimicrobial assessments of fermented rice beverages. Chang *et al*.[5] reported the antimicrobial activity of Taiwanese *Allium fistulosum* (commercial rice wine) using agar disc diffusion method and tube dilution tests and also observed that extracts had antibacterial activity against *Bacillus subtilis, Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa.* Jana *et al*.[42] studied the antibacterial activity of *Handia* (rice beverage of Orissa/West Bengal) against five pathogenic bacterial strains viz., *Vibrio cholerae, Escherichia coli, Micrococcus luteus, Staphylococcus faecalis* and *Staphylococcus aureus* for establishing its ethnomedicinal characteristic and found that the beverage showed average results against all test organisms, for which they interpreted that it may be due to the bacteriocins producing bacteria present in it. The results of Detha and Datta[43] showed that *Sopi* and *Moke* (traditional alcoholic beverages of Indonesia) possessed an acidic pH of 4 and 4.3, making the beverage unfavorable for bacterial growth, thereby releasing bacteriocin like compounds. Hence, *Sopi* and *Moke* could be added to any beverage medium for creating an acidic environment in order to prolong their antimicrobial activities[44]. The level of alcohol, phenolic compounds and acidic pH might develop synergistic effects on the antimicrobial activities of these traditional fermented beverages, thereby prolonging the shelf life of the product[45]. The ethanol content in beverages penetrates easily into the enzyme system of the spoilage microorganisms, especially targeting dehydrogenase and oxidase, thereby causing inhibition of the normal metabolism of the bacteria as well as lowering the bacterial growth and disrupting its reproductive system, respectively[45].

Conclusion

The fermented rice beverage (*Chubitchi*) developed by the laboratory method can be selected at par with the traditionally prepared *Chubitchi* with similar flavour and taste recommended by the native panelists and the entire processing was performed under aseptic conditions. The *Lactobacillus* and the *Saccharomyces* cultures, along with the medicinal plant extract of *S. dulcis* could increase the ACE-inhibition, antioxidant capacity and antimicrobial potential of the laboratory-made rice beverage during the storage study. This research work has a considerable scope to develop the socioeconomic status of the tribal people by providing them with a defined starter culture having beneficial health attributes and standardized protocol for the preparation of rice beverage of Meghalaya.

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Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author Contributions

B K M conceptualized, supervised and helped in writing – reviewing, and editing the manuscript. SD conceptualized the study and investigated the study and also helped in reviewing, and editing the manuscript. JBP supervised the study and edited the manuscript. SH conceptualized and supervised the study, assisted in compiling recent review literature and also helped in reviewing, and editing the manuscript.

References

1 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.

2 Murugan N B, Mishra B K & Paul B, Antibacterial activity of indigenous fermented rice beverage of West Garo Hills, Meghalaya, India. *Int J Ferment Food*, 7 (1) (2018a) 39-44.

3 Murugan N B, Mishra B K & Paul, B, Antioxidant and antibacterial evaluation of medicinal plants used in the starter culture (Wanti) of fermented rice beverage in West Garo

hills, Meghalaya, *J Pharmacogn Phytochem*, 7 (2018b) 1669-1674.
4 Park Y U, Kim M D, Jung D H, Seo D H, Jung J H, Park J G & Shin W C, Probiotic properties of lactic acid bacteria isolated from Korean rice wine Makgeolli, *Food Sci Biotechnol*, 24 (5) (2015) 1761-1766.
5 Bhuyan D J, Barooah M S, Bora S S & Singaravadivel K, Biochemical and Nutritional Analysis of rice beer of Northe East India, *Indian J Tradit Know*, 13 (1) (2014) 142-148.
6 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.
7 Hati S, Patel M, Mishra B K & Das S, Short-chain fatty acid and vitamin production potentials of Lactobacillus isolated from fermented foods of Khasi Tribes, Meghalaya, India, *Ann. Microbiol*, 69 (11) (2019) 1191-1199.
8 Dung N T P, *Defined fungal starter granules for purple glutinous rice wine*, Ph.D. thesis, (Wageningen University, Wageningen, The Netherlands), 2004.
9 Singh P K, & Sigh K I, Traditional Alcholic bevreages, *Indian J Tradit Know*, 5 (2) (2006) 184-190.
10 AOAC, Official methods of analysis of AOAC International, seventeenth Ed, (Gaithersburg, MD, USA: AOAC), 2000.
11 Lab Manual 14, Manual of methods of analysis of food-Microbiological testing. In Microbiology of foods, Food safety and standards authority of India, Ministry of health and family welfare, Government of India, New Delhi, 2012.
12 Donkor O N, Henriksson A, Singh T K, Vasiljevic T & Shah N P, ACE-inhibitory activity of probiotic yoghurt, *Int Dairy J*, 17 (11) (2007) 1321–31.
13 Hati S, Vij S, Singh B P & Kumari V & Mandal S, Antioxidative activity and polyphenol content in fermented soy milk supplemented with WPC-70 by probiotic Lactobacilli, *Int Food Res J*, 20 (5) (2013) 2125e31.
14 Schillinger U, Antimicrobial activity of L. sake isolated from meat, *Appl Environ Microbiol*, 55 (1989) 1901-1906.
15 Jackson R, *Wine Science. Principles and Applications*, (Academic Press Limited, London), 2008.
16 Chay C, Dizon E I, Elegado F B, Norng C, Hurtada W A & Raymundo L C, Isolation and identification of mold and yeast in medombae, a rice wine starter culture from Kompong Cham Province, Cambodia, *Food Res*, 1 (6) (2017) 213-220.
17 Kim E, Chang Y H, Ko J Y & Jeong Y, Physicochemical and Microbial Properties of the Korean Traditional Rice Wine, Makgeolli, Supplemented with Banana during Fermentation, *Prev Nutr Food Sci*, 18 (3) (2013) 203–209.
18 Liu S Q & Pilone G J, An overview of formation and roles of acetaldehyde in winemaking with emphasis on microbiological implications, *Int J Food Sci Technol*, 35 (2000) 49–61.
19 Liu Q, Analysis of volatile compounds and their changes during liquor aging of Chinese liquor 'gujing gongjiu'. All Theses. 1888, 2014. https://tigerprints.clemson .edu/all_theses/1888
20 Xu M L, Yu Y, Ramaswamy H S & Zhu S M, Characterization of Chinese liquor aroma components during aging process and liquor age discrimination using gas chromatography combined with multivariable statistics, *Sci Rep*, 7 (1) (2017) 1-9.
21 Chen S & Xu Y, Effect of wheat Qu on the fermentation processes and volatile flavour-active compounds of Chinese rice wine (Huangjiu), *J Inst Brew*, 119 (2013) 71–77.
22 Dung N T P, Doi N M & Phong H X, Preparation of fungal alcoholic starter using rice malt as substrate, *J Sci*, 1 (2015) 47-52.
23 Chiang Y W, Chye F Y & Ismail M A, Microbial Diversity and Proximate Composition of Tapai, A Sabah's Fermented Beverage, *Malays J Microbiol*, 2 (1) (2006) 1-6.
24 Bhuyan D J, Sakia B M, Sankar B S & Singaravadivel K, Biochemical and Nutritional analysis of rice beer of North East India, *Indian J Tradit Know*, 13 (1) (2014) 142 – 148.
25 Palaniveloo K & Vairappan C S, Biochemical properties of rice wine produced from three different starter cultures, *J Trop Biol Conserv*, 10 (2013) 31-41.
26 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.
27 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.
28 Shrivastava K, Greeshma A G & Srivastava B, Improvement in Traditional Technology of Rice and Millet Based Fermented Beverages of Arunachal Pradesh, North East, India through Scientific Approach, *In Biol Sci*, (2015) 18-19.
29 Kancabaş A & Karakaya S, Angiotensin converting enzyme (ACE) inhibitory activity of boza, a traditional fermented beverage, *J Sci Food Agric*, 93 (3) (2013) 641-645.
30 Vermeirssen V, van der Bent A, Van Camp J, van Amerongen A & Verstraete W, A quantitative in silico analysis calculates the angiotensin I converting enzyme (ACE) inhibitory activity in pea and whey protein digests, *Biochimie*, 86 (3) (2004) 231-239.
31 Jang J H & Lee J S, Antihypertensive angiotensin I-converting enzyme inhibitory activity and antioxidant activity of Vitis hybrid-Vitis coignetiae red wine made with *Saccharomyces cerevisiae*, *Mycobiology*, 39 (2) (2011) 137-139.
32 Hong Y H, Bae S H, Jung E Y, Son H S, Shin K S, Kwon K H & Suh H J, Radical scavenging activities of Korean traditional rice wine, Takju, *J Food Sci Nutr*, 14 (14) (2009) 109-115.
33 Hipol R L B & Alma-in A B, Antioxidant potentials of indigenously produced Benguet tapuy (rice wine), *Int Food Res J*, 25 (5) (2018) 1968-1976.
34 Yang X, Zhou J, Fan L, Qin Z, Chen Q & Zhao L, Antioxidant properties of a vegetable–fruit beverage fermented with two Lactobacillus plantarum strains, *Food Sci Biotechnol*, 27 (6) (2018) 1719-1726.
35 Hur S J, Lee S Y, Kim Y C, Choi I & Kim G B, Effect of fermentation on the antioxidant activity in plant-based foods, *Food Chem*, 160 (2014) 346-356.
36 Berhanu A, Microbial profile of Tella and the role of gesho (Rhamnus prinoides) as bittering and antimicrobial agent in traditional Tella (Beer) production, *Int Food Res J*, 21(1) (2014) 357-365.
37 Abdoul-latif F M, Bayili R G, Obame L C, Bassolé I H N & Dicko M H, Comparison of phenolic compounds and antioxidant capacities of traditional sorghum beers with other alcoholic beverages, *Afr J Biotechnol*, 11 (2012) 14671-14678.
38 Lugemwa F N, Snyder A L & Shaikh K, Determination of radical scavenging activity and total phenols of wine and

spices: A randomized study, *Antioxidants*, 2 (3) (2013) 110-121.

39 Shewakena S, Chandravanshi B S & Debebe A, Levels of total polyphenol, flavonoid, tannin and antioxidant activity of selected Ethiopian fermented traditional beverages, *Int Food Res J*, 24 (5) (2017) 2033-2040.

40 Sindhu S C & Khetarpaul N, Fermentation with one step single and sequential cultures of yeast and lactobacilli: effect on antinutrients and digestibilities (*in vitro*) of starch and protein in an indigenously developed food mixture, *Qual Plant - Plant Foods Hum Nutr*, 58 (3) (2003) 1-10.

41 Rekha R, & Vijayalakshmi G, Biomolecules and Nutritional Quality of Soymilk Fermented with Probiotic Yeast and Bacteria, *Appl Biochem Biotechnol*, 151 (2008) 452–463.

42 Jana D, Ghorai S K, Jana S, Dey P P, Determination of antimicrobial activity of rice based fermented beverage - Haria/Handia, *Int J Curr Res Acad Rev*, 2 (5) (2014) 85-91.

43 Detha AIR & Datta F U, Antimicrobial activity of traditional wines (Sopi and Moke) against Salmonella sp. and Escherichia coli, *J Adv Vet Animal Res*, 3 (3) (2016) 282-285.

44 Klein D W, Lansing M & Harley J, Microbiology, sixth ed, (McGraw-Hill, New York), 2006.

45 Rutala W A & Weber D J, Role of the hospital environment in disease transmission, with a focus on Clostridium difficile, *Healthcare Infection*, 18 (1) (2013) 14-22.