Biochemical and nutritional analysis of rice beer of North East India

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Biochemical properties of five different rice varieties of North East India and a starter culture cake (ASC1) collected from Arunachal Pradesh used in the process of rice beer preparation were analyzed. Further, microbial count of the starter culture cake and nutritional composition of the five laboratory prepared rice beer samples were evaluated. The crude protein, amylose, amylopectin, moisture, crude fat, starch, total soluble sugar, reducing sugar and non reducing sugar contents of rice varieties were estimated. Various biochemical parameters of ASC1 were also determined. Increase with time in the total acidity, volatile acidity, and alcohol % of rice beer was observed. The final recorded alcohol % value of rice beer was in the range of 12-13%. Minerals like calcium, sodium, potassium, iron and phosphorous were estimated with phosphorous having the highest content of 78.108 mg/100 ml. Crude and soluble protein, and vitamin C contents were also analyzed. The antiradical activity (Inhibition %) of the rice beer samples was in the range of 2.479-22.31%. Saccharomyces cerevisiae isolated from ASC1 was found to be the major organism responsible for rice beer fermentation. Highest CFU count of 19 was observed in MRSA medium which also indicates the prevalence of LAB in ASC1.

Keywords: Rice beer, Starter culture, Nutritional composition, Minerals, Saccharomyces cerevisiae

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Rice beer is a traditional alcoholic beverage that plays a major role in the socio-cultural lives of people of North East India. Various ethnic tribes of North East India consume rice beer on a regular basis in different forms and it is believed to possess many therapeutic and medicinal properties1, 2. These products are similar to shaosingiju and laochao of China, sake of Japan, chongju and takju of Korea, brem bali, tape-ketan and tapuy of Indonesia, khaomak of Thailand and tapai pulul of Malaysia3. Rice beer is prepared from rice through fermentation process using yeast and various plant materials. Raw material and microbes involved in the process have wide variability in different places. Literature recorded extensive study on documentation of processing methods of rice beer and starter culture but research on nutritional and medicinal properties of rice beer is still in its infancy. Detailed studies on the nutritive and medicinal values of these products can provide valuable information would prove beneficial in the use of these products on a wider scale4. The present study was undertaken to assess the major nutritional and biochemical parameters for rice varieties and beer samples prepared in optimum laboratory conditions using rice varieties collected from five places and a starter culture cake obtained from Arunachal Pradesh, India in order to explore the possible potential of rice beer as a beneficial fermented beverage. Further the study was also designed to analyze biochemical parameters and microbial count of the starter culture to standardize the production process for universal acceptance.

Methodology

Rice varieties were collected from Assam (AS1), Arunachal Pradesh (AP1, AP2) Nagaland (NL1) and Meghalaya (ML1) states of North East India. Starter culture cake (ASC1) was also collected from Arunachal Pradesh to produce rice beer in the laboratory. The rice samples and starter culture cake were ground to a fine powder with the help of an electrical grinder and stored in desiccators for
analysis. The powdered dry samples were used to determine chemical constituents.

Biochemical analysis of rice varieties and starter culture

**Proximate composition**

Moisture, crude fat, ash contents of collected rice varieties and the starter culture cake were determined by applying the standard method. The total nitrogen content was estimated by Micro-Kjeldahl method and crude protein was calculated by multiplying the total N by 6.25. Crude fibre content was determined using the method as described earlier.

Total soluble sugar was determined by using Anthrone method. The reducing sugar was estimated by standard biochemical method using 3,5-di-nitrosalisylic acid (DNS) reagent with slight modification. The non-reducing sugar content was calculated by subtracting the percentage of reducing sugar from the percentage of total soluble sugar. The starch contents in the rice varieties and the starter culture were estimated by the method as described earlier. The amylose content was determined by standard method and the amylopectin was calculated by subtracting the percentage of the amylose from 100 on moisture free basis.

**Rice fermentation under standardized parameters in laboratory**

Collected Rice varieties were used in the laboratory scale production of rice beer, whereas only one type of starter culture was used during this process. Fifty gm of selected rice and 60 mL of distilled water in a 250 mL conical flask covered by a cotton plug are soaked for 4 hrs at 25°C. After soaking, the rice is steamed in an autoclave for 1hr at 100°C. The gelatinized rice was cooled to 35-40°C, then inoculated with 2 gm of starter culture and mixed. After solid state fermentation for 2 days at 30°C, 70 mL of sterile water was added to the moulded mass to facilitate submerged alcoholic fermentation for 3 days at 30°C under a water lock.

Biochemical analysis of rice beer

**pH, total acidity, volatile acidity and alcohol percentage**

pH of the sample was measured by a pH meter (Eutech Instruments) equipped with glass electrode. Total acidity and volatile acidity are expressed as % of tartaric acid in given sample and volatile acidity as % of acetic acid present in the sample. These were determined by titration test of fermenting rice beer. Alcohol percentage was determined by the specific gravity. Changes in pH, total acidity, volatile acidity and alcohol content of the produced rice beer were analyzed for a period of 5 days.

**Crude protein and soluble protein content**

Crude protein was estimated by Micro-Kjeldahl method. Percentage nitrogen was converted to crude protein and results were expressed in gram crude protein per 100 ml of liquid matter. The soluble protein contents in five different rice beer samples were estimated by using Lowry’s method.

**Minerals and vitamin**

Quantitative analysis of different minerals like Na, K and Ca was done by using Flame photometric analysis. Stock solutions of calcium having 200 ppm, sodium and potassium of 20ppm were prepared by dissolving 0.5 gm of CaCO3, 0.05082 gm of NaCl and 0.03812 gm of KCl in 1000 mL distilled water, respectively. A few drops of (1:1) HCl were added to the dissolved the CaCO3. For calcium estimation, this stock solution, another three solutions having concentration 75 ppm, 100 ppm and 150 ppm were prepared by appropriate dilution. For sodium and potassium estimation, 5 ppm, 10 ppm and 15 ppm concentrations were prepared. The solutions were then placed under Nebuliser and the intensity of emitted lights was measured and the readings were taken in a digital read out. The curve was drawn by plotting concentration versus intensity of emitted light, which will be straight line. The rice beer samples were diluted 100 times by adding 19.8 mL of distilled water to 0.2 mL of samples. Then they were placed under the Nebuliser and readings were taken. The concentrations of calcium, sodium and potassium in the samples were calculated from the standard curve. Iron and phosphorous concentrations were determined by using the standard methods.

Ascorbic acid or Vitamin C content of the rice beer samples were determined by using volumetric method.

**Total antioxidant capacity**

Total antioxidant capacity of the rice beer samples was estimated by using DPPH method as described
earlier with little modification. The samples were centrifuged at 10000 rpm for 15 minutes. 100 µl of aliquot of the supernatants of rice beer samples were taken in a test tubes and 2.9 mL of DPPH reagent (0.5 mM in 99.5% of methanol) was added to each tubes and vortexed vigorously. Then the tubes were incubated in dark for 30 min at room temperature. The discoloration of DPPH was measured against blank at 517nm. DPPH methanolic solution was used as blank.

**Microbial analysis of starter culture cake**

Serial dilution technique and spread plate method were employed to enumerate the microorganisms present in ASC1. Different media like Potato Dextrose Agar (PDA), Yeast Extract Agar (YEA), Nutrient Agar (NA), Plate Count Agar (PCA), MRS Agar (MRSA), Rose Bengal Chloramphenicol Agar (RBCA) were used. Inoculation was carried out using 50 µL of each of the 3 selected dilutions, in duplicate, pipette onto the surface of plates and spread using sterile L-spreader. The plates were then incubated at 27°C and 37°C for 24hrs.

**Results and discussion**

**Biochemical composition of rice varieties**

Rice varieties were found to have crude protein content of 5.833-9.428%. Amylose and amylopectin contents ranged from 1.03-12.30% and 87.7-98.97%, respectively. AS1 had the lowest amylose and highest amylopectin contents of 1.833% and 98.97%, respectively. Many communities in North East India prepare high class rice beer out of bora rice which has high amount of amylopectin content. Generally, food items prepared from high amylopectin rice are cooked easily and preserved for longer time. NL1 showed the highest crude protein content of 9.428% whereas ML1 showed the lowest value of 5.833. The moisture, crude fat, starch, total soluble sugar, reducing sugar and non reducing sugar contents were in the range of 10.63-12.24%, 0.72-2.85%, 68.34-76.38 gm/100gm, 0.87-1.539 gm/100 gm, 0.087-0.703 gm/100 gm, 0.224-0.922 gm/100 gm, respectively (Table 1).

**Biochemical composition of starter culture cake**

ASC1 had amylopectin and amyllose contents of 77.05 and 22.95%, respectively. The starter culture cake was prepared from non glutinous rice. The proximate composition of ASC1 is represented in the (Table 2).

**Biochemical composition of rice beer**

The changes in the various parameters in the laboratory prepared rice beer were analyzed for a period of 5 days under optimum laboratory conditions. The fresh rice beer samples were found to have total acidity value in the range of 0.225-0.637 (% tartaric acid), whereas the five days old rice beer samples showed the values in the range of 0.750-0.975 (Fig. 1). This indicates that the total acidity increases as the fermentation proceeds. Similarly, volatile acidity values were also found to be in increasing order from day 1 to day 5 in all the rice beer samples. The significant increase in volatile acidity value in ML1 was observed from 0.180-0.780 in 5 days (Fig. 2). The pH values were in an acidic range of <4.0 which inhibits the growth of Coliforms and other members of the Enterobacteriaceae. As observed in the fermentation of other indigenous Ethiopian fermented beverages, the Enterobacteriaceae, could participate in the initiation of fermentation and subsequent acid formation, thus reducing the pH until the more acidic forms, the LAB and yeasts, took over. AR2 showed the lowest pH of 3.43 on day 1, while AR1 was found to have lowest pH of 3.45 on day 5 (Fig. 3). Significant increase in the percentage of alcohol (v/v) was noticed from day 1 to day 5. The alcohol content on day 1 was in the range of 1.10-2.28%, while on day 5 it was 10.05-11.28% (Fig. 4).

The alcohol contents of the three months old samples were in the range of 12-13% (v/v) which is significantly higher than the one day old fresh samples. AS1 showed the highest alcohol content of 13% (Table 3).

The calcium, sodium, potassium, iron and phosphorous contents of the samples after 3 months of storage were found in the range of 10.93-34.37 mg/100mL,1.27-3.49mg/100mL, 4.76-9.63mg/100mL, respectively. AP1 had relatively higher phosphorous content of 78.108mg/100mL and AS1 had highest iron content of 3.069 mg/100mL than the other samples. The crude and soluble protein contents of the samples were found in the range of 1.85-2.51 % and 1.163-1.675 mg/100mL, respectively, AS1 showing the highest values (Table 3).

The Vitamin C content of the sample was in the range of 13.332-39.976 mg/100ml with AP2 showing the highest value (Fig. 5).
The antiradical activity values were in the range of 2.479-22.31%, AP1 and AP2 showing the lowest and highest values, respectively (Fig. 6). An antioxidant inhibits the oxidation of lipid or other molecules providing protection against Reactive Oxygen Species. The antioxidant activity of the rice beer samples was most likely to be contributed by the presence of phenolic acids, polyphenols and flavonoids from various indigenous herbs used in the preparation of the starter culture cake. These compounds are known to inhibit the oxidative mechanisms which are responsible for many disorders and diseases in humans such as infections, diabetes, arthritis, cardiovascular diseases, cancer, Alzheimer's diseases, AIDS, etc.

Table 1—Biochemical composition of collected rice varieties

<table>
<thead>
<tr>
<th>Rice Sample Code</th>
<th>Moisture %</th>
<th>Crude fat %</th>
<th>Crude protein %</th>
<th>Amylose %</th>
<th>Amylopectin %</th>
<th>Starch %</th>
<th>Total soluble sugar %</th>
<th>Reducing sugar gm/100gm</th>
<th>Non reducing sugar gm/100gm</th>
</tr>
</thead>
<tbody>
<tr>
<td>AP1</td>
<td>10.94</td>
<td>2.85</td>
<td>5.91</td>
<td>3.42</td>
<td>96.58</td>
<td>68.34</td>
<td>1.539</td>
<td>0.703</td>
<td>0.836</td>
</tr>
<tr>
<td>AP2</td>
<td>12.24</td>
<td>1.0</td>
<td>7.581</td>
<td>3.72</td>
<td>96.28</td>
<td>75.39</td>
<td>1.114</td>
<td>0.344</td>
<td>0.77</td>
</tr>
<tr>
<td>NL1</td>
<td>11.64</td>
<td>1.16</td>
<td>9.428</td>
<td>12.30</td>
<td>87.7</td>
<td>76.38</td>
<td>0.87</td>
<td>0.087</td>
<td>0.783</td>
</tr>
<tr>
<td>ML1</td>
<td>10.63</td>
<td>0.72</td>
<td>5.833</td>
<td>2.68</td>
<td>97.32</td>
<td>71.13</td>
<td>0.914</td>
<td>0.690</td>
<td>0.224</td>
</tr>
<tr>
<td>AS1</td>
<td>11.54</td>
<td>1.20</td>
<td>6.32</td>
<td>1.03</td>
<td>98.97</td>
<td>71.29</td>
<td>1.06</td>
<td>0.138</td>
<td>0.922</td>
</tr>
</tbody>
</table>

AP1, AP2: Arunachal Pradesh rice variety 1 and 2, NL: Nagaland rice variety 1, ML: Meghalaya rice variety 1, AS1: Assam rice variety 1
ASC1: Arunachal Starter culture 1
Values are mean of two replicates

Table 2—Biochemical composition of starter culture collected from Arunachal Pradesh (ASC1)

<table>
<thead>
<tr>
<th>Sample Code</th>
<th>Moisture %</th>
<th>Crude fat %</th>
<th>Crude protein %</th>
<th>Crude fiber %</th>
<th>Ash %</th>
<th>Amylose %</th>
<th>Amylopectin %</th>
<th>Starch %</th>
<th>Total Soluble Sugar %</th>
<th>Reducing Sugar gm/100gm</th>
<th>Non-Reducing Sugar gm/100gm</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASC1</td>
<td>10.72</td>
<td>0.312</td>
<td>6.64</td>
<td>2.153</td>
<td>1.03</td>
<td>22.95</td>
<td>77.05</td>
<td>61.24</td>
<td>1.719</td>
<td>0.161</td>
<td>1.558</td>
</tr>
</tbody>
</table>

Values are mean of two replicates

Table 3—Alcohol, mineral, soluble protein and crude protein contents of rice beer samples

<table>
<thead>
<tr>
<th>Sample Code</th>
<th>Alcohol %</th>
<th>Calcium (mg/100mL)</th>
<th>Sodium (mg/100mL)</th>
<th>Potassium (mg/100mL)</th>
<th>Iron (mg/100mL)</th>
<th>Phosphorous (mg/100mL)</th>
<th>Soluble Protein (mg/100mL)</th>
<th>% Crude protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>AP1</td>
<td>12</td>
<td>12.5</td>
<td>1.90</td>
<td>4.78</td>
<td>0.296</td>
<td>78.108</td>
<td>1.163</td>
<td>1.95</td>
</tr>
<tr>
<td>AP2</td>
<td>12.5</td>
<td>10.93</td>
<td>1.27</td>
<td>4.76</td>
<td>0.497</td>
<td>23.563</td>
<td>1.397</td>
<td>2.33</td>
</tr>
<tr>
<td>NL1</td>
<td>12</td>
<td>15.625</td>
<td>2.22</td>
<td>6.43</td>
<td>0.489</td>
<td>33.711</td>
<td>1.315</td>
<td>2.07</td>
</tr>
<tr>
<td>ML1</td>
<td>12.8</td>
<td>34.37</td>
<td>3.49</td>
<td>9.63</td>
<td>1.4</td>
<td>39.948</td>
<td>1.359</td>
<td>1.85</td>
</tr>
<tr>
<td>AS1</td>
<td>13</td>
<td>26.5</td>
<td>1.91</td>
<td>6.67</td>
<td>3.069</td>
<td>19.123</td>
<td>1.675</td>
<td>2.51</td>
</tr>
</tbody>
</table>

Values are mean of two replicates

The antiradical activity values were in the range of 2.479-22.31%, AP1 and AP2 showing the lowest and highest values, respectively (Fig. 6). An antioxidant inhibits the oxidation of lipid or other molecules providing protection against Reactive Oxygen Species. The antioxidant activity of the rice beer samples was most likely to be contributed by the presence of phenolic acids, polyphenols and flavonoids from various indigenous herbs used in the preparation of the starter culture cake. These compounds are known to inhibit the oxidative mechanisms which are responsible for many disorders and diseases in humans such as infections, diabetes, arthritis, cardiovascular diseases, cancer, Alzheimer's diseases, AIDS, etc.
Total colony count of starter cake

Owing to the variation of concoction practices of making the starter culture cakes and the process, the resultant product differs in quality and often has a short life. Therefore, the diversity of fungi and bacteria associated with ASC1 was investigated by culturing the starters in different selective and nutrient media. These starter cultures cakes are often considered as a rich source for the isolation and selection of microorganisms that can be utilized in the food industry. CFU value of 19 was observed in MRSA medium indicating the prevalence of LAB followed by 6 in the PDA medium which indicates the presence of yeast and moulds in the starter cakes. \textit{Saccharomyces cerevisiae} was found to be the major organism responsible for the alcoholic fermentation of rice beer. Reports also suggest the presence of \textit{Saccharomyces cerevisiae}, \textit{Hanseniaspora} sp., \textit{Kloeckera} sp., \textit{Pischia} sp. and \textit{Candida} sp., with \textit{S. cerevisiae} being the dominant one\textsuperscript{18}. Other microorganisms present in the starter culture also contribute to the overall taste and aroma of the finished product\textsuperscript{19}. Earlier LAB strains like \textit{Pediococcus pentosaceus}, \textit{Lactobacillus plantarum} and \textit{Lactobacillus brevis} were isolated from \textit{Hamei} and \textit{Marcha} which are starter culture cakes for the preparation of rice beer in Manipur and Sikkim states of India, respectively\textsuperscript{20}. Total colony counts of ASC1 collected from Arunachal Pradesh are presented in Table 4.

As the ethnic tribes mostly consume the freshly brewed rice beer which contains very low amount of alcohol on daily basis, the ill effect of consuming higher dose of alcohol is negligible. Moreover, \textit{Hor Acho}, a rice beer produced by \textit{Karbi} tribe of Assam is used as a medicine to cure dysentery and pharyngitis in rural areas. Even during the epidemic of cholera in 1960s and 1970s, \textit{Karbi} people used to rub rice beer on their body as precaution. Preservation of dried fish by using rice beer is also a common practice in tribal communities\textsuperscript{1}. Similar observations have also been reported regarding the local liquor called \textit{Yu} of \textit{Meitei} communities of Manipur where poor health conditions of women due to irregular menstrual flow, obesity, loss of appetite, infertility factors and low
nourishments of food are regulated by consuming Yu with herbs\(^2\). Literature also suggests the use of local rice beer called Rokshi in Sikkim as beauty care product\(^21\).

Traditionally brewed rice beer plays an integral role in the day to day life of several ethnic tribes of North East. Moreover, medicinal and therapeutic properties of this traditional fermented beverage cannot be denied. But only a few modern scientific studies address the benefits of drinking rice beer. Systematic and Scientific approach is needed to get the insights. Standardization of the fermentation process, further investigation of its therapeutic properties and formulation of new techniques to increase their shelf life are very essential to commercialize rice beer in a wider international market just like Japanese sake. The tribal people of North East India have immense knowledge in using microbes and suitable raw materials in the preparation of fermented food and beverages with traditional yet scientifically sound protocols. Minimum self life is one of the major problems associated with these products. Modern scientific and technological approaches could upgrade and refine the indigenous knowledge and open up various possibilities of using these products as sources of different nutraceuticals and novel bioactive compounds.

**Conclusion**

These results indicate that the rice beer produced in North East India is nutritionally rich and have high therapeutic values. The presence of antiradical activity and other earlier evidences also suggest the possible medicinal properties of this traditional drink. The scientific study of raw material as well as processed product will provide database that could throw light to address the various problems associated with rice beer fermentation. There is an ample scope to validate the beneficial properties of rice beer and to standardize various parameters of fermentation for the commercialization of this staple drink.

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**References**

1. Teron R. Hor, the traditional alcoholic beverage of Karbi tribe of Assam, Nat Prod Rad, 5(5) (2006) 377-381.
9 Somogyi M, Notes on sugar determination, J Biol Chem, 195 (1952) 19-23.
10 Chopra SL & Konwar JS, Analytical Agricultural Chemistry, (Kalyani Publisher, Ludhiana), 1976.
18 Tanti B, Gurung L, Sarma HK & Buragohain AK, Ethnobotany of starter culture used in alcohol fermentation by a few ethnic tribes of Northeast India, Indian J Tradit Knowle, 9 (3) (2010) 463-466.