Chapter 2: Biochemical and microbiological characterization of rice beer and starter cultures produced in different regions of Assam

Chapter 2: Biochemical and microbiological characterization of rice beer and starter cakes produced in different regions of Assam and Northeast India

2.1 Introduction

All of the tribes in Assam prepare their indigenous alcoholic beverages at home using round to flattened solid ball-like mixed dough inocula or starter [1,2]. The starters are prepared by grinding of softened rice with various parts of different plant species. The paste thus obtained is sometimes mixed with old powdered starters and made into dough out of which round flattened cakes of uniform sizes are made. These are fermented for some days and then dried using various methods in order to obtain the starter cake [1,3]. The fermentation is usually carried out in earthen pots at room temperature and takes about 5 to 7 days for completion of the entire process of preparation. The fermented mass is further diluted with water in appropriate ratio and strained with cloth in order to get the rice beer in liquid form. The methodology of fermentation carried out by different tribes is almost the same, except that the difference comes from the different types of plant species used in starter culture preparation [4]. Various plants have been reported to be used in the preparation of rice beer starter cultures in North-East India by various authors. Some are *Albizia myriophylla* by the *Maiteis* in the state of Manipur [5], *Amomum aromaticum* by the *Jaintia* tribe of Meghalaya [6], *Plumbago zeylanica, Buddleja asiatica, Vernonia cinerea* and *Gingiber officinale* in the state of Sikkim [7], *Glycyrrhiza glabra* by the *Dimasas* in Assam [8], *Ananas comosus, Artocarpus heterophyllus*, *Calotropis gigantea*, *Capsicum frutescens* etc. by the Rabha tribe of Assam [9] and sprouted rice grains by the *Angamis* in Nagaland [16].

During the fermentation process, a succession of microbes with a delicate balance between different kinds is observed along with changes in biochemical parameters especially in sugar contents [3]. The starter cakes consists of a consortium of different groups of microflora like moulds [2], yeast [1,7] and lactic acid bacteria (LAB) [11]. The use of this kind of mixed cultures for fermentation also contributes to the synthesis of various esters and alcohols [12]. The amylolytic microbes *M. Circinelloides, R. chinensis, S. fibuligera, S. capsularis* and *P. burtonii* have been isolated from the starter culture *marcha* used in Sikkim. Whereas, ethanol production was shown by the isolated strains *S. bayanus, C. glabrata* and *P. anomala* [7,11]. The lactic acid bacteria *Lactobacillus plantarum, Lactobacillus brevis* and *Pediococcus pentosaceus* have been isolated from samples of starter cultures used in the states of Sikkim and Manipur [2].

The process of manufacturing rice beer consists of saccharification of the rice starch by fungal enzymes followed by alcoholic fermentation by yeasts supplied by the starters. This process is unique and the product differs from commercial malt beer or wine. Even though the methodology of production has resemblance with malt beer, however, there is difference in the saccharification process of both. In malt beer, the enzymes for conversion of starch to sugars (α and β amylases) and proteases are produced during the germination process of the barley grains. Whereas, in case of rice beer, these enzymes are produced by fungus supplied externally. The whole process of preparation involves saccharification of the starch present in steamed or boiled rice by fungal enzymes followed by alcoholic fermentation by yeasts. These causative organisms are supplied by traditional starters which are usually in the form of dry powder or hard balls or cakes. Various plant materials are used in the preparation of these starters.

In this chapter, the key ingredients used in the preparation of rice beer starter cultures in Assam, India and the fermentation technologies followed by the indigenous people was studied. The presence of various components which might contribute to the unique characteristics and nutritional aspects rice beer prepared in Assam was studied. The comparative evaluation has been done based on the composition of different organic acids, carbohydrates, amino acids and aromatic compounds. It was also seen that detailed differentiation in between these starter cakes and the rice beer produced with these cakes have not been reported earlier. Hence, it was aimed to bring about a clear distinction in between these cakes and the rice beer produced from them by examining their physical, microbiological and biochemical parameters.

2.2 Materials and Methods

2.2.1 Materials

Samples of rice beer and starter cakes were collected from four different states of Northeast India, namely, Assam, Nagaland, Arunachal Pradesh and Meghalaya during. Collection was made from the locations which were predominantly involved in the process of making rice beer, either for self consumption or for commercial purposes. All the samples were collected in replicates of three in 500 mL sterile glass sample bottles (Borosil, India), marked according to the place of collection, brought to the laboratory under refrigerated condition on the same day and stored at 4 ^{o}C. Both the microbiological and biochemical examination of the

samples were started within 24 h of storage. A non-glutinous variety of rice (*Oryza sativa*) named *Mahsuri*, collected from Assam Agricultural University, Jorhat, Assam was used as a substrate for the preparation of rice beer in the laboratory. The chemicals and standards were obtained from HiMedia (India) and Sigma-Aldrich Corporation (USA).

2.2.2 Field survey

A field survey was carried out in the villages and rural areas of the states of Assam, Nagaland, Arunachal Pradesh and Meghalaya. The areas were selected based on the information available upon the prevalence of traditional methods of rice beer preparation. Information was collected from the producers predominantly involved in the process of making rice beer. The women in all the communities visited were mostly involved and they were inquired about their practices for preparation such as making of starter cakes along with plants and their parts added, fermentation procedure, duration and uses of the beverage. Some of the nearby fields and forests were visited along with local help and the available plant samples were collected and stored in plastic bags and sealed. Later on, these samples were dried and made into herbarium as per the guidelines given by Anderson [13]. Further identification of the collected plant species, the plant samples and herbariums were done by Department of Agronomy, Assam Agricultural University, Jorhat, Assam and Department of Botany, Darrang College, Tezpur, Assam.

2.2.3. Biochemical analysis of the collected rice beer samples

2.2.3.1 Colour measurement

The colour measurement of the starter cakes and rice beer was done by analyzing the samples in a Hunter Lab Color Quest (Ultrascan Vis, HunterLab, USA). The measurement was done without altering the original shape or size of the cakes. The results were expressed in Commission Internationale de I'Eclairage L, a and b (CIELAB) systems in which L indicates the degree of lightness or darkness (L=0 indicates perfect black and L=100 indicates most perfect white); “a” indicates degree of redness (+) and greenness (-) and “b” indicates degree of yellowness (+) and blueness (-).

2.2.3.2 Sample preparation for biochemical analysis

Initially 20 mL of each of the samples in three replicates were taken and made CO_2 free for carrying out HPLC and biochemical analyses, except for the study of volatile compounds. This was done by transferring the test samples to a large flask and shaking, first gently and then vigorously in a refrigerated incubator shaker (Excella E24R, NBS, USA), maintaining the temperature at 20-25 ^{o}C as per the AOAC Official Method 920.49 (14)[20].

2.2.3.3 pH, acidity measurement Alcohol content estimation

The pH and acidity were determined according to AOAC Official Methods 945.10 and 950.07 respectively [14]. The undiluted test portions were tested in a digital pH meter (pH510, Eutech Instruments) and the indicator titration method was used to obtain the total acidity of the samples. The results were reported as % of lactic acid (1 mL of 0.1M alkali = 0.0090 g lactic acid). The alcohol content (by weight) was measured by the specific gravity method under AOAC Official Method 935.22 [14]. A 100 mL calibrated pycnometer was used to find the specific gravity and then the corresponding percentage of alcohol by volume and weight was calculated.

2.2.3.4 HPLC analysis of organic acids

Extraction: The samples were first mixed with a mixture of acetonitrile and type I water (70:30) and then stirred continuously for 2 h in a shaker. The mixture was then centrifuged for 10 min at 10,000 rpm. The supernatant was then filtered through Whatman No. 4 filter paper and the filtrate was again subjected to solid phase extraction using Sep-Pak® C18 cartridge. This extract was used for analysis of organic acids [15].

Analytical conditions: The analysis of organic acids was carried out in a HPLC system (Ultimate 3000, Dionex, Germany) equipped with an autosampler. The injection volume was 20 µL and the detector used was Ultimate 3000 Variable Wavelength detector at 210 nm (UV range). The column used was Acclaim OA® (5µm beads size, 4.0 x 250 mm, Thermo Scientific). The mobile phase was 0.2 M sodium sulphate solution (pH adjusted to 2.68 with methane sulphonic acid). An isocratic run was used with a constant flow rate of 0.6 mL min^{-1} at a temperature of 30 ^{o}C.

2.2.3.5 HPLC analysis of carbohydrates

Extraction: Prior to the analysis of carbohydrates, the samples were once again degassed for 15 min in an ultrasonic bath (RZ 08892-26, Cole Parmer, USA) in order to remove any residual gases. It was then passed through 0.22 µm pore size organic syringe filter of 30 mm diameter (SF2-1, HiMedia) and then through a Sep-Pak® C18 cartridge which had previously been activated with 10 mL of methanol, followed by 10 mL of type I water [16,17].

Analytical conditions: The analysis of carbohydrates was carried out in a HPLC system (Ultimate 3000, Dionex, Germany) equipped with an autosampler. The injection volume was 20 µL and Shodex RI-101® Refractive Index detector was used with plus polarity at 512 µRIU recorder and 500 µRIU/V integrator ranges. The column used was a Hamilton HC-75® Ca++ column. The mobile phase used was type I water with an isocratic flow rate of 0.6 mL min^{-1} at a temperature of 80 ºC.

2.2.3.6 HPLC analysis of amino acids

Acid hydrolysis: The samples were taken in a hydrolysis tube, dried, mixed with 6 M HCl containing 0.1% phenol, sealed and then hydrolyzed at 110 ºC for 24 h in vacuum. Following hydrolysis the residual acid was dried off in a vacuum oven. The samples were then suspended in 100 mM HCl and passed through 0.22 µm size syringe driven filter (18).[24]

Derivatization procedure: The derivatization of amino acids was done by modification of the method of Bank et al. [19]. Sample (100 µL), 900 µL of borate buffer (1 M, pH 6.2) and 1 mL of fluorenylmethyloxycarbonyl chloride (FMOC-Cl) were taken and vortexed. It was then kept for 2 min and then 4 mL of n-pentane was added, followed by vortexing for 45 min. The upper layer was discarded and the lower layer was used for injection after passing through 0.22 µm size syringe driven filter.

Analytical conditions: The analysis of amino acids was carried out in a HPLC system (Ultimate 3000, Dionex, Germany) equipped with an autosampler. The injection volume was 20 µL and the Ultimate 3000 Variable Wavelength detector was used at 265 nm (UV range). The column used was Acclaim 120® (C18, 5 µm beads size; 4.0 x 250 mm, Thermo Scientific) and the column oven temperature was maintained at 30 ºC. The mobile phase used was (A) acetate buffer: acetonitrile (9:1) and (B) acetate buffer: acetonitrile (1:9) with a flow rate of 1.0 mL min^{-1}. The flow gradient for the two solvents is shown in Fig. 1.

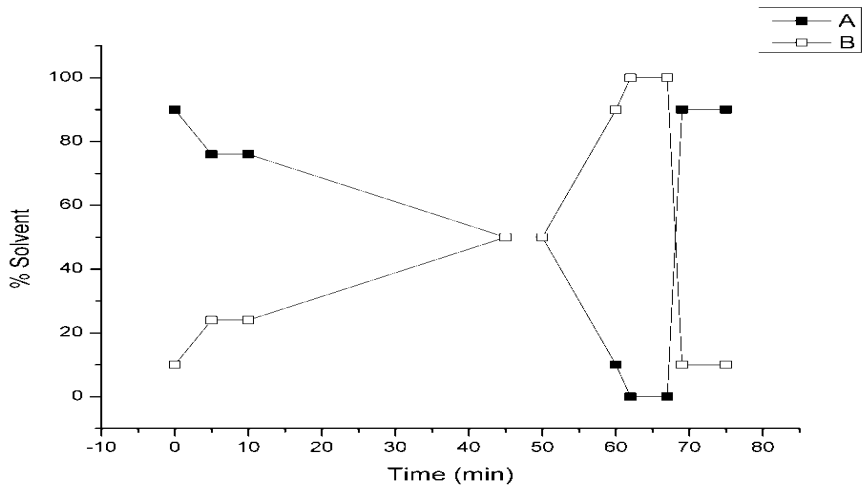

Fig 2.1 Flow gradient used in the analysis of amino acids by HPLC. (A) acetate buffer: acetonitrile (9:1) and (B) acetate buffer : acetonitrile (1:9)

2.2.3.7 GC-MS analysis of aromatic components

Extraction: The aromatic compounds present in the rice beer samples were extracted by slight modification of the method of Patakova-Juzlova et al. [20]. Samples (100 mL) were first filtered through Whatman No. 4 filter paper and then distilled in a rotary evaporator (8763.RV0.000 Roteva, Equitron, India) until 20 % of the total volume had been collected as distillate. This distillate was then extracted with 10 % dichloromethane in a separating funnel by shaking for 10 min. The DCM extract was filtered through 0.2 µm pore size filter driven syringe and stored at 4 ^{o}C until analysis.

GC-MS analysis: The GC-MS system (Clarus 600 Gas Chromatograph and Clarus 600C Mass Spectrometer, PerkinElmer, USA) was equipped with thermal conductivity detector (TCD) and mass spectrometer detector (Photomultiplier Tube Detector). The mass range selected was from m/z 50-500. A split type injector was used. The injection volume was 1 µL. The carrier gas was helium (1 mL min^{-1}). The oven temperature was at 50 ^{o}C for 2 min and then programmed to 250 ^{o}C for 10 min at a range of 10 ^{o}C min^{-1} and held for 10 min. The column used was Elite 5MS®. The stationary phase of the column was 5 % phenyl and 95 % methyl-polysiloxane.

2.2.3.8 Mineral elements analysis by atomic absorption spectroscopy

All glassware and digestion tubes were soaked in 10% nitric acid for 24 h and then rinsed several times with Milli-Q water prior to use. The whole digestion was carried out in an acid digestion system (KES 06L, Pelican Equipments, India) operated at 350 ºC. 5 ml of sample was taken in a kjeldahl flask and boiled down to a small bulk with HNO_3. Then added few glass beads, 10 ml of H_2SO_4 and 10 ml of HNO_3.Then heated gently until the liquid appreciably darkens in colour. Then added HNO_3 in small proportions (1 ml) and continued heating until darkening again takes place. Continued addition of acid and heating to fuming for 10 mins until the solution fails to darken. The allowed the solution to cool and added 10 ml of Milli-Q water and boiled gently to fuming. Then allowed the solution to cool again and added 5 ml of Milli-Q water and boiled gently to fuming. Finally cooled and made up the volume to 50 ml with Milli-Q water [21].

The quantitative analysis was carried out in an AAS system (iCE 3000, Thermo Scientific, USA) operated in a double beam mode. The analysis was carried out in flame mode with a 100 mm burner and the fuel used was a mixture of acetylene and air. The measurement mode was absorbance and the lamps used were hollow cathode lamps, each with distinctive emission wavelengths. Background correction was accomplished with a deuterium lamp in analyses where emission wavelength was less than 300 nm. All the standards used were AAS grade and purchased from Sigma Aldrich, USA. The software used for analysis of data SOLAAR.

2.2.4 Effect of the microbial starters cakes on some quality attributes of rice beer

2.2.4.1 Physical analysis

2.2.4.1.1 Volume and density measurement of the starters cakes (SC)

Certain volume of toluene was measured in a 1000 ml graduated measuring cylinder and the SC (whose mass had already been recorded) were placed in the cylinder and completely submerged. The difference between the measurements in the cylinder before and after placing of the starters gave the volume (cm^3) of the SC. The true density was calculated by dividing the mass with the actual volume (g/cm^3) [22].

2.2.4.1.2 Texture analysis of the SC

This analysis was carried out in a texture analyzer (TA-HD Plus 5187, Stable Micro Systems, UK). A p75 probe was used with a 100 kg load cell and a heavy duty platform (HDP/90). The pre test, test and post test speed were set at 1.00 mm/sec, 0.50 mm/sec and 5.00 mm/sec respectively. The trigger force used was 20 g.

2.2.4.1.3 Colour measurement

This was done according to section **2.2.3.1**

2.2.4.2 Production of rice beer (RB) in the laboratory

The rice was first boiled in distilled water for 10 min. This was followed by cooling the rice to room temperature. The starter cakes (SC) were powdered in a clean mortar and pestle and then mixed with the boiled rice at a ratio of 5 g per kilogram of rice. This mixture was transferred to sterile glass containers Fermentation was allowed to take place at 30 C in an incubator for eight days. After the completion of fermentation, the produce was strained using a muslin cloth and the filtrate was further diluted with distilled water in 1:1 ratio. This procedure was adapted from the traditional methodology for preparation of rice beer followed by the indigenous people of Northeast India. Nine types of rice beers (RB) were thus produced which were further used for analysis. The local names of the starter cakes and rice beers and the different codes used for them are shown in Table 1.

2.2.4.3 Biochemical analysis

2.2.4.3.1 Sample preparation for biochemical analysis

This was done according to section **2.2.3.2**

2.2.4.3.2 pH, acidity and alcohol content estimation

This was done according to section **2.3.3.3**

2.2.4.3.3 Total soluble solids (TSS) measurement

This estimation was carried out in a digital Abbe refractometer (DR-A1, Atago, Japan) at room temperature.

2.2.4.3.4 Estimation of proximate composition, reducing sugars, starch and amylose

All of these were done according to standard AOAC official methods [14].

2.2.4.3.5 Total polyphenols content (TPC) estimation

The concentration of total phenolic compounds was determined according to Bray and Thorpe [23]. The sample extracted was treated with Folin-Ciocalteu reagent and the absorbance was read at 650 nm in a UV-Vis spectrophotometer (Spectrascan UV- 2600, Thermo Scientific).

2.2.4.3.6 Radical scavenging activity estimation

This experiment was performed according to 2, 2-diphenyl-1-picrylhydrazyl (DPPH) cation free radical scavenging activity method of Brand-Williams et al. [24].

2.2.4.4 Microbial analysis

Plate count agar (PCA) was used for general aerobes, potato dextrose agar (PDA) supplemented with tartaric acid and Rose Bengal chloramphenicol agar (RBCA) for yeasts and moulds respectively, media of deMan, Rogosa and Sharpe (MRS) supplemented with $CaCO_3$ and bromocresol purple indicator for lactic acid bacteria (LAB), Salmonella Shigella agar (SSA) for *Salmonella* and *Shigella* species, Baird Parker agar (BPA) for coagulase positive *Staphylococcus* species, Eosin Methylene blue (EMB) agar for enterobacteriaceae and Modified MYP Agar for *Bacillus cereus*. The PDA and RBCA plates were maintained at 27 ^{o}C, the PCA, SSA and BPA plates at 37 ^{o}C and the MRS and EMB plates in an anaerobic gas pack system at 37 ^{o}C. The results obtained were expressed as log of colony forming units (CFU) per gram of sample.

2.2.5 Statistical analysis

This was carried out using the software Origin Pro (Version 8.0). Values were taken as mean of three replicates and standard deviation (SD) was calculated. The Fisher`s Least Significant Difference (LSD) was taken at $p<0.05$ and different superscripted alphabets has been used to represent the difference along a column.

2.3 Results and Discussions

2.3.1 Observations on the methodologies followed by different tribes in the preparation of rice beer

2.3.1.1 *Hor-alank* – Karbi tribe

The *Karbis* are one of the major tribes of Assam and are settled mostly in the districts of Karbi Anglong and North Cachar Hills. They prepare a traditional alcoholic beverage called *hor-alank.* This beverage is used as a refreshing drink and also bears significance in many social ceremonies and events. For preparation of *hor-alank* the yeast starter culture called *thap* first needs to be prepared. For preparing *thap* (Fig 2.2), rice is soaked in water for 1 day. The soaked rice is then mixed with leaves of *marthu* (*Croton joufra*), *janphong* (*Artocarpus heterophyllus*), *jockan* (*Phlogocanthus thysiflorus*), *hisou-kehou* (*Solanum viarum*) and barks of *themra* (*Acacia pennata*) plant. The mixture is grinded together in a wooden mortal called "*long*" with a pestle called "*lingpum*" in order to make a paste. This paste is then made into small flat shaped cakes of about 6 cm in diameter and 0.5 cm in thickness. These are overlaid with powder of previous *thaps* and kept in a bamboo sieve called "*ingkrung*" and dried for about three days under the sun or above the fire place. These can be stored for about 1 year for further use. For preparing beer, rice is first boiled, then spread and allowed to cool. It is followed by mixing with powdered *thaps* (5 Kg rice + 7 *thaps*). The whole mixture is kept in a large container and covered, first with plastic bags and then with sack. It is left to ferment for a period of 2 days at room temperature. After that it is mixed with water and further fermented for 2 (summer) to 4 (winter) days.

Fig 2.2 A *thap*

2.3.1.2 *Sujen* – Deori tribe

Being one of the oldest settlers of Assam, the Deoris are mostly inhabitant of Lakhimpur, Sibasagar, Dibrugarh, and Tinsukia districts of Assam, India. The indigenous rice beer of the Deoris is known as *sujen* (Fig 2.3). The starter material is known as *perok kushi* (Fig 2.4). The plant materials used for preparing *perok kushi* are leaves *of bhatar duamali* (*Jasminum sambac*), *thok thok* (*Cinnamomum byolghata*), *tesmuri* (*Zanthoxylum hamiltonianum*), *zing zing* (*Lygodium flexuosum*), *zuuro* (*Acanthus leucostychys*), *bhilongoni* (*Cyclosorus exlensa*), *sotiona* (*Alstonia scholaris*) and roots of *dubusiring* (*Alpinia malaccensis*) and the stem and rhizome of the plant *jomlakhoti* (*Costus speciosus*). All these are washed and cut into small pieces. They are then grinded in a specialized wooden grinder called as *dheki*. The mixture is then soaked in water in a vessel until the water becomes coloured. The whole mixture is added to grinded rice in a vessel in order to make dough. Round balls of about 4 cm diameter is made out of this and dried either in the sunlight or over the fire hearth by placing in a bamboo mat called as *aaphey*. After getting dried they are placed in a bamboo container called as *kula* (Fig 2.5) the inside of which is laid with *kher* (paddy straw). Its mouth is again covered with *kher* and is kept over the hearth for storage. They can be kept in this manner for many months and can be used as and when required. For fermentation of *sujen,* an earthen pot (*disoh*) is first sterilized by washing it with ash and placing it over the hearth for drying and fumigation. Rice is first boiled and then allowed to cool by spreading on banana leaves placed above an *aaphey*. This is followed by addition of powdered *perok kushi* to the cooled rice (1 starter per 3 Kg of rice). The mixture is kept in a *disoh*, the mouth of which

is sealed with *kol pat* (banana leaves) and left for fermentation to take place for about 4 to 5 days. It can then be diluted and filtered (Fig 2.6). It is said that the fermented mass in the *disoh* can be stored for up to 1 to 2 months at room temperature.

Fig 2.3 *Sujen* served on a brass cup

Fig 2.4 A *perok kushi*

Fig 2.5 A *kula*

Fig 2.6 A Deori woman filtering *sujen*

2.3.1.3 *Xaj pani* – Ahom community

The Ahoms or Tai-Ahoms are an ethnic group settled in Assam and are of Tai origin. They are a part of the Assamese society and are found all over Assam. The Ahoms prepare rice beer in their own traditional way and name it as *xaj pani* or *koloh pani.* The starter cake is known as *vekur pitha* (Fig 2.7) and consists of various parts of several plant species. The mainly used are leaves *of banjaluk* (*Oldenlandia corymbosa*), *kopou lota* (*Lygodium* sp.), *horuminimuni* (*Hydrocotyle sibthorpioides*), *bormanmunii* (*Centella asiatica*), *tubuki lota*

(*Cissampelos pareira*) and seeds of *jaluk* (*Piper nigrum*). All these are washed and dried well and then grinded in an *ural* (wooden mortar) with a pestle and mixed with grinded rice and a little water in a vessel and made into a paste. From this, oval shaped balls of about 4.5 cm x 3 cm are made and placed on *kol pat* [banana (*Musa* sp.) leaves] and dried either in the sun or over the fire place by taking care not to bring them not to close to the fire. After a period of about 5 days they become hard and are ready to be used. This *vekur pitha* can be stored for up to a year and used when needed. For preparing *xaj pani, rice* (either glutinous or non-glutinous) are half cooked and spread on banana leaves to cool it down. It is then mixed with powdered *vekur pitha* (1 per Kg of rice) and again spread for some time. The mixture is kept on a *koloh* (earthen pot) and the mouth is sealed. This is kept in a closed room for a period of 3 to 5 days. After this some amount of water is added to the fermented mass and left for about 10 minutes. Filtration is done by straining the mass by using a cloth (Fig 2.8).

Fig 2.7 A *vekur pitha*

Fig 2.8 An Ahom woman filtering *xaj pani*

2.3.1.4 *Apong* – Mising tribe

Although inhabiting in many districts of Assam, the Misings are concentrated mostly in the districts of Dhemaji, Lakhimpur and Jorhat. They are said to have migrated to Assam from the state of Arunachal Pradesh. The rice beer prepared by the Misings is known as *apong* and the starter cake is called as *apop pitha* (Fig 2.9). The different leaves needed for preparing *apop pitha* are of the plants *bormanimuni* (*Centella asiatica*), *horumanimuni* (*Hydrocotyle sibthorpioides*), *banjaluk* (*Oldenlandia corymbosa*), *kuhiar* (*Saccharum officinarum*), *dhapat*

tita (*Clerodendrum viscosum*), *bhilongoni* (*Cyclosorus exlensa*), *bam kolmou* (*Ipoemea* sp.), *senikuthi* (*Scoparia dulcis*), *lai jabori* (*Drymeria cordata*), *jalokia* (*Capsicum annuum*), *anaras* (*Ananas comosus*) and *kopou dhekia* (*Lygodium flexuosum*). All these leaves are cleaned and dried by placing on a bamboo mat called *opoh*. They can be either used freshly or dried in the sun before addition. Soaked rice and the leaves are grinded separately in a *kipar* (wooden grinder) and they are mixed together in a vessel with little water. From the dough, oval shaped balls of about 6 cm x 3 cm are made and dried in the sun. Before starting the fermentation process, the *kiling* (earthen pot) used for fermentation is first fumigated by placing it on a *torap* (a bamboo frame constructed over the fire place) until the pot turns blackish (Fig 2.10). After that boiled rice is spread on a *kol pat* (banana leaf) and allowed to cool. To this powdered *apop pitha* is added (1 *apop pitha* for 1 kg of rice) and the whole mixture is kept inside the *kiling* and the mouth of the pot is covered with banana leaves or leaves of *bhilongoni* (Fig 2.11). This is left for fermentation to take place for a period of about 5 days. A little water is added to the fermented product and is filtered to get the *apong* (Fig 2.12). The Misings also prepare another kind of rice beer and it is known by the name *sai mod.* In this method, hay and husk are half burned till they become black in colour. This ash is mixed in equal amount with boiled rice and to it the *apop pitha* is added. In this case, the amount of *apop pitha* added in double quantity with respect to *apong* preparation. The mixture is compactly packed in a *killing* and fermented for about 15 days. It is filtered in the same way as *apong*.

Fig 2.9 An *apop pitha*

Fig 2.10 A *kiling* before fumigation

Fig 2.11 Fermentation in a *killing*

Fig 2.12 A Mising woman filtering *apong*

2.3.1.5 *Jou bishi* – Bodo tribe

The *Bodos* are one of the largest linguistic groups in North-East India and among the earliest settlers of Assam. They inhabit most of the regions in Assam but resides mostly in the Bodoland regions. The local rice beer prepared by the Bodos is known as *jou bishi* and the starter cakes are known as *ankur* (Fig 2.13). For preparing *angkur*, different plant materials are said to be used based on their availability in different regions. However, the most common species are leaves of *agarsita* (*Xanthium strumarium*) and *dongphang rakhep* (*Scoparia dulcis*) and either roots or leaves of *lokhunath* (*Clerodendrum viscosum*). These plants are first washed properly and allowed to dry in the air. Rice grains are soaked for about 5 hours in normal temperature water and allowed to soften. This is then mixed with the plants and grinded together in a wooden mortar with a pestle and this set of apparatus is called *wayal.* Dough is made by adding a little water to the mixture. They are then made into round cakes of about 5.5 cm diameter and 0.5 to 1 cm thickness and covered with powder of the mixture to which water is not added. This is followed by covering with *gigab* (paddy straw) and allowed to dry for a period of 3–4 days. These can be stored in moisture free places for more than a year. For preparing the beer, either glutinous or non-glutinous rice can be used. When glutinous rice is used the product is known as *maibra jou bishi* and when non-glutinous rice is used it is known as *matha jou bishi.* The rice is first boiled with care not to allow it to overcook. It is then cooled and allowed to dry. To this powdered *ankur* is added (about one *ankur* for 1 kg of rice) and mixed well. This mixture is put inside a plastic bag and kept closed for one night. After this a little water is added to it and left in a *baiphu* (earthen pot) covered with banana leaves for a

period of at least 3 days. The fermented mass if further mixed with water and strained in order to get the liquid *jou bishi.*

Fig 2.13 An *ankur*

2.3.1.6 *Judima* - Dimasa tribe

The Dimasas are one of the earliest indigenous ethnic groups of North-Eastern India. They are mostly found in the North Cachar Hills of Assam and Dimapur in Nagaland. The starter cake for preparing *judima* is called as *umhu* or *humao* (Fig 2.14) and is a mixture of rice and bark of *thempra* (*Acacia pennata*) plant. The barks are cut into small pieces and dried in the sun. Rice is soaked in water until it is softened. It is then grinded in a wooden or metallic mortal pestle called *rimin* along with the barks of *thempra* plant. A little water is added in order to make a paste. They are then made into cakes of appropriate sizes and allowed to dry for a period of one week. They can be stored for many months. For preparing *judima,* rice is boiled and allowed to cool. It is mixed with powdered *humao* (one large sized *humao* is sufficient for 5 Kg of rice) and kept in a large container which is covered with jute gunny bags. After about a week, slightly yellowish juices come out of the mass which indicates the completion of fermentation. This can further be diluted with water and filtered for consumption (Fig 2.15).

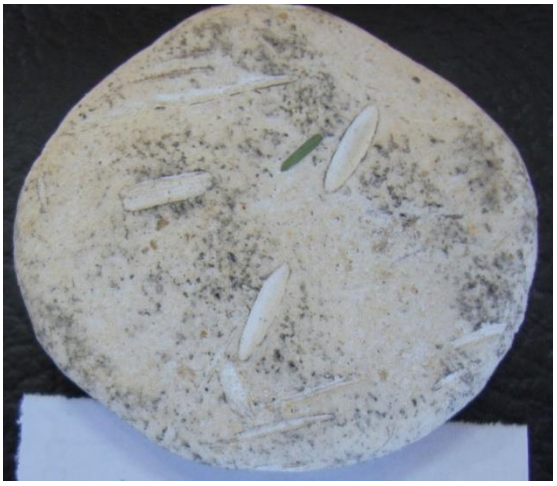

Fig 2.14 A *humao*

Fig 2.15 A Dimasa woman serving *judima*

2.3.1.7 *Zutho* – Angami tribe

Nagaland is chiefly a mountainous state and is inhabited by many different Naga tribes. Each of these tribes has some common culture and traditions and they are all regarded as to having warrior background. The local brew prepared by the Angami tribe is known as *zutho* (Fig 2.16). This starter material used in the preparation of *zutho* is known as *piazu*, which is basically sprouted rice. For preparing *piazu,* un-hulled rice is first soaked in water for a period of about 3-4 days. After this, some of the water is drained out and the grains are allowed to germinate. This may sometimes take about a week depending on the prevailing temperature. After being dried in the air, the sprouted grains are pounded on a wooden mortar with a pestle. The powder obtained is known as *piazu*. For preparing *zutho,* rice is first boiled and then allowed to cool by spreading on a bamboo mat. To this rice, *piazu* (about 10 g for 1 kg of rice) is added and mixed well. The amount of *piazu* added is needed more (almost double) during the months of winter. The mixture is then left to ferment in a closed earthen or wooden vessel for about 4 days in summer and about a week in winter. After completion of fermentation, some amount of water is added to the rice and is filtered by using a bamboo or plastic mesh and usually served in bamboo cups (Fig 2.17). The Ao tribe of Nagaland also prepared this brew in a similar manner and they call it as *litchumsu*.

Fig 2.16 *Zutho* after fermentation

Fig 2.17 *Zutho* served in bamboo mugs

2.3.1.8 *Opo* - Adi-Galo tribe

Located in the far North-East India, Arunachal Pradesh is inhabited by many different tribes and each of these bears their own cultural resemblance. This study was done in Pasighat sub- division of East Siang district and the contribution came from the *Adi-Galo* tribe residing in that area. The local rice beer prepared by this tribe is called as *opo* and the starter cake is known as *siiyeh* (Fig 2.18) or *opop.* For preparing *opop*, leaves and barks of the plants *dhapat* (*Clerodendron viscosum*) and *Lohpohi* (*Veronia* sp.) are washed, sun dried and then made into powder. This is then mixed with powdered rice and a little bit of previously prepared *opo* in order to make a paste. From this flat cakes of about 10–11 cm diameter are made and placed upon bamboo mats. The mats are then kept in the hearth for about 3–4 days, when the cakes become hardened. These can be stored for many months. For preparing *opo*, rice husk called *ampe* is half burnt till they become black in colour. After that, rice is boiled and then spread on a bamboo mat called as *peche.* After the rice gets cooled, it is mixed with the burnt husk in 1:1 ratio. To this powdered *opop* is added (about 100 g of the starter for 10 kg of the mixture) and mixed well. This mixture is then put in a plastic container, the walls of which are covered with leaves of a locally available plant called as *oko (*Zingiberaceae family*).* The mouth is also sealed with *oko* leaves and is left undisturbed for about 5 days. After this the contents are mixed well and are again left in the same manner for a longer duration. The product becomes ready after about 20 days of fermentation. It is also kept for longer durations for production of more alcohol. For filtration, a special type of funnel called as *perpur* is used where *oko* leaves are used as the filter. The fermented mass is first placed on the *perpur* and then hot water is poured

over it slowly in order to obtain the *opo* as the filtrate (Fig 2.19). The quantity of water poured depends on the desired concentration of the final product.

Fig 2.18 A *siiyeh*

Fig 2.19 An Adi-Galo woman filtering *opo*

2.3.1.9 *Sadhiar* - Khasi tribe

The Khasis are an indigenous group of tribal people, the majority of whom live in the State of Meghalaya. They are also found in small populations in Assam, and in parts of Bangladesh. This study was done among the Khasi community of Shillong in Meghalaya. The rice beer prepared by the Khasis is called as *sadhiar* and the starter is known as *thiat*. For making *thiat*, dried leaves of *khawiang* (*Amomum aromaticum*) are ground in a wooden mortar pestle. Also *khoso* (local variety of rice) is ground in the mortar pestle into a fine powder. These two are then mixed and a little spring water is added to the mixture to make dough from which the round flattened cakes are made. These are dried on a *malieng* (bamboo basket) which is kept over the hearth until the cakes get hardened. For brewing *sadhiar*, 4-5 Kg of *khoso* is mixed with spring water and cooked in a metallic vessel with continuous stirring. The cooked rice is then spread on a *malieng* for cooling and drying. Then to this 2-3 finely crushed cakes of *thiat* is mixed. The mixture is then put in a cone shaped basket called *shang*. The whole basket is covered with a cloth and left for 2-3 days. The fermented mash known as *jyndem*, from which the off-whitish rice beer (*sadhiar*) is filtered out.

2.3.2 Biochemical analysis of the rice beer samples

2.3.2.1 General characteristics of rice beer samples

Details of rice beer samples collected across the Northeastern states of India including its origin of preparation are presented in Table 2.1. The alcohol content in all the samples was mild and ranged from 3.99% (sample AsB2) to 5.09% (sample AsB3). Acidic pH was recorded in all of the samples and varied from 4.16 in sample NaB1 to 4.81 in sample MeB1. Sample NaB1 showed the highest value of "L" (74.97) indicating it to be the whitest, while sample AsB5 showed the lowest value of L (1.11). The "a" values ranged from 0.43 (AsB5) to 0.88 (AsB4), and hence redness was more prevalent than greenness. The "b" values varied among the samples and the highest value was recorded in ArB1 (14.01) and lowest in AsB2 (1.77), showing more and less yellowness respectively.

The biochemical examination of the samples revealed that there are significant differences in quality among them. One of the plausible reasons for this variation might be attributed to differences in the methodology of making each product, and especially the kind of starter cultures used. For instance, in the samples NaB1 and AsB3 collected from Dimapur and Diphu respectively, the main component of the starter was the bark of the plant *Acacia pennata*. However, in case of the sample NaB2 collected from Chumukedima the starter was only the powder of sprouted paddy. Whereas, the starters for all the other samples contained roots, leaves or whole plant of various herbs and shrubs. In addition, the strain of microbes carrying out the fermentation process also differs from product to product [1,2,7,11].

Table 2.1 Details of the nine rice beers samples collected from various regions of Northeast India

Code	Local name	Community	Place of collection	Alcohol content (%)	pH	Colour		
						L	a	b
AsB1	*Jou-bishi*	Bodo	Gossaigaon, Assam	4.33±0.07	4.22±0.04	4.28±0.12	0.71±0.13	2.01±0.09
AsB2	*Sujen*	Deori	Lakhimpur, Assam	3.99±0.03	4.56±0.02	3.11±0.21	0.44±0.06	2.63±0.09
AsB3	*Hor-alank*	Karbi	Diphu, Assam	5.09±0.24	4.72±0.01	2.56±0.42	0.63±0.09	1.77±0.15
AsB4	*Xaj-pani*	Ahom	Sibsagar, Assam	4.12±0.01	4.39±0.01	1.98±0.05	0.88±0.04	4.63±0.32
AsB5	*Apong*	Mising	Lakhimpur, Assam	4.77±0.04	4.31±0.08	1.11±0.09	0.43±0.21	2.59±0.54
NaB1	*Judima*	Dimasa	Dimapur, Nagaland	4.13±0.09	4.16±0.04	56.35±0.76	0.54±0.09	7.34±0.14
NaB2	*Jutho*	Angami	Chumukedima, Nagaland	4.68±0.10	4.24±0.05	13.77±0.62	0.77±0.11	2.65±0.12
ArB1	*Opo*	Adi-Galo	Passighat, Arunachal Pradesh	4.59±0.05	4.56±0.03	22.58±0.20	0.54±0.22	14.01±0.28
MeB1	*Sadhiar*	Khasi	Shillong, Meghalaya	4.45±0.11	4.81±0.03	6.20±0.21	0.78±0.06	2.56±0.41

2.3.2.2 Analysis of organic acids

The result of high performance liquid chromatography (HPLC) analysis for the content of different organic acids present in the various samples is shown in Table 2.2. Lactic acid was found to be predominant among all the other acids and its concentration varied significantly. It was recorded the highest (9119.42 mg/L) in AsB4 and lowest in NaB2 (618.76 mg/L). Propionic acid was recorded in less amount in NaB2 (0.19 mg/L) and not found in rest of the samples. Oxalic acid was present in seven of the samples and there was no significant difference among the samples NaB2, AsB1 and MeB1, and the highest concentration was shown by NaB1 (727.04 mg/L). Three samples evinced the presence of citric acid; they were NaB1 (292.70 mg/L), AsB1 (492.34 mg/L) and AsB3 (361.96 mg/L). The highest concentration of tartaric acid was recorded in NaB2 (2618.37 mg/L) and lowest in MeB1 (37.32 mg/L). Out of all the tested samples, five of them showed the presence of tartaric acid. Succinic acid was found in the samples NaB2, AsB2, AsB3 and ArB1 in varying concentrations from 543.94 mg/L to 914.68 mg/L. Pyruvic acid was present in six of the samples and NaB1 (215.09 mg/L) had the highest concentration followed by AsB5 (33.84 mg/L) and rest of the samples did not reveal significant difference. Except NaB1 and MeB1 all the samples showed the presence of formic acid and the highest concentration was shown by AsB5 (500.92 mg/L). Acetic acid was found in AsB1 (1172.84 mg/L), AsB5 (531.70 mg/L) and MeB1 (1331.30 mg/L).

A variety of organic acids were present in the samples and most of them are natural products of microorganisms or intermediates in their major metabolic pathways [25]. The high concentration of lactic acid found in all the samples of the present study can be attributed to the lactic acid fermentation of sugars undertaken by the group of LAB present in all of the rice beers [26]. These organic acids contribute to the unique and distinctive tartness and flavours of beers and are also responsible for the organoleptic properties apart from aiding in preservation process. Analysis of the organic acid content of Italian lager beers using HPLC methodologies showed that the total content of organic acids was in between 451 mg/L and 712 mg/L which corroborate our results. The most common organic acid found in the Italian beers was lactic acid (128 mg/L), followed by citric (116 mg/L), acetic (108 mg/L), succinic (68 mg/L), malic (63 mg/L), pyruvic and formic acid (44 mg/L) [27]. The occurrence of acetic, lactic, citric, malic, pyruvic and succnic acid in 58 samples of lager beer representing 20 different brands from different parts of the world have also been reported by Nord et al. [28]. Lee et al.[29] found that the static fermentation of brown rice produced high contents of acetic, oxalic,

tartaric, and malic acids with increasing concentration of the starter *nuruk*. They also found that acetic, tartaric and malic acid contents were higher in static culture than agitated culture. The presence of these organic acids helps in increasing the shelf life of the products by inhibiting the growth of spoilage bacteria like *Escherichia coli* and *Salmonella* spp. [30,31]. Such inhibition of microbial growth by organic acids is affected by several factors such as inhibition of essential metabolic reactions [32], membrane disruption [33], stress on intracellular pH homeostasis [34] and the accumulation of toxic anions [35].

Table 2.2 Organic acid profile of the rice beer samples

Sample	Organic acid concentration (mg/L)								
	Lactic acid	Propionic acid	Oxalic acid	Citric acid	Tartaric acid	Succinic acid	Pyruvic acid	Formic acid	Acetic acid
NaB1	4430.79 ±18.68^{d}	0.0^{a}	727.04 ±11.99^{e}	292.70 ±7.61^{b}	83.81 ±4.52^{c}	0.0^{a}	215.09 ±15.56^{c}	0.0^{a}	0.0^{a}
NaB2	618.76 ±6.28^{a}	0.19 ±0.06^{b}	0.17 ±0.04^{a}	0.0^{a}	2618.37 ±10.07^{e}	914.68 ±10.24^{d}	7.65 ±1.12^{a}	63.05 ±6.24^{b}	0.0^{a}
AsB1	6431.44 ±28.44^{g}	0.0^{a}	4.15 ±0.15^{a}	492.34 ±7.20^{d}	0.0^{a}	0.0^{a}	1.76 ±1.15^{a}	102.18 ±8.14^{c}	1172.84 ±10.01^{c}
AsB2	4529.34 ±15.85^{e}	0.0^{a}	0.0^{a}	0.0^{a}	80.72 ±4.31^{c}	686.73 ±17.03^{c}	0.0^{a}	54.70 ±5.33^{b}	0.0^{a}
AsB3	4076.94 ±14.03^{c}	0.0^{a}	0.0^{a}	361.96 ±13.39^{c}	0.0^{a}	543.94 ±19.02^{b}	0.0^{a}	208.65 ±13.63^{d}	0.0^{a}
AsB4	9119.42 ±13.13^{i}	0.0^{a}	255.98 ±6.95^{d}	0.0^{a}	0.0^{a}	0.0^{a}	3.02 ±1.17^{a}	92.56 ±6.35^{c}	0.0^{a}
AsB5	6024.76 ±7.48^{f}	0.0^{a}	130.08 ±5.41^{b}	0.0^{a}	428.58 ±14.28^{d}	0.0^{a}	33.84 ±3.56^{b}	500.92 ±11.83^{e}	531.70 ±30.44^{b}
ArB1	1811.65 ±9.56^{b}	0.0^{a}	151.54 ±8.11^{c}	0.0^{a}	0.0^{a}	912.59 ±17.35^{d}	0.0^{a}	91.32 ±9.07^{c}	0.0^{a}
MeB1	7438.05 ±5.46^{h}	0.0^{a}	5.36 ±1.10^{a}	0.0^{a}	37.32 ±2.47^{b}	0.0^{a}	2.88 ±0.66^{a}	0.0^{a}	1331.30 ±29.57^{d}

Note: Values are mean of three replicates ± Standard Deviation (SD). Means followed by different superscripted alphabet differs significantly (p<0.05).

2.3.2.3 Analysis of carbohydrates

The HPLC analysis of carbohydrates profile of the nine rice beer samples (Table 2.3) revealed that glucose was present in six samples and NaB1 evinced the highest value (3675.88 mg/L) and concentration in all the samples differed significantly. On the other hand, fructose was present in AsB3 and ArB1 and its concentration did not differ significantly. Raffinose - a trisachharide of galactose, fructose and glucose was present in seven samples and AsB2 showed the highest concentration of 1413.00 mg/L. Trehalose was detected in two samples namely AsB4 and AsB5. Another monosaccharide (5C) i.e. arabinose was found in six samples

and its concentrations ranged from 18.84 mg/L to 49.26 mg/L. Xylose which is also one of the important pentose monosaccharides was detected in AsB1 (1881.85 mg/L) and AsB3 (45.01 mg/L). The hexose monosaccharide galactose was present in five samples and differed significantly in its concentration, and the sample NaB1 evinced the highest value (3023.80 mg/L). A naturally occurring deoxy sugar rhamnose was found only in MeB1 at (2828.63 mg/L). The less known classical sugar inositol (or cyclohexane-1,2,3,4,5,6-hexol) was also present in six samples in low concentration. Although most of the common carbohydrates were present, the disaccharides namely sucrose, maltose and melibiose were not found in the present study.

Carbohydrates which were found in varying quantities in all the samples might attribute as the major sources of energy and the monosaccharides contribute to the sweetness of the product. The presence of simple sugars like glucose represents the easily metabolized carbohydrate, whereas other complex forms like raffinose can act as dietary fibers which have several health benefit effects such as prevention of heart diseases, diabetes, obesity and certain gastrointestinal diseases [36]. The quantification of carbohydrates in pilsner beer (a type of pale lager beer) by HPLC revealed that fructose, glucose, maltose, maltotriose and maltotetraose were present in 2.4 to 2.6 g/L, 4.2 to 406 g/L, 0.35 to 38.5 g/L, 1.3 to 8.1g/L and 1.1 to 2.1 g/L respectively [17].

Table 2.3 Carbohydrate profile of the rice beer samples

Sample	Carbohydrate concentration (mg/L)												
	Glucos-e	Fructo-se	Sucro-se	Raffin-ose	Mal-tose	Trehal-ose	Arabi-nose	Xylos-e	Galacto-se	Rham-nose	Melib-iose	Lacto-se	Inosi-tol
NaB1	3675.88 ±17.08^{g}	0.0^{a}	0.0^{a}	230.16 ±22.50^{b}	0.0^{a}	0.0^{a}	18.84 ±0.51^{b}	0.0^{a}	3023.80 ±9.01^{f}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}
NaB2	1744.91 ±5.90^{d}	0.0^{a}	0.0^{a}	503.81 ±7.03^{f}	0.0^{a}	0.0^{a}	49.26 ±1.48f	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	20.02 ±1.62^{e}
AsB1	0.0^{a}	0.0^{a}	0.0^{a}	450.75 ±12.96^{e}	0.0^{a}	0.0^{a}	20.45 ±0.78^{b}	1881.85 ±9.57^{c}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	23.87 ±4.82^{f}
AsB2	319.36 ±10.49^{b}	0.0^{a}	0.0^{a}	1413.00 ±14.29^{g}	0.0^{a}	0.0^{a}	31.20 ±1.72^{c}	0.0a	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	15.14 ±3.45^{d}
AsB3	1855.23 ±10.00^{e}	20.03 ±0.08^{b}	0.0^{a}	395.79 ±5.69c	0.0^{a}	0.0^{a}	0.0^{a}	45.01 ±3.53^{b}	497.11 ±13.58^{c}	0.0^{a}	0.0^{a}	0.0^{a}	13.57 ±1.23^{cd}
AsB4	3647.89 ±25.17^{f}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	979.79 ±13.62^{c}	46.84 ±1.53^{e}	0.0^{a}	1540.12 ±13.99^{d}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}
AsB5	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	608.98 ±5.10^{b}	34.81 ±0.74^{d}	0.0^{a}	2565.76 ±4.92^{e}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}
ArB1	950.55 ±2.11^{c}	21.83 ±3.47^{b}	0.0^{a}	433.41 ±4.62^{de}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	253.20 ±5.23^{b}	0.0^{a}	0.0^{a}	0.0^{a}	11.09 ±1.4c
B1	0.0^{a}	0.0^{a}	0.0^{a}	431.01 ±9.28^{d}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	2828.63 ±8.66^{b}	0.0^{a}	0.0^{a}	4.79 ±0.29^{b}

Note: Values are mean of three replicates ± Standard Deviation (SD). Means followed by different superscripted alphabet differs significantly (p<0.05).

2.3.2.4 Analysis of amino acids

The amino acid profile of the samples is presented in Table 2.4. All the samples contained arginine, serine, aspartic acid, glutamic acid, glycine, tyrosine, proline, valine, phenylalanine, isoleucine, leucine, histidine and lysine and concentration differed significantly. Alanine was present in only NaB2 and AsB3 and methionine in only AsB1, AsB3 and AsB4. Aspartic acid was found in high concentration (1091.81 to 14626.35 mg/L), and rest of the amino acids varied with samples. Results revealed that most of the essential amino acids were present in the tested rice beer samples. In addition, some of the conditionally essentially amino acids (amino acids which are to be supplied exogenously with diet for some specific population) [37] were also found e.g. arginine, glycine, glutamic acid, histidine, proline, serine and tyrosine.

The amino acids obtained in relatively high amounts in the present study signify that rice beer can be a good source of essential nutrients and energy for body metabolism. Nord et al. [28] also reported the occurrence of all these amino acids in 58 samples of lager beer representing 20 different brands. Kabelová et al. [38] also measured the content of 16 free amino acids in 35 beers commercially available in Czech Republic using HPLC method. Proline was found to be the most common amino acid with a concentration of 40 to 250 mg/L. Proline has been described as the chief amino acid present in wines by Ough [39], who found that the average value of proline content in California wines was 869 mg/L. The proteins rich in proline are also responsible for producing the haze (turbidity) in beer by combining with the polyphenols [40]. The presence of these amino acids indicates the presence of low molecular weight peptides in rice beer with bioactive and sensory active properties as have been described by Han and Xu [41].

Table 2.4 Amino acid profile of the rice beer samples

Sample	Amino acid concentration (mg/L)														
	Arginine	Serine	Aspartic acid	Glutamic acid	Glycine	Alanine	Tyrosine	Proline	Methi-onine	Valine	Phenyl-alanine	Isoleucine	Leucine	Histidine	Lysine
NaB1	1104.53 ±25.04^{f}	349.24 ±24.69^{a}	6855.74 ±31.86^{c}	1964.80 ±37.99^{e}	1059.51 ±45.07^{c}	0.0^{a}	7354.06 ±49.50^{f}	463.01 ±5.96^{f}	0.0^{a}	435.58 ±22.03^{e}	349.67 ±13.51^{c}	233.07 ±3.76^{cd}	434.88 ±10.76^{d}	1472.20 ±78.14^{a}	365.05 ±20.64^{b}
NaB2	473.02 ±23.66 ^{b}	519.62 ±11.95^{b}	3935.96 ±31.57^{b}	1171.45 ±61.21^{b}	866.29 ±11.41^{a}	2093.85 ±6.18^{c}	338.98 ±5.58^{a}	320.64 ±9.35^{d}	0.0^{a}	351.07 ±8.55^{d}	381.97 ±12.69^{d}	247.42 ±11.81^{de}	386.57 ±1.49^{c}	3848.21 ±33.40^{f}	491.34 ±6.13^{d}
AsB1	847.51 ±4.36^{d}	1034.78 ±33.17^{e}	12415.78 ±96.10^{f}	2890.68 ±39.14^{h}	1240.41 ±37.56^{d}	0.0^{a}	399.78 ±18.82^{b}	515.58 ±15.17^{g}	108.22 ±4.48^{c}	454.12 ±31.01^{e}	572.33 ±5.96^{g}	431.80 ±4.85^{f}	692.46 ±14.09^{h}	6763.49 ±11.49^{h}	775.15 ±18.04^{f}
AsB2	453.33 ±23.17 ^{b}	572.75 ±20.55^{b}	11273.79 ±66.43^{e}	2341.91 ±42.16^{f}	836.63 ±33.29^{a}	0.0^{a}	489.17 ±12.82^{c}	375.23 ±6.09^{e}	0.0^{a}	508.59 ±14.47^{f}	416.65 ±6.19^{e}	378.90 ±21.48^{e}	533.64 ±9.75^{f}	4729.79 ±28.42^{g}	575.56 ±12.44^{e}
AsB3	1147.56 ±45.84^{g}	760.85 ±34.23^{c}	9380.68 ±55.96^{d}	2558.65 ±26.90^{g}	1054.61 ±49.72^{bc}	1131.59 ±27.25^{b}	1113.79 ±61.43^{e}	450.40 ±9.70^{f}	96.45 ±8.40^{b}	512.25 ±10.95^{f}	472.09 ±7.24^{f}	356.07 ±10.18^{e}	566.17 ±12.26^{g}	2663.85 ±10.37^{c}	454.21 ±28.44^{c}
AsB4	977.10 ±19.77^{e}	1214.89 ±79.71^{f}	14626.35 ±355.97^{h}	2540.44 ±55.31^{g}	1381.06 ±24.83^{e}	0.0^{a}	721.19 ±19.10^{d}	380.81 ±13.44^{e}	95.85 ±3.29^{b}	251.94 ±5.91^{c}	466.69 ±18.25^{f}	264.25 ±27.32^{d}	464.91 ±11.86^{e}	3047.17 ±32.59^{e}	363.94 ±6.89^{b}
AsB5	519.39 ±20.61^{c}	788.55 ±24.24^{cd}	12434.13 ±53.70^{f}	1341.38 ±23.44^{c}	886.43 ±11.43^{a}	0.0^{a}	526.57 ±15.50^{c}	238.67 ±15.14^{b}	0.0^{a}	157.05 ±21.14^{b}	172.44 ±6.57^{a}	163.73 ±6.82^{b}	284.91 ±21.80^{b}	1930.43 ±49.65^{b}	285.10 ±1.72^{a}
ArB1	281.36 ±15.56 ^{a}	849.01 ±17.54^{d}	12948.99 ±53.48^{g}	1636.62 ±38.65^{d}	876.81 ±17.99^{a}	0.0^{a}	494.86 ±17.81^{c}	266.54 ±11.21^{c}	0.0^{a}	247.24 ±12.14^{c}	156.93 ±14.63^{a}	217.93 ±17.13^{c}	283.36 ±14.46^{b}	4019.48 ±6.62^{f}	427.57 ±13.75^{c}
MeB1	278.60 ±11.77 ^{a}	2120.16 ±44.36^{g}	1091.81 ±12.15^{a}	850.08 ±33.94^{a}	1005.41 ±23.43^{b}	0.0^{a}	374.87 ±4.78^{ab}	173.04 ±14.63^{a}	0.0^{a}	120.77 ±8.36^{a}	216.09 ±16.42^{b}	125.16 ±3.78^{a}	211.25 ±2.88^{a}	4761.02 ±50.27^{g}	357.55 ±16.03^{b}

Note: Values are mean of three replicates ± Standard Deviation (SD). Means followed by different superscripted alphabet differs significantly (p<0.05).

2.3.2.5 Analysis of aromatic compounds

GC-MS analysis is a very precise and time efficient method for analyzing the volatile and semi volatile compounds and has been used for this purpose by several other workers [42-46].The volatile and semi-volatile compounds are considered the major components responsible for imparting the distinctive flavour to wines and beers [42-44]. The occurrence of different volatile organic compounds in the rice beer varieties, under study, is presented in Table 2.5. The relative peak areas of the compounds are listed as percentage of areas occupied by the individual peak out of the total area of all the detected peaks. Various alcohols and esters were detected in all the samples. It has been observed that phenylethyl alcohol was present in all the varieties of rice beer under investigation. All the samples contained significant amounts except NaB1 and ArB1. The compound 2,2´-oxybis-ethanol was detected in six rice beer varieties with maximum peak area up to 37.28 %. In case of butanedioic acid diethyl ester, only four samples showed the presence of the compound with peak area in the range of 0.66-3.85 %. The compound 3-methyl-1-butanol was detected only in AsB2, ArB1 and MeB1. However, the presence of the compound was negligible in AsB2. Ethyl acetate (in ArB1 and MeB1), 1,4-butanediol (in ArB1 and MeB1), and 6-methylheptyl ester-2-propenoic acid (in NaB1 and NaB2) were present only in two samples each. NaB2 contained the compounds namely (S)-(+)-5-methyl-1-heptanol, 2-(1,1-dimethylethyl)-cyclobutanone and (S)-(+)-6-methyl-1-octanol whereas, NaB1 contained 2-methyl-dipropanoate-1,3-propanediol, 5-methyl-2-(1-methylethyl)-1-hexanol and 2-isopropyl-5-methyl-1-heptanol. On the other hand, AsB1 (5-methyl-4-octanol and hexyl ester chloroacetic acid) and AsB3 (4-butoxy-butanoic acid and didodecyl phthalate) revealed the presence of two compounds each. Samples ArB1 and AsB5 contained 3,3-dimethyl-4-methylamino-butan-2-one and 3-hydroxy-butanal respectively. The occurrences of these compounds was less than 8% except 2-(1,1-dimethylethyl)-cyclobutanone which showed 18.04 %.

The present study revealed varying patterns in the content of volatile aromatic compounds. The production of similar groups of aromatic compounds by different strains of yeasts in fermentation mixture of *sochu* (a Japanese alcoholic drink) have also been reported by Yamamoto et al. [47] who studied the fermentation process at different temperatures. Many volatile compounds have been characterized as odour active compounds in rice beer and these are considered to provide alcohol like, sweet, fruity, buttery and pungent aromas [46]. A large number of volatile and aromatic compounds in rice beer have also been earlier identified by

Isogai et al.[44] using GC-MS methods. Many volatile and non-volatile components contribute to the distinctive flavour of beer [48] and a diverse group of volatile and semi-volatile aromatic compounds were detected in the samples. However, the influence of these volatiles on the actual aroma profile of the rice beer will depend on their threshold values. Phenylethyl alcohol is the most abundant compound in the samples studied and is an important constituent in many essential oils, flavours, and perfumery and moreover it has antimicrobial properties [49]. Lee et al. [29] also found high content of phenylethyl alcohol in agitated fermentation of brown rice to produce vinegar using the starter *nuruk.* The occurrence of the group of higher alcohol like 1-butanol, 1-hexanol, 2,3-butanediol, phenylethyl alcohol, 2-butanol, ethyl alcohol and 3-ethoxy-1-propanol have also been reported in alcoholic beverage prepared from fruits and these may contribute distinctive flavour to the beverage [50].

Table 2.5 Aromatic compounds in the rice beer samples detected by GC-MS analysis

Organic compounds	Retention time (min)	Rice beer varieties/Sample								
		NaB1	NaB2	AsB1	AsB2	AsB3	AsB4	AsB5	ArB1	MeB1
Ethyl acetate	2.0	x	x	x	x	x	x	x	√ (25.12)	√ (0.12)
1,4-butanediol	2.1	x	x	x	x	x	x	x	√ (20.26)	√ (11.01)
3-methyl-1-butanol	2.9	x	x	x	√ (0.36)	x	x	x	√ (39.37)	√ (32.46)
(S)-(+)-5-methyl-1-heptanol	3.0	x	√ (3.75)	x	x	x	x	x	x	x
5-methyl-4-octanol	3.2	x	x	√ (3.31)	x	x	x	x	x	x
2-methyl-dipropanoate-1,3-propanediol	3.5	√ (16.46)	x	x	x	x	x	x	x	x
2,2`-oxybis-ethanol	3.9	√ (15.97)	√ (1.25)	√ (1.86)	x	√ (3.09)	√ (37.28)	x	x	√ (20.61)
3-amino-2-methyl-butanoic acid	5.6	x	x	x	x	x	x	x	√	x
2-(1,1-dimethylethyl)-cyclobutanone	6.9	x	√ (18.04)	x	x	x	x	x	x	x
5-methyl-2-(1-methylethyl)-1-hexanol	7.90	√ (14.62)	x	x	x	x	x	x	x	x
3,3-dimethyl-4-methylamino-butan-2-one	7.92	x	x	x	x	x	x	x	√ (7.37)	x
4-butoxy-butanoic acid	7.93	x	x	x	x	√ (2.31)	x	x	x	x
(S)-(+)-6-methyl-1-octanol	8.1	x	√ (2.95)	x	x	x	x	x	x	x
Phenylethyl alcohol	8.9	√ (7.67)	√ (67.52)	√ (93.03)	√ (96.19)	√ (86.91)	√ (67.72)	√ (71.96)	√ (4.48)	√ (35.79)
Diethyl ester butanedioic acid	9.8	x	√ (3.85)	√ (0.66)	√ (1.61)	√ (1.86)	x	x	x	x
6-methylheptyl ester-2-propenoic acid	10.5	√ (39.67)	√ (2.65)	x	x	x	x	x	x	x
Hexyl ester chloroacetic acid	12.6	x	x	√ (1.14)	x	x	x	x	x	x
2-isopropyl-5-methyl-1-heptanol	12.9	√ (5.62)	x	x	x	x	x	x	x	x
3-hydroxy-butanal	13.1	x	x	x	x	x	x	√ (6.92)	x	x
Didodecyl phthalate	19.2	x	x	x	x	√ (3.27)	x	x	x	x

Note: "x" denotes the absence of the compound and "√" denotes the presence of the compound along with the relative peak area in %.

2.3.2.6 Presence of different mineral elements

Humans require more than 22 mineral elements, all of which can be supplied by an appropriate diet. However, however improper diet plans very often lead to a deficiency of minerals such as iron, zinc, calcium, magnesium, copper, or selenium. Also other trace elements such as copper and zinc are essential for and human and animal nutrition [51]. The content of some of the mineral elements detected in the beers is shown in Table 2.6. The highest content in all the beers was that of Mg and it ranged in between 14.34 mg/L in MlB1 to 28.89 in AsB1. Na, K and Ca were also detected in good amount in all the beers. The content of Cu, Fe and Mn remained below 1 mg/L in all the beers, whereas content of Zn was in between 0.54 mg/L to 1.57 mg/L. The minerals in alcoholic beverages can be attributed to many factors, such natural sources including raw materials, soil, water and yeast, and to contamination during the making process, transport and storage. Their levels in beer can be a significant parameter affecting its consumption. They have certain beneficial effects on human body, e.g. Fe is an essential constituent of hemoglobin, myoglobin, and other enzymes, Zn, Mg and Cu are also essential for numerous enzymes and Cu is also a constituent of hair, bone, and other body organs [52]. However, high intake of any mineral may have negative effect on human health. Hence, in this study, the amount of elements detected in all the rice beers signifies a safe level for human consumption.

In similar studies, Pan et al. [52] analyzed trace elements in Chinese rice wine by ICP–OES and found them to be in the range of 0.088–0.106 mg/L for Co, 0.097–0.164 mg/L for Cr, 0.085–0.126 mg/L for Cu, 0.181–0.308 mg/L for Fe, 0.179–0.311 mg/L for Mg, 0.102–0.184 mg/L for Mn, 0.219–0.349 mg/L for Se, and 0.090–0.139 mg/L for Zn. He et al. [57] also studied the mineral content in North China rice wines and found that the amount of K was the largest (667.430 mg/kg) followed by Mg (305.578 mg/kg), Na (199.004 mg/kg), and Ca (167.231 mg/kg). Among the trace elements Fe (9.925 mg/kg) was found in the highest content.

Table 2.6 Mineral content in the rice beer samples

Sample	Concentration in mg/L							
	Cu	Na	Zn	K	Ca	Fe	Mg	Mn
AsB1	0.24	10.48	0.98	13.36	12.30	0.82	28.89	0.29
AsB2	0.14	11.89	1.31	12.39	5.47	0.53	25.62	0.18
AsB3	0.14	8.88	0.93	11.30	3.42	0.39	24.05	0.10
AsB4	0.12	8.98	0.93	13.08	3.92	0.56	26.28	0.24
AsB5	0.05	10.31	1.06	12.89	4.06	0.48	26.04	0.23
NaB1	0.15	12.87	1.57	13.37	6.06	1.42	25.52	0.23
NaB2	0.17	13.38	1.16	12.75	3.07	0.46	23.74	0.19
MlB1	0.00	13.82	0.54	8.09	2.76	0.25	14.34	0.07
ArB1	0.09	11.06	0.69	17.13	13.78	0.26	27.63	2.50

2.3.3 Effect of the microbial starters on the quality of rice beer

The various starter cakes (SC) used, their codes and the code names for the rice beers prepared in the laboratory are shown in Table 2.7.

Table 2.7 Various codes for the samples

Microbial starter cake (SC)		Rice beer prepared in laboratory (RB)
Local name	Code name	Code name
Angkur	AsS1	AsR1
Perok-kushi	AsS2	AsR2
Thap	AsS3	AsR3
Vekur-pitha	AsS4	AsR4
Apop-pitha	AsS5	AsR5
Umhu	NaS1	NaR1
Piazu	NaS2	NaR2
Siiyeh	ArS1	ArR1
Thiat	MeS1	MeR1

2.3.3.1 Physical properties of the SC

Some physical characteristics of the SC are shown in Table 2.8. The shape of six of the samples was similar i.e. round and flat, while three others were oval balls. Sample ArS1 was the largest, with a total volume of 142.15 cm^3 while sample AsS4 was the smallest with a

volume of 13.65 cm^3. The highest densities were observed in the oval shaped samples i.e. AsS5, AsS5 and MeS1 with values of 0.75, 0.74 and 0.70 g/cm^3 respectively with no significant difference (at $p<0.05$), while the densities of all the other samples varied significantly.

Significant differences in hardness, springiness, cohesiveness and gumminess was observed among most of the SC. AsS4 was found to be the softest with a value of 7.2 Kg force and AsS2 was the hardest with a value of 113.39 Kg force. AsS4 also had the least values of springiness, cohesiveness and gumminess with values of 0.23 mm, 0.02 and 0.15 Kg force respectively. NaS2 had the highest values of springiness (0.62 mm) and cohesiveness (0.30) and AsS2 had the highest values of gumminess (31.94 Kg force).

Table 2.8 Some physical characteristics of the microbial starter cakes

Sample code	Parameters						
	Shape	Total volume (cm^3)±SD	True density (g/cm^3)±SD	Hardness (Kg)±SD	Springiness (mm)±SD	Cohesiveness ± SD	Gumminess (Kg)±SD
NaS1	Round flat	$16.91±3.50^{ab}$	$0.53±0.01^{ab}$	$21.44±0.31^{c}$	$0.29±0.01^{b}$	$0.11±0.01^{d}$	$2.23±0.09^{c}$
NaS2	Round flat	$78.02±22.9^{e}$	$0.65±0.11^{cd}$	$36.57±0.15^{f}$	$0.62±0.02^{g}$	$0.30±0.02^{h}$	$11.02±0.45^{e}$
AsS1	Round flat	$35.66±2.51^{cd}$	$0.60±0.03^{bc}$	$73.34±0.30^{h}$	$0.34±0.01^{c}$	$0.19±0.01^{f}$	$13.38±0.78^{f}$
AsS2	Round flat	$33.63±2.54^{bcd}$	$0.55±0.07^{ab}$	$113.39±0.34^{i}$	$0.39±0.004^{d}$	$0.28±0.004^{g}$	$31.94±0.6^{g}$
AsS3	Round flat	$19.84±2.29^{abc}$	$0.74±0.01^{e}$	$72.44±0.41^{g}$	$0.43±0.02^{e}$	$0.09±0.01^{c}$	$7.04±0.72^{d}$
AsS4	Oval	$13.65±4.34^{a}$	$0.75±0.03^{e}$	$7.2±0.06^{a}$	$0.23±0.01^{a}$	$0.02±0.004^{a}$	$0.15±0.01^{a}$
AsS5	Oval	$24.89±3.68^{abcd}$	$0.74±0.01^{e}$	$27.83±0.28^{e}$	$0.40±0.004^{d}$	$0.02±0.001^{a}$	$0.57±0.04^{ab}$
ArS1	Round flat	$142.15±19.08^{f}$	$0.52±0.02^{a}$	$23.67±0.29^{d}$	$0.25±0.004^{a}$	$0.05±0.004^{b}$	$1.16±0.07^{b}$
MeS1	Oval	$40.08±5.69^{d}$	$0.70±0.03^{de}$	$18.31±0.18^{b}$	$0.47±0.02^{f}$	$0.13±0.004^{e}$	$2.38±0.13^{c}$

2.3.3.2 Colour in CIELAB expression

The results (Table 2.9) were expressed in Commission Internationale de I'EclairageL, a and b (CIELAB) systems. L indicates the degree of lightness or darkness (L=0 indicates perfect black and L=100 indicates most perfect white); “a” indicates degree of redness (+) and greenness (-) and “b” indicates degree of yellowness (+) and blueness (-).A direct correlation was observed in between the colour of the SC and that of the respective RB. Starter NaS1 with the highest value of L (74.97) produced the beer with the highest value of L (78.58), while starter AsS4 with the lowest value of L (50.08) also produced the beer with the lowest L value (0.83). Similarly, the starter AsS4 with an a value of 5.10 produced the beer AsR4 with an a value of 0.13 and the starter AsS3 with an a value of 1.02 produced the beer AsR3 with an a

value of 1.80. The same trend was also observed in case of b values with starter NaS1 (b = 15.25) producing beer NaR1 (b = 23.47) and starter AsS1 (b = 10.99) producing beer AsR1 (b = 1.21). Thus it was observed that the colour of the starters greatly influence the colour of the final product, even if added at a very small ratio. Since the colour further influences the sensory characteristics of the RB, a proper combination of SC and substrate is needed to be maintained.

Table 2.9 Colour of the microbial starter cakes and rice beer in CIELAB expression

Sample code	Colour					
	L±SD		a±SD		b±SD	
	SC	RB	SC	RB	SC	RB
NaS1/ NaR1	74.97 ± 2.20^{c}	78.58 ± 0.26^{h}	2.29 ± 0.25^{cd}	0.55 ± 0.04^{bc}	15.25 ± 0.63^{c}	23.47 ± 0.35^{f}
NaS2/NaR2	73.96 ± 6.65^{c}	2.07 ± 0.06^{d}	2.16 ± 0.69^{bcd}	0.70 ± 0.02^{bc}	14.82 ± 1.24^{c}	$2.65\pm0.06c^{d}$
AsS1/AsR1	59.62 ± 1.65^{b}	1.16 ± 0.04^{b}	1.91 ± 0.27^{bc}	0.57 ± 0.02^{bc}	10.99 ± 0.24^{a}	1.21 ± 0.17^{a}
AsS2/AsR2	52.46 ± 1.76^{a}	5.38 ± 0.10^{f}	3.12 ± 0.26^{f}	0.40 ± 0.02^{ab}	13.14 ± 0.43^{b}	2.91 ± 0.61^{d}
AsS3/AsR3	83.05 ± 0.92^{d}	1.99 ± 0.01^{d}	1.02 ± 0.16^{a}	0.13 ± 0.03^{a}	10.87 ± 0.43^{a}	2.26 ± 0.02^{c}
AsS4/AsR4	50.08 ± 5.74^{a}	0.83 ± 0.03^{a}	5.10 ± 0.63^{g}	1.80 ± 0.54^{d}	15.20 ± 1.03^{c}	1.53 ± 0.02^{ab}
AsS5/AsR5	51.18 ± 1.72^{a}	0.93 ± 0.05^{a}	1.45 ± 0.33^{ab}	0.67 ± 0.02^{bc}	13.28 ± 0.90^{b}	1.39 ± 0.01^{a}
ArS1/ArR1	72.08 ± 1.97^{c}	14.50 ± 0.07^{g}	2.88 ± 0.16^{df}	1.74 ± 0.05^{d}	15.19 ± 0.20^{c}	10.19 ± 0.18^{e}
MeS1/MeR1	62.67 ± 1.56^{b}	1.43 ± 0.04^{c}	2.14 ± 0.65^{bcd}	0.83 ± 0.03^{c}	12.38 ± 0.94^{b}	1.83 ± 0.03^{b}

2.3.3.3 Proximate composition

The proximate composition of the samples is given in Table 2.10. All the results have been expressed on wet basis. Low content of moisture was found in the SC and varied from 8.96 % (AsS4) to 10.55 % (NaS1). Except for NaS1, there were no significant differences among the other samples. Among the rice beers, the moisture content varied from 90.30 % (NaR1) to 98.50 % (ArR1).

The ash content ranged in between 0.43 % to 1.79 % for the SC and in between 0.02% and 0.37% for the rice beer. These results tally with the ash content of *Ou* (Thai rice wine) samples which have also been found to range in between 0.1 % to 0.3 % [46]. Other reported values of ash content on a dry matter basis in RB are 5.1 % [53] and 1.7 % [54]. Tamang and Sarkar (11) also studied various characteristics of *marcha* cakes and found them to contain 13 % w/w moisture and 0.7 % w/w ash (dry weight basis). The SC with the highest content of ash viz. AsS3, AsS4 and AsS5 were also responsible for producing the beer with the highest content of ash viz. ASR3, AsR4 and AsR5.

Crude fibre was present in varying content in all the starter and beer samples, with the samples NaS1 (2.48 %) and AsR5 (0.29 %) having the highest content. The content of fats was minimal in all the SC samples, however with significant differences. Among the RB samples, except AsR1 and MeR1 there was significant difference in fats content among all the other samples. NaR2 and AsR4 were found to have the highest content with 0.76 % and 0.86 % respectively. The fat content of *kodo ko jaanr* (fermented finger millet beverage) [53] was however found to be higher (2 % DM) than all the samples studied by us.

Table 2.10 Proximate composition of the microbial starter cakes and the prepared rice beer on wet basis

Sample code	Moisture (%)±SD		Ash (%)±SD		Crude fiber (%)±SD		Fats (%)±SD		Protein (%)±SD		Carbohydrates (%)±SD	
	SC	RB*	SC	RB	SC	RB	SC	RB	SC	RB	SC*	RB
NaS1/ NaR1	10.55 ±1.42^{a}	90.30	0.82 ±0.02^{b}	0.17 ±0.003^{e}	2.48 ±0.34^{de}	0.09 ±0.01^{ab}	1.27 ±0.63^{c}	0.06 ±0.01^{a}	4.35 ±0.21^{de}	0.90 ±0.05^{g}	80.53	8.47 ±1.75^{c}
NaS2/N aR2	13.17 ±1.06^{b}	97.19	0.77 ±0.05^{ab}	0.02 ±0.01^{a}	2.47 ±0.50^{de}	0.23 ±0.04^{d}	2.75 ±0.21^{d}	0.76 ±0.02^{g}	4.48 ±0.27^{def}	0.51 ±0.06^{e}	76.36	1.29 ±0.11^{a}
AsS1/A sR1	10.53 ±1.66^{a}	98.14	1.26 ±0.09^{c}	0.17 ±0.01^{e}	2.74 ±0.04^{e}	0.24 ±0.02^{d}	0.28 ±0.01^{ab}	0.36 ±0.02^{e}	4.26 ±0.12^{d}	0.45 ±0.05^{d}	80.93	0.63 ±0.18^{a}
AsS2/A sR2	10.08 ±0.77^{a}	98.42	0.93 ±0.52^{bc}	0.16 ±0.01^{d}	1.89 ±0.12^{bc}	0.31 ±0.03^{f}	1.29 ±0.07^{c}	0.11 ±0.02^{b}	4.81 ±0.34^{f}	0.47 ±0.04^{d}	81.00	0.83 ±0.05^{a}
AsS3/A sR3	9.83 ±0.65^{a}	97.84	1.79 ±0.17^{d}	0.24 ±0.02^{f}	2.23 ±0.08^{cd}	0.18 ±0.01^{c}	0.28 ±0.07^{ab}	0.16 ±0.02^{c}	2.59 ±0.11^{b}	0.77 ±0.06^{f}	83.28	0.80 ±0.04^{a}
AsS4/A sR4	8.96 ±1.89^{a}	93.68	1.76 ±0.16^{d}	0.33 ±0.01^{g}	1.53 ±0.09^{b}	0.12 ±0.03^{b}	0.23 ±0.02^{a}	0.86 ±0.02^{h}	1.90 ±0.27^{a}	1.01 ±0.09^{h}	85.62	4.00 ±0.09^{b}
AsS5/A sR5	9.56 ±1.45^{a}	97.58	1.78 ±0.13^{d}	0.37 ±0.01^{h}	1.46 ±0.39^{b}	0.29 ±0.02^{ef}	0.63 ±0.04^{b}	0.43 ±0.02^{f}	1.65 ±0.07^{a}	0.35 ±0.03^{c}	84.92	0.98 ±0.08^{a}
ArS1/A rR1	9.91 ±2.02^{a}	98.50	0.43 ±0.12^{a}	0.14 ±0.01^{c}	0.79 ±0.25^{a}	0.06 ±0.02^{a}	0.24 ±0.03^{a}	0.32 ±0.02^{d}	3.68 ±0.09^{c}	0.28 ±0.04^{b}	84.95	0.70 ±0.02^{a}
MeS1/ MeR1	9.02 ±0.91^{a}	97.69	1.75 ±0.12^{d}	0.04 ±0.01^{b}	1.84 ±0.18^{bc}	0.27 ±0.02^{de}	0.47 ±0.09^{ab}	0.37 ±0.01^{e}	4.68 ±0.02^{ef}	0.25 ±0.03^{a}	82.24	1.37 ±0.34^{a}

* Calculated after subtracting the sum of other compositions from 100

Protein was found to be present in all the SC and there was significant difference in its content among most of the samples. The highest content was found in MeS1 (4.68 %) and AsS5 (1.65 %) had the lowest content. In the RB samples, protein was found to be present in the range of 0.25 % (MeR1) to 1.01 % (AsR4). There was no significant difference among NaR2, AsR1 and AsR2, and among AsR5, ArR1 and MeR1. However, no correlation was observed in the protein content of the SC and the RB produced from them. The results can be corroborated to the protein content of *Ou* samples which vary from 0.45 to 0.99 % [46]. The protein content of similar products reported on a dry basis was 9.5 % [54] and 9.3 % [53].

Since rice, a starch rich substrate is the major constituent of both the SC and the RB, high content of carbohydrates was found in the SC as well as RB. Carbohydrate was the major constituent in the starters and AsS4 (85.62 %) was found to have the highest content, while NaS2 (76.36 %) had the lowest content. In case of RB, NaR1 had the highest content of carbohydrates with 8.47 %, followed by AsR4 with 4.0 %. All the other RB samples had concentration in the range of 0.70 % - 1.37 % and showed no significant difference.

2.3.3.4 Biochemical attributes of the RB

The values of some of the biochemical attributes of the RB samples are shown in Table 2.11. These readings were helpful in understanding the general quality aspects of the rice beer from this region.

The pH of all the samples was found to be low. AsR1, AsR3 and AsR4 showed no significant difference in their pH.MeR1 had the lowest value (pH 3.35), whereas ArR1 had the highest (pH 5.11). The low pH has been reported in other types of beer like *jutho* (pH 3.6) [10], *kodo ko jaanr* (pH 4.1) [43], *poko* (pH 3.2 – 3.0) [3]. The pH of different varieties of *tapuy* (Philippine rice beer) has also been found to range in between 4.6 to 5.0 [55]. The total acidity of the samples was expressed as % of lactic acid. There was no significant difference among the samples NaR2, AsR1 and AsR5, in between NaR1 and AsR2 and in between NaR2 and AsR5. The highest value (0.77 %) was found in MeR1 and lowest (0.33 %) in ArR1. The acidity values were in line with *yakju* (Korean rice beer) brewed with different wild type yeast strains. [56] The values were however more than that of *bhaati jaanr* during whose fermentation the titrable acidity was found to increase from 0.01 % to 0.2 % till the 4th day, and remained at a level of 0.17 % till the end [54].

All the samples had similar alcohol by weight content within the range of 3.93 - 4.39 % and there was very little significant difference. The alcohol contents were found to be similar to that of *zutho* [10], *bhaati* jaanr [54] and *kodo ko jaanr* [53] which were 5.0 %, 5.9 % and 4.8 % respectively, and more than that of *poko* [3] which had1.0-1.6 %. This content was less than that found in samples of *yakju* of Korea [56], *Ou* of Thailand [46] and *tapuy* of Philippines [55]. There was significant difference in the TSS value of all the samples except in between AsR2 and AsR3. The values of total titritable acidity, pH, alcohol were similar with the findings of Akpinar-Bayizit et al. [57], who studied the changes in total titritable acidity, pH, alcohol, organic acid profiles and sensory properties during the fermentation of boza, an alcoholic beverage produced from rice, maize, millet and wheat flours in Turkey.

The TSS value of the samples ranged from a minimum of 2.63 ^{o}Bx in MeR1 to a maximum of 16.27 ^{o}Bx in NaR1. NaR1 had the highest content of reducing sugars with 3.47 %, followed by AsR4 (1.67 %). All the other samples had concentration in the range of 0.20 % - 0.32 % and showed no significant difference. The concentration of reducing sugars in samples of *tapuy* has been reported to be in between 0.07 % - 0.21 % [55] and that in *zutho* to be 6.3 mg/ml) [10].

Table 2.11 Biochemical attributes of the rice beer prepared under laboratory condition

Sample code	Parameters								
	pH ±SD	Acidity (%)±SD	Alcohol (%)±SD	TSS (^{0}Bx)±SD	RS (g/100g) ±SD	Starch (g/100g) ±SD	Amylose (g/100g) ± SD	TPC (mg/100g) ±SD	RSA (%)±SD
NaR1	4.06 ±0.01^{c}	0.47 ±0.01^{c}	3.93 ±0.01^{a}	16.27 ±0.06^{i}	3.47 ±0.19^{c}	0.85 ±0.04^{bc}	0.48 ±0.05^{a}	10.06 ±0.18^{g}	45.28 ±0.61^{a}
NaR2	4.63 ±0.01^{f}	0.50 ±0.01^{d}	4.24 ±0.06^{b}	3.20 ±0.10^{b}	0.21 ±0.01^{a}	1.38 ±0.08^{e}	0.52 ±0.05^{a}	1.83 ±0.15^{b}	81.11 ±1.51^{e}
AsR1	4.28 ±0.01^{e}	0.50 ±0.01^{d}	4.30 ±0.02^{b}	6.20 ±0.50^{g}	0.25 ±0.07^{a}	1.07 ±0.02^{d}	0.52 ±0.05^{a}	2.19 ±0.20^{c}	90.95 ±0.39^{f}
AsR2	4.72 ±0.01^{g}	0.40 ±0.01^{b}	4.26 ±0.05^{b}	4.03 ±0.06^{d}	0.23 ±0.02^{a}	1.07 ±0.12^{d}	0.55 ±0.05^{ab}	2.00 ±0.02^{bc}	90.29 ±0.54^{f}
AsR3	4.23 ±0.01^{d}	0.75 ±0.01^{f}	4.39 ±0.36^{b}	4.27 ±0.12^{e}	0.32 ±0.13^{a}	0.88 ±0.03^{c}	0.82 ±0.06^{cd}	5.05 ±0.002^{f}	69.93 ±0.68^{d}
AsR4	4.27 ±0.01^{e}	0.75 ±0.01^{f}	4.00 ±0.01^{a}	13.40 ±0.10^{h}	1.67 ±0.07^{b}	0.94 ±0.05^{c}	0.69 ±0.07^{bc}	4.71 ±0.02^{e}	63.70 ±2.49^{c}
AsR5	3.60 ±0.01^{b}	0.58 ±0.01^{e}	4.37 ±0.02^{b}	3.40 ±0.23^{c}	0.20 ±0.05^{a}	1.36 ±0.05^{e}	0.68 ±0.07^{bc}	0.93 ±0.08^{a}	47.21 ±2.93^{a}
ArR1	5.07 ±0.01^{h}	0.32 ±0.01^{a}	4.35 ±0.02^{b}	6.03 ±0.06^{f}	0.21 ±0.01^{a}	0.76 ±0.04^{ab}	1.21 ±0.05^{e}	2.62 ±0.13^{d}	56.89 ±2.57^{b}
MeR1	3.35 ±0.01^{a}	0.76 ±0.02^{f}	4.35 ±0.03^{b}	2.63 ±0.06^{a}	0.20 ±0.004^{a}	0.74 ±0.05^{a}	0.84 ±0.04^{d}	0.91 ±0.04^{a}	44.38 ±0.41^{a}

Note: TSS: Total Soluble Solids, RS: Reducing Sugars, RSA: Radical Scavenging Activity

Starch content ranged in between 0.74 g/100g (MeR1) and 1.38 g/100g (NaR2) and there was less significant difference among the samples. The amylose content also varied depending on the content of starch. The partial sweetness of the products is explained by the presence of carbohydrates, especially the reducing sugars in them. The presence of starch and amylose in the final product indicates the partial conversion of starch to sugars and it is also due to the straining practice followed instead of proper filtration.

The TPC and the antioxidant activity of the samples are presented in Table 2.11. The sample NaR1 had the highest content of polyphenols (10.06 mg/100g) followed by AsR3 (5.05 mg/100g) and AsR4 (4.71 mg/100g) and there was significant difference among most of the samples. All the samples showed moderate to high 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity (RSA). Both AsR1 and AsR2 showed the highest activity with

values of 90.95 % and 90.29 % RSA respectively. The RSA of other samples varied and remained in the range of 44.38 % RSA (MeR1) to 81.11 % RSA (NaR2). The presence of polyphenols may account for the high antioxidant activity exhibited by most of the samples, which in turn includes health benefits such as prevention of diseases like cancer and coronary heart disease. The antioxidant property may also be attributed to the various plants used in preparing the SC. High content of polyphenols and antioxidant activity was also observed by Zujko and Witkowska [58] in different type of wines and beer and concluded that the antioxidant potential of the foods tested was related to the total polyphenol contents.

2.3.3.5 Microbiological profile of the samples

The count of five different groups of microbes viz. plate counts, LAB, yeasts, moulds and *Staphylococcus* sp. observed in the SC samples is given in Table 2.12. All the analyses were done in three replicates and the mean values were taken.

The plate count of heterotrophic bacteria includes all bacteria that use organic nutrients for growth. They are present in all types of water, food, soil, vegetation, and air. They were found in high numbers in both the SC and the RB samples. In case of SC there was no much significant variation in their counts. NsS2 (8.58 log CFU g^{-1}) had the highest count, while AsS5 (5.03 log CFU g^{-1}) had the lowest count. However, significant variation in counts was observed in case of the RB samples, with AsR1 (10.28 log CFU g^{-1}) having the highest count and AsR3 (2.54 log CFU g^{-1}) with the lowest count. Similar count of mesophilic aerobes in fermented poko, a rice based fermented food of Nepal have been reported by Shrestha et al. [3]. They found the count to start from $7.9x10^7$ CFU g^{-1} on the first day of fermentation and decrease to a count of $1x10^7$ CFU g^{-1} on the fifth day. Thapa and Tamang [53] have also reported the total count of aerobes in *kodo ko jaanr*, a fermented finger millet beverage to be around 7.4 log CFU g^{-1}.

Lactic acid bacteria (LAB) are a group of fermentative bacteria and are abundant in nutrient rich environments where carbohydrates and proteins are usually present. They have remarkable selective advantages in diverse ecological niches due to the efficient use of nutrients and the production of lactic acid during growth [59]. The LAB were found to be present in all the samples in considerable high number and their count varied significantly in all the samples studied. Among the SC, NaS2 had the highest numbers with a count of 7.76 log CFU g^{-1}, while lowest numbers were found in AsS5 with a count of 3.71 log CFU g^{-1}. Tamang et al. [60] also reported the average population of LAB in *hamei* (starter cake used in Manipur,

India) to be log 6.9 CFU g^{-1} and *marcha* (starter cake used in Sikkim, India) to be log 7.1 CFU g^{-1}. The isolates from *hamei* were identified as *Lactobacillus plantarum* and that from *marcha* as *Lactobacillus brevis.* Among the RB samples, NaR2 had the maximum population with a count of 7.55 log CFU g^{-1} and AsR3 had the minimum with a count of 4.32 log CFU g^{-1}. Similar results were obtained by Thapa and Tamang [53], who found the count of LAB in *kodo ko jaanr* (fermented finger millet beverage) to range from 4.1 to 6.5 log CFU g^{-1}. Shrestha et al.[3] also found the population of LAB in the fermentation mixture of *poko* (fermented rice product) to increase from an initial value of $3.5x10^6$ CFU g^{-1} on day 1 to a value of $5x10^7$ CFU g^{-1} on day 5. The dominance of lactic acid bacteria (LAB) in fermented products results in the inhibition of the growth of pathogens and spoilage microbes [61] and most of the LAB are also probiotic in nature [62], which adds to the uniqueness of this type of beer.

Table 2.12: Microbiological profile of the samples

Sample code	Log CFU g-1±SD									
	Plate counts		LAB		Yeasts		Moulds		*Staphylococcus* sp.	
	SC	RB	SC	RB	SC	RB	SC	RB	SC	RB
NaS1/ NaR1	8.32 ±0.16^{bc}	9.51 ±0.03^{f}	7.56 ±0.29^{e}	5.43 ±0.03^{c}	7.14 ±0.70^{c}	6.55 ±0.06^{b}	5.72 ±0.50^{bc}	0.0^{a}	0.0^{a}	0.0^{a}
NaS2/NaR2	8.58 ±1.19^{c}	7.28 ±0.09^{c}	7.76 ±0.14^{e}	7.55 ±0.03^{i}	8.78 ±0.76^{f}	8.34 ±0.08^{h}	5.82 ±0.81b^{c}	0.0^{a}	0.0^{a}	4.30 ±0.21^{d}
AsS1/AsR1	6.28 ±0.75a^{b}	10.28 ±0.12^{g}	7.29 ±0.36^{e}	6.81 ±0.03^{f}	8.39 ±0.40^{ef}	7.73 ±0.03^{f}	7.67 ±0.26^{d}	0.0^{a}	0.0^{a}	2.27 ±0.07^{b}
AsS2/AsR2	6.83 ±1.87 ^{abc}	9.36 ±0.11^{f}	6.51 ±0.29^{d}	7.15 ±0.05^{h}	3.11 ±0.10^{a}	8.32 ±0.08^{h}	6.46 ±0.35^{c}	0.0^{a}	0.0^{a}	4.81 ±0.14^{e}
AsS3/AsR3	6.82 ±0.59 ^{abc}	2.54 ±0.12^{a}	6.68 ±0.39^{d}	4.32 ±0.07^{a}	7.77 ±0.52^{cde}	7.24 ±0.05^{d}	7.58 ±0.55^{d}	0.0^{a}	0.0^{a}	0.0^{a}
AsS4/AsR4	6.43 ±1.14a^{bc}	8.49 ±0.19^{e}	5.62 ±0.45^{c}	4.61 ±0.12^{b}	4.63 ±0.42^{b}	6.68 ±0.05^{c}	5.26 ±0.35^{b}	0.0^{a}	0.0^{a}	0.0^{a}
AsS5/AsR5	5.03 ±0.85^{a}	7.40 ±0.23^{c}	3.71 ±0.09^{a}	6.92 ±0.03^{g}	3.33 ±0.34^{a}	7.56 ±0.04^{e}	4.15 ±0.26^{a}	0.0^{a}	0.0^{a}	3.19 ±0.10^{c}
ArS1/ArR1	7.21 ±1.50^{abc}	6.72 ±0.15^{b}	4.62 ±0.28^{b}	6.06 ±0.05^{d}	8.2 3±0.40^{def}	6.43 ±0.08^{a}	5.49 ±0.33^{b}	0.0^{a}	0.0^{a}	0.0^{a}
MeS1/MeR1	6.44 ±0.3^{abc}	7.79 ±0.02^{d}	6.62 ±0.25^{d}	6.53 ±0.06^{e}	7.47 ±0.45c^{d}	8.05 ±0.04^{g}	5.35 ±0.93^{b}	0.0^{a}	0.0^{a}	0.0^{a}

Amylolytic yeasts and moulds accomplish starch hydrolysis and fermentation in a wide range of traditional alcoholic foods and beverages [63]. Yeasts were the dominant microbes in all the samples. There was significant difference in their count among the SC samples and varied from 3.11 log CFU g^{-1} in AsS2 to 8.78 log CFU g^{-1} in NaS2. In case of the RB samples, there was significant difference in their count except NaR2 and AsR2. Their counts ranged from 6.43 log CFU g^{-1} in ArR1 to 8.34 log CFU g^{-1} in NaR2. The count of yeasts in *bhaati jaanr*, which is a type of rice beer made in the Eastern Himalayas was found to increase from 10^5 CFU g^{-1} on day 1 of fermentation to 10^8 CFU g^{-1} on day 2, and then gradually decreased to

level of 10^5 CFU g^{-1} on day [54]. Shrestha et al. [3] have also found an increase in the population of yeasts from $1.8x10^6$ to $1.3x10^8$ CFU g^{-1} from the first to the fifth day of fermentation of *poko.* The presence of yeasts in considerable high counts in all the samples also confirms that they are the primary organisms responsible for the alcoholic fermentation of RB. The count of moulds in the SC was more consistent among all and remained in the range of 4.15 log CFU g^{-1} to 7.67 CFU g^{-1}. The moulds were however found to be absent from all the RB samples. The mucorales have roles (amylolytic or proteolytic enzyme activities) in the initial phase of fermentation; mostly in saccharification and their disappearance from the final product have been reported by others [53,54].

Jeyaram et al. [1] studied the fungal species associated with *hamei* and found yeasts in the range of 8-9 log CFU g^{-1} and moulds from 5-7 log CFU g^{-1}. The population of LAB and yeasts in *makgeolli*, a naturally fermented Korean rice beer was studied by Yoon [64]. They found that *Saccharomyces cerevisiae* was predominant in the samples with an average count of 4.6×10^7 CFU/ml. Whereas, *Lactobacillus plantarum* and *Weissella cibaria* were the predominant LAB species with an average count of 1.7×10^7 CFU/ml.

Staphylococcus species were absent from all the SC samples but were present in four of the RB samples viz. NaR2, AsR1, AsR2 and AsR5 in counts ranging from 2.27 to 4.95 log CFU g^{-1} . Their count in all the four samples differed significantly. The most possible source for this group of microbes in four of the rice beer may be the air. However, the presence of S*taphylococcus* species is not of much concern as most of them are harmless and reside normally on the skin and mucous membranes of organisms [65]. Members of the common food contaminating groups, viz. Enterobacteriaceae, *Salmonella* and *Shigella* species and *Bacillus* sp. were not detected in any of the samples. The absence of such contaminants may be attributed to the low pH and high acidity of the products and also the antagonistic effect of the LAB group [59].

2.4 Conclusion

It was observed that the process of rice beer preparation followed by different ethnic tribes residing in different states of North-East India is more or less similar. The only difference is the ingredient in the form of different parts of various plants species. The tribes in different regions use different plant species based on their availability. The knowledge of the indigenous people in the use of the starter cultures as a source of yeast is very interesting. The local brews such as rice beer bears very significant resemblance of the culture and traditions of the tribal

people residing in this part of the country. Each of the beverages prepared is rooted with the socio-cultural practices of the individual tribes and also on various environmental factors. It has been found that the preparation of rice beer is considered as sacred by all the tribes and it occupies special recognitions in many of the occasions like rituals, festivals, marriages and communal gathering. The consumption of mild amount of alcohol in the form of rice beer gives some relaxation to the hard working population of these states and practically has no side effect on their health. Apart from imparting colour, flavour and sweetness to the beer, the various plants used in the starter culture are also said to have many medicinal properties. Also some of the plant extracts may also provide certain nutrients for the survival of the microflora present in the starter cakes. The quality of the starter culture is said to be dependent on the variety of plant parts used and also on the maintenance of proper sanitary conditions. The preference of the variety of rice used for fermentation also differs from communities to communities. However, it is seen that glutinous rice is preferred more than non-glutinous rice, owing to the taste and alcohol content of the product.

The study revealed that all the rice beer samples collected from the Northeastern states of India are a potential source of nutrition owing to their various biochemical compositions like carbohydrates, amino acids, organic acids, aromatic compounds etc at appreciable amounts. The average consumption of rice beer by tribal communities of Northeast India is around 2 glasses (400 mL approx.) in the evening for 3 to 4 days in a week and no any health complication related to the consumption of rice beer has been reported. The level for consumption of alcohol also varies among individuals. The results revealed that the average content of alcohol is around 4.5 % which is less as compared to 5-10 % (avg. 7.5 %) in malt beers. Therefore, consumption of rice beer at this level could be considered as safe for human health. This level is also within the limit issued by the World Health Organization (WHO). Their guidelines define one unit of alcohol as the equivalent of 8g of ethanol and the "responsible" or "low", level of risk for men as "3 units per day and 21 per week" and for women as "2 units per day and 14 per week" spread throughout the week (including 2 alcohol free days per week). The presence of alcoholic groups such as phenylethyl alcohol and some other esters contributed to the appealing flavour and smell of all studied varieties of rice beer. Out of all samples, the AsB4 variety (locally called *Xaj – pani*, collected from Sibsagar district of Assam) prepared by the Ahom community showed relatively higher content of lactic acid, carbohydrates like glucose, trehalose and galactose and almost all the amino acids as compared to the other samples. In addition, the alcohol content was also in medium range and this sample might be considered relatively more nutritious as compared to the others.

It was also observed that, even though used for the same purpose, differences in terms of physical, chemical and microbial properties were observed among the various starter cakes. Under the same conditions of fermentation and substrate type, there was significant difference in quality among all the types of rice beer and their quality was found to be affected by the type of starter cake. The plausible reason for this variation might be attributed to differences in the plants and rice used in preparing the starters, their ratio and also the differences in the microbial consortium. A direct correlation between the colour of the starters and the rice beer was observed. The low moisture in the starters contributes to their shelf life and the presence of ash (viz. minerals), protein, and fats in minimal amount in the rice beer makes this kind of product a balanced nutritional drink. The low pH and high acidity may prove beneficial in controlling the growth of spoilage microbes. Also phenolic compounds were found in all the rice beers and these may contribute to the high antioxidant activity exhibited by most of them. The load of microbes belonging to the LAB group was also high in both the starters and the rice beer, and these may act as potential probiotic organisms present in the drink.

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