Assessment of Traditional Rice Beer Quality Manufactured by Different Ethnic Tribes of Assam, India

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Ethnic communities of North-East India consume various traditional beverages which are mostly prepared by fermentation of rice of their own. Many ethnic communities reside in Assam, India and the consumption of fermented drinks is always considered to be their culture and existence of the ethnic tribes. In present work, eight different rice beer samples are collected from different parts of Assam representing Ahom, Mising and Karbi community. The samples are characterized with respect to various physico-chemical analysis and microbiological parameters. In present work, the methodologies for the process of preparation of rice beers by different ethnic communities are also carefully observed and properly documented. It has been observed that all the samples are acidic in nature and the pH values are ranged from 2.83-5.05, while conductance ranged from 0.028-1.86 ms/cm. The amount of free carbon dioxide varies from 0.105 g/L to 6.81g/L. The amount of hardness varied from 0.012 g/L to 0.38 g/L. The sugar content of various rice beers ranged from below detection limit (BDL) to 4.4 g/100 mL while the percentage of ethanol varies from 2.2 to 5.7 g/100 mL. The correlation matrix study also shows a good positive correlation between conductance and TDS, hardness, free CO₂ and bicarbonate alkalinity. In this study, no coliform bacterial contamination appeared while the colony forming unit ranged from 0.3×10^5 to 1.68×10^5 cfu mL⁻¹.

Keywords: Rice beer, Ethnic tribes, Assam.

INTRODUCTION

The North-East India comprises the Sikkim and the seven sister states namely Assam, Arunachal Pradesh, Nagaland, Meghalaya, Mizoram, Manipur and Tripura. Nearly 40% of the total geographical area of this region is covered by evergreen forest. Each state of the North-east is inhabited by number of ethnic tribes characterized by their native languages, rituals, costumes and housing patterns [1]. Many of the tribes prepare their own local beverage, mostly using rice grains as the substrate [2]. The tribal communities residing in North-Eastern States of India has a common practice to consume homemade rice beer [2-4]. Consuming rice beer in different occasions such as ritual ceremonies, festivals, marriages and even death ceremonies is a mark of socio-cultural life of tribal people. The climatic conditions and the easy accessibility of the natural ingredients may be one of the prime reason for preparing and consuming beverages by ethnic tribes in North-East India [1,5]. Since the various medicinal plants are used in the preparation of rice beer, hence it is believed that rice beer has different therapeutic and medicinal values and it should be consume on regular basis for fitness of health [6,7].

It is reported that rice beer is effective against insomnia, headache and body ache, inflammation of body parts, diarrhea and urinary problems, expelling worms and as a treatment of cholera [8-10]. It has also been reported that 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 pulal of Malaysia [6,11]. Most of the tribes prepare their own traditional rice beer at home using starter, which contain amylolytic and alcohol producing yeasts, starch degrading moulds and lactic acid bacteria [3,7,12,13].

In addition each of the tribes also prepares their own unique starter cultures (cakes) to carry out fermentation [2]. The yeast starter cakes are prepared by mixing a wide variety of natural

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plant ingredients with boiled rice [14]. The principal yeast species responsible for fermentation is *Saccharomyces cerevisiae*, which has been used for thousands of years in the production of alcoholic beverage [14]. The metabolic pathway involves in this ethanol fermentation process is known as glycolysis [14].

Some people of these communities sell the starter culture as well as the fermented beverage for their livelihood. However, in most of these indigenous preparations, various plants and plant parts are commonly used as ingredients for the preparation of starter cake and fermentation process. The procedure adopted by different tribes for fermentation is almost same for different rice beers but difference occurs only in the use of different plant species during starter culture preparation [15]. Apong, Xaj and Hor are the traditional alcoholic beverage produced by the Mising, Ahom and Karbi communities of Assam state of India, respectively. The present investigation was performed to study the quality of rice beers produced by the three main ethnic tribes of Assam state of India.

EXPERIMENTAL

Description of tribes and preparation of their respective bevarages

Ahoms tribe: The Tai-Ahoms or Ahoms are one of the ethnic groups settled in Assam and are of Tai origin [8]. They are a part of the Assamese society and settled mainly in the Brahmaputra valley of Assam [13]. The Ahom tribes are belong to Indo-Mongoloids racial group and entered Assam through the upper courses of river Irrawaddy of Myanmar in 12th century A.D. The traditional beverage of the Ahom people is called Xaj-pani or Kolohi-pani.

Preparation of xaj: It is produced by fermenting rice with their starter culture (cake) known as vekur-pitha made

from powdered rice grain and various other plants. Various plants used in the preparation of Xajpani along with their medicinal properties are presented in Table-1. The rice grain and various other plants are properly washed and dried well and then grinded in wooden mortar with a pestle and the mixture then mixed with a little water in a vessel and made into a paste [8]. The oval shaped balls are prepared from the paste and then placed on banana leaves. It is 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 is known as vekur pitha and can be stored for up to a year [8].

Xaj pani is prepared by spreading half cooked rice (either sticky or non-sticky) on banana leaves for cooling. The half cooked rice is then mixed with powdered vekur pitha (1 per kg of rice) and again spreading on banana leave for some time [8]. This mixture is kept on an earthen pot and the mouth of the pot properly sealed. It 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 min. Filtration is done by draining the mass by using a cloth and the filtrate is ready for consuming [2].

Mising tribe: Mising is a tribal community belonging to Mongoloid group - a multitude of people that followed Austro-Asiatic races to India [16,17]. The rice beer prepared by Missing community is named as Apong and become an integral part of the socio-cultural as well as ritual sentiments of the Mising people [18-21].

Preparation of Apong: The rice cake for making Apong is called E'pob. It involves multiple steps and require many days for completion. E'pob is prepared from various plants in addition to rice grains. Various plants used in the preparation of Apong are presented in Table-2. The plant leaves are properly

TABLE-1
VARIOUS PLANTS AND HERBS ALONG WITH THEIR MEDICINAL PROPERTIES
USED IN PREPARATION OF XAJPANI BY AHOM TRIBES [Ref. 15]

Local name	Scientific name	Family	Parts used	Medicinal properties		
Bar manimuni	Centella asiatica	Apiaceae	Whole plant	Whole plant used in chronic dysentery, liver disorder, cuts and wounds, nervous disability, roots used in stomach ache; leaves used in dyspepsia, gastric trouble		
Patihanda	Cinnamomum bejolghota	Lauraceae	Leaves	Leaves are used as antidiabetic agent		
Tubuki lota	Cissampelos pareira	Menispermaceae	Leaves	Roots used in diarrhoea, piles, dysuria, bone fracture, to control labor pain, fruits used in liver disorder		
Dhapat tita	Clerodendrum viscosum	Verbenaceae	Young leaves, shoot, flower, bark, root	Leaf juice used in fever, cough, dysentery, stomach pair ulcer due to scabies. Bark, flower and roots used in ski diseases, malaria		
Lota mahudi	Croton caudatus	Euphorbiaceae	Leaves	Leaves used in urinary Trouble		
Saru manimuni	Hydrocotyle sibthorpioides	Apiaceae	Whole plant	Whole plant used in amoebic dysentery, malaena and for improving memory; leaves in dysentery and hypertension		
Kapou dhekia	Lygodium Flexuosum	Lycopodiaceae	Leaves	Rhizome used for skin disease and in rheumatism; leaves used for treating female infertility, fix fractured bones		
Gorob-choi	Naravelia zeylanica	Ranunculaceae	Leaves	Stem is used in tooth Ache		
Dhan	Oryza sativa	Poaceae	Rice grain	Rice-wash water used in diarrhoea and dysentery		
Bihlongoni	Pteridium Aquilinum	Pteridaceae	Fronds and roots	Leaves are antibacterial and germicidal		
Jaluk	Piper nigrum	Piperaceae	Seeds	Seeds used in indigestion, body ache, bone fracture, post labor ailment		
Sonbarial	Sida rhombifolia	Malvaceae	Leaves	Decoction of tender leaf is given to cure hypertension		
Tikoni barual	Smilax perfoliata	Smilaceae	Leaves	Roots used in post natal care and in odorous urine, stem used in swelling of gum		

Madhuriam

Posotia

TABLE-2										
VARIOUS PLANTS AND HERBS ALONG WITH THEIR MEDICINAL PROPERTIES										
USED IN PREPARATION OF APONG BY MISING TRIBES [Ref. 15]										
Local name Scientific name Family Parts used Medicinal properties										
Titabahak	Adhatoda vasica	Acanthaceae	Leaves and Shoot	Juice of leaves used as expectorant, cough relief						
Ananas	Ananas comosus	Bromeliaceae	Leaves, bark	Juice of leaves for amoebic dysentery, intestinal worm						
Kathal	Artocarpus heterophyllus	Moraceae	Leaf, shoot, bark	Leaves are antihelmenthic						
Satmul	Asparagus racemosus	Liliaceae	Tuberous root	Root decoction diuretic, phthalmic, galactagogue						
Tezpat	Cinnamomum tamala	Lauraceae	Leaves	Leaves useful in rheumatism, diarrhea, enlargement of spleen and diabetes						
Jolokia	lokia Capsicum annuum Solanaceae Fruit Roots used in asthma; fruits used for stor									
Barmanimuni	Centella asiatica	Apiaceae	Whole plant	Leaves used in amoebic dysentery or liver problem						
Jo mLakhuti	Costus speciosus	Liliaceae	Leaves, Barks	Rhizome paste used for jaundice treatment						
Durun	Leucas plukenetii	Lamiaceae	Leaf	Leaf juice used in sinusitis						
Kapou dhekia	Lygodium flexuosum	Lycopodiaceae	Leaves	Rhizome used for skin disease and in rheumatism; leaves used for treating female infertility, fix fractured bones						
Bhimkol	Musa balbisiana	Musaceae	Leaves	Young pseudo stems used as medicine against tuberculosis						
Pipoli	Piper longum	Piperaceae	Leaves	Fruits and roots are eaten in respiratory disorders, muscular pains, epilepsy and drowsiness.						
Jaluk	Piper nigrum	Piperaceae	Seeds	Seeds used in indigestion, body ache, bone fracture and post labour ailment						
Titaphool	Phlogacanthus thyrsiformis	Acanthaceae	Flower	Used for rheumatism, anemia and cough						

Leaves

Root, twigs, Leaves

Myrtaceae

Verbenaceae

TABLES

washed and sun dried and then powdered with uniform size. The leave powder is then mixed with rice powder of 'Bao' or 'Sali' variety of paddy and adequate quantity of water is added to make a paste. The mixture is again crushed in Ki.per with E'gi (with addition of water in between) till a sticky paste is obtained. As a source of microbial spawn, little amount of good quality old E'pob is added to the mixture and left for drying. It requires 3-5 days for proper drying depending upon the prevailing ambient temperature. Finally, the cakes are prepared and stored in an earthen pot whose mouth is covered with Rukii leaves and kept near a fire place for future use. For fermentation process, the boiled rice is cooled down on banana leaf and powdered starter cake is mixed with it. Then the fermentation process is carried out in the earthen pot (kiling) which is disinfected by placing on a bamboo frame constructed over the fire place (torap) till the pot turns into black colour. This is left for a period of about 5 days to take place proper fermentation [8]. After proper fermentation, small amount of water added to the fermented mixture and it is strained to get Apong [8]. The prepared Apong is diluted with water before consumption [16].

Psidium guajava

Vitex negundo

Karbi tribes: The Karbis are one of the major tribes of Assam. They are mostly concentrated in two hill districts of Assam-Karbi Anglong and North Cachar hills [2]. The region is largely mountainous with rough terrains and dense forests and therefore ethno botanically least explored. The Karbis are Mongoloid in origin group and speak a dialect 'Karbi', a Tibeto Burman language more particularly the Kuki-chin sub-group of languages [8]. The alcoholic beverage of the Karbis is known as Hor.

Preparation of Hor: The starter cake is prepared by mixing leaves of the plants with 24 h pre-soaked rice in a wooden mortar and pestle [8]. The various plants used in the preparation

of Hor are presented in Table-3. This paste is then converted into small flat shaped cakes. These are overlaid with powder of previous thaps and kept in a bamboo sieve and dried for about 3 days under the sun or above the fire place. These can be stored for about 1 year for further use [13]. The starter cake is then wrapped with banana leaf and kept for sun drying for 3 days [8]. For beverage preparation boiled rice is cooled by spreading. After cooling, boiled rice is mixed with thap. The mixture is kept in a large storage container for 2 days at room temperature. It is covered with plastic bags initially and afterwards with the help of gunny bags. Then a little amount of water is added and kept for 2 days of fermentation in summer and 4 days during winter [8]. Hor-alank obtained is concentrated and sweet in taste [12]. Before Hor-alank is filtered or strained, water is added until a diluted form is obtained. This diluted rice beer from Hor-alank is called Horpo.

Tender leaves are used in amoebic dysentery

Leaves & roots are used as febrifuse and tonic

Collection of samples: The samples were collected from different brewers. Xajpani and Apong were collected from Sivasagar district of Assam state whereas various kinds of Hor were collected from Karbi Anglong district of Assam state. Total eight samples were collected (Table-4) out of which six are undistilled and two are distilled comprising of two samples from each community, respectively. The samples are designated as A1 and A2, M1 and M2, K1 and K2 and two distilled samples KD1 and KD2. A meaningful chemical analysis mostly depends on the sampling procedure. The samples for physico-chemical analysis was collected in pre-cleaned 1 L polyvinyl containers and brought immediately to the laboratory for analysis. Separate samples were collected in sterilized bottles for biological analyses with necessary precautions for sampling and preservation.

Analytical methods: All the chemical analysis was carried out as per standards procedure laid in APHA (2005) [22]. The

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TABLE-3 VARIOUS PLANTS AND HERBS ALONG WITH THEIR MEDICINAL PROPERTIES USED IN PREPARATION OF HOR BY KARBI TRIBES [Ref. 15]									
Local name Scientific name Family Parts used Medicinal properties									
Marthu	Croton joufra	Euphorbiaceae	Leaves	Leaf juice used to cure eye problem, seeds used for indigestion, stem bark used to cure boils					
Kathal	Artocarpus heterophyllus	Moraceae	Young leaf, shoot, bark	Leaf juice used to cure eye problem, seeds used in indigestion, stem bark used to cure boils					
Thempra	Acacia pennata	Fabaceae	Barks	Barks Leaves used for cholera treatment, indigestion, headache, body pain and even to cure snake poisoning, stem bark is anti-Inflammatory and spasmolytic, roots used in dysentery and urinary Discharge.					

TABLE-4 DETAILS OF VARIOUS RICE BEER SAMPLES COLLECTED FROM DIFFERENT TRIBES										
Different tribes Sample (code) Distilled/undistilled Sample collection details										
Ahom	Xajpani (A1)	Undistilled	District: Sibasagar							
	Xajpani (A2)	Undistilled	Name of Village: Manipuri Gaon							
			Name of House Hold: Bikram Phukan (A1)							
			Name of Village: Hachara							
		Name of House Hold: Birinchi Dehingia (A2)								
Mising Apang (M1)		Undistilled	District: Sibasagar							
	Apang (M2)	Undistilled	Name of Village: Lapai Gaon							
			Name of House Hold: Naba Doley (M1)							
		Name of Village: Desangmukh								
			Name of House Hold: Jugal Pegu (M2)							
Karbi Horpo (K1)		Undistilled	District: Karbi Anglong							
Hor-alank (K2)		Undistilled	Name of Village: Dengaon							
	Hor-arak (KD1)	Distilled	Name of House Hold: Ka-et Rongpharpi							
Pangbuitang (KD2) Distilled										

basic parameters such as pH, electrical conductivity (EC), total dissolved solids (TDS) were measured with the help of Digital pH meter and conductivity meter (Hanna make). The hardness, free CO_2 , total alkalinity and total acidity were determined using titration method [23]. In the present study, anionic constituent phosphate, sugar and ethanol contents were measured with UV-visible Spectrophotometer (Agilent Cary-60). Total coliform bacteria as maximum probability number (MPN) and cfu/mL were counted using standard method [22].

RESULTS AND DISCUSSION

Physico-chemical characteristics: The physico-chemical characteristic parameters along with basic statistics and drinking water standards are presented in Tables 5 and 6. The pH content of a rice beer is a very significant parameter as it affect its stability, acidity, freshness, taste and flavor [16,24]. The pH of the samples varies from 2.83-5.05. The highest pH was found for

the beer Hor-Arak prepared by Karbi Tribe while Xaj Beer prepared by Ahom tribe Xaj beer has the lowest pH value. The variation in pH might be related to the different degree of utilization of the carbon substrate *i.e.* the different rice varieties used in the fermentation by different tribes, yeast cells have evolved so that they can thrive in more acidic environments better than many competing organisms. Yeast cells consume their nitrogen from plant source and liberate hydrogen ions decreasing the pH of the solution [25].

The presence of ionic components ranged from $28.09-1718~\mu\text{S/cm}$. The variation was significantly high in different samples and the highest conductance appeared for the sample Apong 1 and the lowest in the Hor Arak (distilled rice beer). The large variation was also observed for total dissolved solid (TDS), which ranged from 40 ppm (Hor Arak) to 1665 ppm (Apang II). TDS is an indicator of brewing water quality such as mineral composition of water, which affects mash pH during brewing process.

	TABLE-5										
	BASIC PHYSICAL CHARACTERIZATION OF VARIOUS ETHNIC RICE BEERS										
-	District Deliver Control of Village Billion										
					Different eth	nic rice beers					
	Parameters	Xajpani (A1)	Xajpani (A2)	Apang (M1)	Apang (M2)	Horpo (K1)	Hor-alank (K2)	Hor-arak (KD1)	Pangbuitang (KD2)		
-	Ctata	Ctoto Limid Limid Limid				T i and d					
	State	Liquid	Liquid	Liquid	Liquid	Liquid	Liquid	Liquid	Liquid		
	Colour	White	White	White	White	Pale Yellow	White	White	White		
	Taste	Sweet,	Sweet,	Sweet,	Sweet,	Sweet,	Sweet,	Sweet,	Sweet,		
		Tongue	Tongue	Tongue	Tongue	Tongue	Tongue	Tongue	Tongue		
		Sensitizing	Sensitizing	Sensitizing	Sensitizing	Sensitizing	Sensitizing	Sensitizing	Sensitizing		
	Opacity	Opaque	Opaque	Opaque	Opaque	Opaque	Opaque	Clear	Clear		

TABLE-6								
BASIC STATISTICS OF PHYSICO-CHEMICAL CHARACTERIZATIONS OF VARIOUS RICE BEER								
Domonostono	Unit -			This study				
Parameters	Unit -	Min	Max	Mean	SD	FSSAI		
pН	-	2.8	5.0	3.6	0.67	3.3-4.8		
Conductance	ms/cm ⁻¹	0.028	1.86	1.26	0.76	-		
TDS	g/L	0.009	0.93	0.63	0.38	-		
CO ₃ alkalinity	mg/L	0	0	0	0	-		
HCO ₃ alkalinity	g/L	0.06	2.6	1.26	0.87	_		
Free CO ₂	g/L	0.105	6.81	3.87	2.38	1.8-3.6		
Total acid-Tartaric acid	g/100 mL	0.02	1.4	0.64	0.47	_		
Total acid-Acetic acid	g/100 mL	0.018	1.12	0.52	0.38	_		
Hardness	g/L	0.012	0.38	0.25	0.15	-		
Phosphate as PO ₄ ³⁻	mg/L	0.28	1.2	0.59	0.29	-		
Sugar	g/100 mL	0.0	4.4	2.05	1.47	-		
Ethanol	% (v/v)	2.1	5.7	3.9	1.4	0.5-5.0		
Total colony count	cfu/mL	0.3×10^{5}	1.68×10^{5}	_	_	< 10		
Coliform	MPN/100 mL	Absent	Absent	Absent	Absent	Absent		

The amount of carbonate alkalinity (CO₃²⁻) was found to be nil in all the samples while the amount of bicarbonate alkalinity (HCO₃) varied from 60 ppm to 2600 ppm. The Karbi undistilled rice beer Hor-Alank has the highest amount of bicarbonate alkalinity while Ahom Xajpani has the lowest one. Alkalinity is a measurement of the concentration of all alkaline substances dissolved in the brewing water such as carbonates and bicarbonates which buffer pH in the calcium concentrations must be balanced with low carbonate-bicarbonates levels as they have countering effect on calcium. These ions should be kept to less than 50 ppm. Bicarbonates, being strong alkaline buffers, may raise the pH of the mash to unacceptable levels, if available in large amounts [26]. The free carbon dioxide content ranged from 105.6 mg/L (Har Arak) to 6811 mg/L (Horpo). The hardness of brewing water caused by the presence of multivalent metallic cations and highly caused by calcium and magnesium. The presence of hardness was ranged from 12 mg/L (Pangbuitang) to 380 mg/L (Horpo). The distilled samples (KD1 & KD2) have significantly low hardness. Calcium is considered as the major ion which determines water hardness. It can overcome the buffering capacity of malt phosphates, lower the mash pH to acceptable range and promote clarity, flavor and stability of the finished beer [27]. The ideal calcium concentration in the brewing water lies between 50 and 150 ppm. Magnesium also contributes to water hardness and therefore, affects the mash pH but to a lesser extent in comparison to calcium [2]. The sugar content of rice beer varied from 1.8 mg/mL to 4.4 mg/mL. The highest sugar content was obtained for the xajpani (A2) while the lowest for the Apang (M1). The sugar content was below detectable level for the two distilled rice beer (KD1 & KD2). In the case of yeast, NADH is regenerated by the reduction of pyruvic acid to ethanol [28]. The total acidity of the rice beer ranged from 0.02 g/100 mL tartaric acid to 1.4 g/100 mL, tartaric acid and 0.018 g/100 mL acetic acid to 1.11 g/100 mL acetic acid. The ethanol content of the rice beer ranged from 2.1% (K1) to 5.7% (KD1). It is to be noted that both the distilled rice beer [KD1 & KD2] has maximum percentage of ethanol content.

The phosphate content of the rice beer varied from 0.28 mg/L (A1) to 1.2 mg/L (A2). Maximum phosphate concen-

tration is may be due to the solar radiation, which might have encouraged the biological degradation of the organic matter [29]. In the present work, the coliform bacteria in terms of most probable number (MPN) was absent for all the samples however, the colony forming unit (cfu) was ranged from 0.3×10^5 cfu/mL to 1.68×10^5 cfu/mL, where the karbi rice beer Horpo(K1) consisted the highest value while it was lowest in the distilled karbi rice beer Pangbuitang (KD2).

Conclusion

In present work, it has been observed that the rice beer prepared by different ethnic tribes such as Ahom, Mising and Karbi is nutritionally rich and have high therapeutic values and diversity in terms of quality. These traditional rice beers are consumed as a staple drink and considered as most promising beverages having high medicinal and nutritional values, which perhaps could be a health benefit for the consumers. This can be attributed due to the use of various plants in starter culture, which have the various medicinal and nutritional properties. Moreover, during the preparation of rice beer, the old traditionally designed manufacturing units are used which lack of in depth scientific and quality standards. Therefore, the proper scientific inputs towards manufacturing as well as comprehensive study towards the characterization of phytochemicals, brewing water, substrate and microbial consortia used in the starter culture are highly essential. There will be a plenty of scope to validate the beneficial properties of rice beer and it may be a promising strategy toward its potential as a commercial beverage.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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