



Comparative study on traditional beverage preparation of certain ethnic communities from Assam state of India

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Abstract

Traditionally fermented beverages are used as health drink by most of the ethnic tribes. Rice beer is also a traditionally prepared beverage from North-east India and the tribal people prepared their own way of preparation. Because of presence of reducing sugar, carbonic acids and bioavailability of nutrients, traditionally prepared rice beer played an important role to improve healthy life. Along with maintenance of hygiene, pH played an important role during preparation.

Key words: Fermented beverage, rice beer, ethnic tribes, Northeast India.

Introduction

Fermented beverages constitute one of the widely accepted health drink all over the world. Archaeological evidence indicates that fermented beverages are as old as the earliest civilization and legally consumed in most culture¹. The preparation of ethnic beverages using the fermentation process by the tribal people of India is documented by several workers^{2,3}. It is reported that more than 350 types of major traditional beverages are prepared using indigenous knowledge. These homemade beverages prepared informally at family level or region-specific and prepared manually by mixing old starter culture with the raw material⁴. World Health Organization⁵ has also reported about the importance of traditional beverages in cultural and social events. It is important to mention that these traditional alcoholic beverages also contain extract of plant parts and a good source of minerals and bioactive compounds beneficial for health⁶⁻⁹.

Northeastern India, particularly Assam, is homeland of various ethnic communities like Bodo, Karbi, Mishing, Rabha, Deuri, Tiwa, Dimasa, Hajong, Garo, Khasi, Jaintia, Mesh, Sonowalkachari, Zeme Naga, Rengma Naga, Kuki, Hmar etc¹⁰. Preparation and consumption of traditional rice beer (fermented drink) is a common practice among all those tribes. The preparation processes are varied, simple and authentic, but are mostly region-specific and ethnic group as well. In addition to rice, it is a practice to use different plants and their parts that are locally available and those used as folk medicine for treatment of various diseases¹¹. Apart from the variations in the composition, the methods of preparation of traditionally fermented rice beers also vary among different ethnic groups. Different tribes employ different techniques and use different plant species based on their availability, necessity and traditional knowledge. Traditional healers use these beers and medicinal plants to cure different diseases like malaria, jaundice, cholera, rheumatism, clearing the intestinal parasitic worms etc.^{12,13}. Hariya, xaj, Jaad and jou are very popularly homebrewed rice beer and an integral part of socio-cultural life of the Adivasi,

Ahom, Nepali and Bodos, respectively. Raw rice fermented beverage is a semi transparent liquid. The starter culture has been prepared by involving different plant species. The rich nutritional content in rice beer has various medicinal values and ill effect of consuming higher dose of freshly brewed rice beer is negligible.

The manufacture of this beer in some households is done under poor hygienic conditions which result in product with short shelf-life and variable quality and low alcohol content. So, understanding the changes in physico-chemical properties is essential to upgrade the traditional processing to commercial scale and also would reveal facts about concentration of products and different nutritional component present in rice beer¹⁴. Time and temperature have a profound effect on the final biochemical and microbial properties of the beer. The optimal conditions for the production of a probiotic beer using starchy substrates and the specific fermenting microbes were established, and these conditions were successfully applied in the production of beer. These optimal conditions obtained from research could be successfully applied in the production technology for a wide variety of beers from starchy substrates¹⁵. Since various plant species have high medicinal value and are being used in the preparation of rice beer cake, it is of utmost importance to record the probability of having medicinal property of rice beer¹⁶. So, preservation of the indigenous knowledge based on indigenous culture and healthcare system has become an urgent need for society.

The information generated from the study will be beneficial in the field of ethno botanical research in future. With this backdrop, the present study was undertaken on the fermented product-rice beer like Hariya, Xaj, Chhaang and Jou of four tribes of Assam *i.e.* Adivasi, Ahom, Nepali and Bodo, respectively, which are very popularly homebrewed rice beer and an integral part of socio-cultural life.

Methods

Prior to production of traditional rice beer, rice cake is essential component. For the present study, four ethnic tribes like Adivasi, Ahom, Nepali and Bodo were selected. Already prepared rice cakes were collected from the mentioned ethnic communities along with their ingredients name. Among the ingredients, along with rice-floor, some plant materials are found common. Following are the raw materials for preparing rice cake by different tribes (Table 1). The production of rice beer, prepared by those ethnic communities, involves the following basic steps: collection of plant samples and preparation of rice cake, cooking of rice, crushing of rice starter cake, mixing of rice cake with cooked rice and allowing of fermentation at room temperature in earthen pot. Although the process is simple, it requires aseptic and controlled environment. For laboratory experiment, rice cake was collected from the ethnic community areas where the starter cake is predominantly available. Moreover, in our experiments, only sticky rice samples were collected from farmers for alcoholic beverage preparation. Earthen pots with lid were collected from market.

Preparation: Rice cake is the main ingredient or starter for the fermentation of rice into alcoholic beverage. Collected rice cakes were allowed to dry in the sunshine for three hours at 30°C with 75% relative humidity.

Cooking of rice: The dehusk sticky rice sample was cooked properly and allowed to cool and sun dried for one hour to reduce moisture content up to @ 75% relative humidity, so that rice cake can be mixed well and thoroughly.

Grinding and mixing of the rice cake: The rice cake was grinded properly with the help of mortar and pestle to the fine particles to form of powder. Then cooled cooked rice was mixed with rice cake powder properly so that no portion of the rice remained unmixed.

Insertion of mixed rice: The mixed rice was put into earthen pot. Before keeping inside the pitcher, it was rinsed with hot water so that the vessel remained free from any type of contamination. Then the pot was sealed to maintain the anaerobic condition.

Fermentation: The pots were kept in an incubator at 30°C for 72 h. The fermentation will take place within the time by yeast containing in rice cake powder. However, there remain some chances of growing moulds inside the vessel if the conditions are not aseptic which means the media or the substrate is contaminated.

Extraction: The fermented rice beer was ready for extraction after 3 days. Extraction was done in a 1000 ml beaker with the help of silk cloth with very carefully so that only extractant will passed into the beaker. Then extracted rice beer was covered with aluminium foil and kept in the refrigerator at 4°C for further use. The extracted rice beer was used to analyzed the following physico-chemical parameters.

Determination of physicochemical parameters

Test for sugar: Benedict solution was used to test for the presence of sugar. In this procedure, 3 ml of the sample of different

preparations were taken into test tube and 2 ml of Benedict reagent was added to each tube. The test tubes were placed in water bath at 80°C for 5 min and formation of reddish colour confirmed the presence of sugar in soft drinks.

Test for reducing sugar: The presence of reducing sugar was tested using Fehling solution. In this test, 3 ml of the sample was taken in a test tube and 2 ml of a mixture of Fehling's A and Fehling's B solution in equal amount was added. The test tube was heated in a water bath for 10 min and the appearance of brown precipitation confirmed the presence of reducing sugar.

Test for alcohol: Of sample 3 ml for each preparation of soft drink was transferred into a separate test tube. Of iodine 1 ml was added, followed by 1 ml of potassium iodine and 1 ml of sodium hydroxide (NaOH) solution. The test tube were boiled at 100°C in a water bath for 30 min. Appearance of yellow colour precipitation confirmed the presence of alcohol in soft drinks.

Test for carbon dioxide: As soon as the stock bottles were opened, 3 ml of the sample for each preparation of soft drinks was added to 2 ml of lime water (calcium hydroxide). The change of lime water from colourless to milky confirmed the presence of dissolved carbon dioxide in the soft drinks.

Quantification of acid concentration: The acidity of the soft drinks was determined by acid titration method. 0.1 M sodium hydroxide was taken into a 25 ml capacity burette. 10 ml of the prepared sample along with 2 drops of 1% phenolphthalein was taken for titration until the end point was attained marked by a colour changed from colourless to pink and the acid concentration was determined.

Determination of pH: pH of the rice beer sample was determined by digital pH meter.

Acidity experiment: Fermented beverage exhibited acidic nature as because during the process, it produces organic acids like acetic acid, succinic acids, other metabolites like glycerol ester and ketone too. In our experiment, the entire fermented beverage exhibited acidic pH.

Determination of alcohol content of traditional beverage by dichromate oxidation and redox titration: Alcohol content of traditionally prepared beverages was estimated using titration¹⁷. To determine the oxidation reaction of prepared beverage, pipette out 10 ml in a conical flask where 25 ml standard potassium dichromate solution added very slowly. After screw cap, the arrangements were kept in water bath at 60°C for 30 min. With equal amount of distilled water and potassium dichromate solution, a blank was also prepared which also kept into water bath. The solution was allowed to cool. The burette was filled with standard ferrous ammonium sulphate solution and titrate both blank and sample solution separately until getting emerald green colour. Then adding 5 drops of indicator solution i.e. 1,10 phenanthroline ferrous sulphate solution and continuing titration for getting blue green to brown colour. Burette reading for blank sample was recorded as V_B and beverage sample as V_A . Calculation was as follows:
% alcohol: $25-25(V_A/V_B)$.

Table 1. Studied tribe names, name of their beer, local and scientific name of the plant, and used plant parts.

Ethnic groups	Beverage name	Raw materials		
		Vernacular name	Scientific name of plants	Parts used
Adivasi	Handia	Kapou Dhekia	<i>Lygodium flexuosum</i> (L.) Sw.	leaf
		Bon Jaluk	<i>Oldenlandia diffusa</i> (Willd.)Roxb.	Whole plant
		Jackfruit	<i>Artocarpus heterophyllus</i> Lam	leaf
		Tejpat	<i>Cinnamomum tamala</i> (Buch.-Ham.) T.Nees & Eberm	leaf
		BiyoniHakota	<i>Desmodium velutinum</i> (Willd.)DC	Shoot portion
Nepali	Chhaang	Dhopat-tita	<i>Clerodendrum infortunatum</i> L.	Leaf
		Pineapple	<i>Ananas comosus</i> (L.)Merr.	Leaf
		Jackfruit	<i>Artocarpus heterophyllus</i> Lam	Leaf
		Sugarcane	<i>Saccharum officinarum</i> L.	Leaf
		Duron	<i>Leucas cephalotes</i> (Roth)Spreng.	Leaf
Bodo	Jou	Dhopat-tita	<i>Clerodendrum infortunatum</i> L.	Leaf
		Pineapple	<i>Ananas comosus</i> (L.)Merr.	Leaf
		Sugarcane	<i>Saccharum officinarum</i> L.	Leaf
		Jackfruit	<i>Artocarpus heterophyllus</i> Lam	Leaf
Ahom	Xaj	Kapou Dhekia	<i>Lygodium flexuosum</i> (L.) Sw.	Leaf
		Dhopat-tita	<i>Clerodendrum infortunatum</i> L.	Leaf
		Bon Jaluk	<i>Oldenlandia diffusa</i> (Willd) Roxb.	Shoot portion
		Madhoi maloti	<i>Combretum indicum</i> (L.) DeFilipps	Leaf

Results

For preparation of rice cake, the ingredients along with rice-floor, some plant materials are found common for all four (Table 1). However, *Cinnamomum tamala*, *Desmodium velutinum* by Adivasi tribes and *Combretum indicum* by Ahom tribes uses these plants along with others during their rice cake preparation.

Beer pH: pH plays an active role in fermentation process. Conversion of the most starch to alcoholic beverage required in the range of pH 5.0 to produce a clearer beer with better flavor and bitterness. Moreover, pH 5.0 normally doesn't degrade beer quality as quickly over time. The pH of the rice beer of four samples were 5.09, 4.89, 5.25 and 4.69 from Advasi, Nepali, Bodo and Ahom respectively (Table 2).

Beer TSS (Total soluble solid): TSS states the amounts of soluble solids in fermented products. TSS value affects the taste of the product, because it can indicate the level of sweetness of the beverage. In our experiment, it was observed that fermented rice beverage had 14, 12, 12 and 10 g/100 ml for Adivasi, Nepali, Bodo and Ahom tribes, respectively (Table 2).

Percentage of alcohol (%v/v) in beer: The alcohol contents in the rice beer was determined using dichromate oxidation method,

Table 2. Determination of pH, TSS and alcohol (%) of studied rice beers.

Parameters	Values obtained			
	Adivasi	Nepali	Bodo	Ahom
pH	5.09 (±0.03)	4.8 (±0.02)	5.25 (±0.03)	4.69 (±0.02)
TSS (g/100ml)	14 (± 0.3)	12 (± 0.2)	10 (±0.15)	10 (± 0.15)
Alcohol, %	3.25 (± 0.15)	3.75 (±0.2)	4.0 (±0.3)	3.5 (±0.2)

Table 3. Biochemical test for four studied traditional rice beers.

Parameters	Values obtained			
	Adivasi	Nepali	Bodo	Ahom
Sugar	+	+	+	+
Reducing sugar	++	++	++	++
Colour	Pale yellow	Pale yellow	Pale yellow	Pale yellow
Alcohol	++	++	++	++
Carbon dioxide	+	+	+	+
Acidity	+	+	+	+

titrated against ferrous ammonium sulphate and expressed in (% v/v). In our experiments, the alcoholic beverage exhibited 3.25%, 3.75%, 4.0% and 3.0% from Adivasi, Nepali, Bodo and Ahom tribe, respectively (Table 2).

Sugar content of beer: Sugar content in fermented beverage plays an important role. Generally, beer contains no sugar or very little and the appearance is clear. In our experiments on fermented rice, the beer obtained is milky-white colour. Sugar content was qualitatively determined by standard biochemical procedures and exhibits of occurrence (Table 3).

Reducing sugar of beer: Reducing sugar intake lowers specifically the risk of developing overweight and obesity and in turn in developing diabetes. It also has a significant effect on lowering dental caries. In our experiment, reducing sugar was quantitatively determined by standard biochemical method and exhibited presence of reducing sugar (Table 3).

Carbon dioxide in beer: A low level carbon dioxide presence in fermented product is leading to produce carbonic acids and giving slightly bitter taste. In our experiments, the traditionally prepared beverage exhibited presence of carbon dioxide (Table 3).

Discussion

The ethnic tribes of the NE region used traditional fermented beverage as their health drink since time immemorial. Rice beer is safe and nutritionally beneficial in many aspects. Biochemical properties of the fermented alcohol beverages play a great role in the human physiology when consumed and results of the physico-biochemical properties and major nutritional compositions. The product obtained after fermentation is a light alcoholic drink with an attractive test, texture, and pale yellow in colour and also varying pH in Adivasi (Table 3).

Basumatary *et al.*¹⁴ reported about pH 4.6 in Jou (Bodo), however, in the initial stage pH is a bit high but in the later days it decreased due to more fermentation whereas the experimental pH was 5.25. They also reported about significant increase in

percentage of total alcohol content with time allowed for fermentation and alcohol content ranging from 5.30- 22.05% v/v, whereas in the experiment performed by us the total alcohol content was 4.0%. According to report, the colour of the product was light golden brown and detected presence of carbohydrate and reducing sugar whereas the fermented product by us was pale yellow (Fig. 1) and confirmed the presence of carbohydrate and reducing sugar.



Figure 1. Sample of rice beer.

The pH of rice beer plays a great role in determining the degree of acidity of a beer which affects its stability during ageing (the greater the level of acidity the more it can be aged) as well as its freshness and colour. Total acidity increases as the fermentation proceeds. The performed test showed that the samples i.e. the rice beers contained sugar, reducing sugar, phosphates and the pH was 5.25 in Bodos, 4.69 in Ahoms, 5.09 in Adivasi and 4.8 in Nepali. These experiments were followed by the determination of concentrations of the alcohol. The concentrations of the alcohol in four tribes viz. Bodo, Ahom, Adivasi and Nepali were 4.0% , 3.5%, 3.25% and 3.75%, respectively. This finding is corroborating the facts of metabolic enrichment during fermentations for various participating microorganism in the starter culture.

The amount of carbohydrate varies with the usage of different rice varieties (glutinous, non glutinous, inferior quality etc.) and also plant species that are used as a substrate for starter culture. Use of different rice varieties has also been associated with the production of beer with different taste and flavor. The reducing sugar level in the initial fermentation stage increases but decreases slowly with increase in fermentation time. On the contrary, the concentration of alcohol is low at the initial stage of fermentation but it increases with fermentation aged. Similar discussion from other studies report that the initial sugar level plays a significant role in the final alcohol content of the product of fermentation. The sugar level decreased with fermentation time probably due to mainly microbes; but discussion of the paper is limited only to biochemical and the related microbial works are discussed elsewhere. The presence of sugar is quite useful in energy metabolism as it can meet energy requirement of the cell to a certain extent.

Conclusions

The traditional rice beer fermentation is a process of enrichment

of food with protein, amino acid and vitamin. The manufacture of this rice beer in some households is generally done under poor hygienic conditions which results in product with short shelf-life and variable quality and low alcohol content. However, in the present days due to commercialization, the traditional way of fermenting is somehow ruined with addition of some other organic materials which results in the good satisfaction of the customers but on the other hand affecting the real taste and nutritious value of the beer. Systematic and scientific approach in the process of fermentation is needed for commercialization of the product at wider scale by enhancing and upgradation of traditionally prepared rice beer but maintaining their way of brewing. Also the approach for aseptic laboratory conditions should be made for the process of brewing and fermentation to be completed aseptically and yield a non-contaminated product. Phytochemical analysis of the substrate used in the preparation (starter cakes) used in rice beer fermentation may further reveal the presence of useful components which might have beneficial effects for health as well as tremendous therapeutic potential in curing various ailments. This study broadened the prospects of the particular bacterial and yeast strains to be utilized in the preparation of fermented alcoholic beverages and optimized methodology for the production of a good quality rice beer has techno-economic feasibility and the potential to promote entrepreneurship.

Declarations

Ethics approval and consent to participate: Not applicable. Manuscripts reporting studies involving No human participants, human data or human tissue and need not for approval from ethical committee.

Consent for publication: The manuscript does not contain any individual person's data in any form and all the coauthors are agreed with communicating author for possible publication.

Availability of data and materials: The data generated during analysed of the current study are available in the manuscript. Not applicable. Manuscript does not contain any secondary data. The material in the form of 'rice cake' is available with respective ethnic groups.

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Authors' contributions:

Corresponding author have done experimental work along with co-author Mohammad Aziz and Ashish Kr Basumaotary. Another co-author Dulumoni Saharia prepared manuscript.

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