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Effect of various microbial starters for amylolytic fermentation on some quality attributes of rice beer

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Abstract

Rice beer was prepared, using nine different starter cakes predominant among various tribal communities of Northeast India. A comparative study was done based on their physicochemical, biochemical and microbiological properties. The starter cakes were tested for their physicochemical properties such as density, hardness, colour and significant variation was found. The proximate composition analysis revealed that carbohydrate was the major part and in addition it has low moisture. The pH, titrable acidity, alcohol content, sugars and starch did not vary much among the prepared rice beer. Polyphenols were present in the final product in various concentrations and the rice beers also evinced considerably high antioxidant activity. Yeasts and lactic acid bacteria were dominant in all the samples and spoilage microbes were absent. The study revealed significant variations of the physicochemical, biochemical and the microbiological properties among the starter cakes and the final product.

Introduction

The production of beer from rice is very common in the Asian countries and is known by different names such as *shaosingiju* and *lao-chao* of China, *sake* of Japan, *chongju* and *takju* in Korea, *tapuy* in Phillipines, *brembali* and *tape-ketan* in Indonesia, *khaomak* in Thailand, *rou nep than* in Vietnam and *tapai-pulul* in Malaysia (Aidoo *et al*., 2006). Northeast India is characterized by a vast assortment of tribal communities, each of which belongs to the aboriginal race of the region. These people of Northeast India have a strong correlation with nature, which is revealed by the fermentation technologies practised and their expertise in assessment of the microbial benefits (Das and Deka, 2012). Among these is the production and consumption of rice beer which also bears significance in social occasions such as festivals, marriages and even death ceremonies (Das *et al*., 2012).

The process of manufacturing rice beer consists of saccharification of the rice starch by fungal enzymes followed by alcoholic fermentation by yeasts supplied by traditional starters. These 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 (Das *et al*., 2012; Shrestha *et al*., 2002; Jeyaram *et al*., 2008). 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 (Shrestha *et al*., 2002). The product is mildly alcoholic and is sweet flavoured (Thapa and Tamang, 2004). Various organic acids, carbohydrates, amino acids and volatile aromatic compounds have been detected in some rice beer of Northeast India (Das *et al*., 2014). Various kinds of microflora have been isolated from the starter cakes of this region and their identification has been done viz. moulds from *marcha* of Sikkim (Tamang and Sarkar, 1995), yeast strains from marcha (Tsuyoshi *et al*., 2005) lactic acid bacteria from *hamei* of Manipur and *marcha* (Tamang *et al*., 2007) and yeasts from Hamei (Jeyaram *et al*., 2008).

Even though reports are available on the microbiological and biochemical properties of the starter cakes used in this region, yet detailed differentiation in between these starter cakes and the rice beer produced with these cakes have not been reported earlier. This work aims 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.

Materials and Methods

Materials

Nine varieties of starter cakes (SC) were

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collected from households of four different states of Northeast India viz. Assam, Nagaland, Meghalaya and Arunachal Pradesh during the months of November, 2010 to February, 2011. All the samples were collected in three replicates in sterile bottles, marked according to the place of collection, brought to the laboratory under refrigerated condition on the same day and stored at 4°C. Both the microbiological and biochemical examination of the samples were started within 24 h of storage. The chemicals were obtained from HiMedia (India) and Sigma-Aldrich Corporation (USA).

Production of rice beer in laboratory

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 (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 ratio of 1:1 ratio. This procedure was adapted from the traditional methodology for preparation of rice beer followed by the indigenous people of Northeast India (Das *et al.*, 2012). Nine types of rice beers 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.

Volume and density measurement

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) (Webb, 2001).

Texture analysis

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.

Colour measurement

The colour measurement was carried out by analyzing the samples in a Hunter Lab Color Quest (Ultrascan Vis, HunterLab, USA).

Sample preparation for biochemical analysis

All the RB samples were made CO_2 free prior to analysis. This was carried out by transferring the test samples to a large flask and shaking, first gently and then vigorously, maintaining the temperature at 20-25°C (AOAC, 2010).

Estimation of proximate composition, alcohol content, pH, acidity, reducing sugars (RS), starch and amylose

All of these were done according to standard AOAC official methods (2010).

Total soluble solids (TSS) measurement

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

Total polyphenols content (TPC) estimation

This was determined according to Folin-Ciocalteu reagent method of Bray and Thorpe (Bray *et al*., 1954).

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.* (1995).

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°C, the PCA, SSA and BPA plates at 37°C and the MRS and EMB plates in an anaerobic gas pack system at 37°C. The results obtained were expressed as log of colony forming units (CFU) per gram of sample.

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.

Results and discussions

Physical properties of the SC

Some physical characteristics of the SC are shown in Table 2. 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).

Colour in CIELAB expression

The results (Table 3) 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.

Proximate composition

The proximate composition of the samples is given in Table 4. 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% (Chuenchomrat *et al*., 2008). Other reported values of ash content on a dry matter basis in RB are 5.1% (Thapa and Tamang, 2004) and 1.7% (Tamang and Thapa, 2006). Tamang and Sarkar (1995) 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) (Thapa and Tamang, 2004) was however found to be higher (2% DM) than all the samples studied by us.

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

Table 1. Various codes for the samples with place of collection and rice beer prepared in laboratory condition

Tribe/ Community	Place of collection	Microbial starter cake		Rice beer	
		Local name	Code name	Local name	Code name
Kachari	Nagaland	*Umhu*	NaS1	*Judima*	NaR1
Ao/Angami	Nagaland	*Piazu*	NaS2	*Litchumsu*	NaR2
Bodo	Assam	*Amou*	AsS1	*Jou-bishi*	AsR1
Deori	Assam	*Perok-kushi*	AsS2	*Sujen*	AsR2
Karbi	Assam	*Thap*	AsS3	*Hor-alank*	AsR3
Ahom	Assam	*Vekur-pitha*	AsS4	*Xaj-pani*	AsR4
Mising	Assam	*Apop-pitha*	AsS5	*Apong*	AsR5
Adi-Galo	Arunachal Pradesh	*Siiyeh*	ArS1	*Opo*	ArR1
Khasi	Meghalaya	*Thiat*	MeS1	*Kiad*	MeR1

Table 2. 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}

Table 3. 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±0.06^{cd}
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^{def}	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}

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% (Chuenchomrat *et al*., 2008). The protein content of similar products reported on a dry basis was 9.5% (Tamang and Thapa, 2006) and 9.3% (Thapa and Tamang, 2004).

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.

Biochemical attributes of the RB

The values of some of the biochemical attributes of the RB samples are shown in Table 5. 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) (Teramoto *et al*., 2002), *kodo ko jaanr* (pH 4.1) (Thapa and Tamang, 2004), *poko* (pH 3.2 – 3.0). (Shrestha *et al*., 2002). The pH of different varieties of *tapuy* (Philippine rice beer) has also been found to range in between 4.6 to 5.0 (Sanchez *et al*., 1988). 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 (Kim *et al*., 2010). 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 (Tamang and Thapa, 2006).

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* (Teramoto *et al*., 2002), *bhaati jaanr* (Tamang and Thapa, 2006) and *kodo ko jaanr* (Thapa and Tamang, 2004) which were 5.0%, 5.9% and 4.8%, respectively, and more than that of poko (Shrestha *et al*., 2002) which had1.0-1.6%. This content was less than that found in samples of *yakju* of Korea (Kim *et al*., 2010), *Ou* of Thailand (Chuenchomrat *et al.*, 2008) and *tapuy* of Philippines (Sanchez *et al.*, 1988). 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.* (2010), 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% (Sanchez *et al*., 1988) and that in *zutho* to be 6.3 mg/ml) (Teramoto *et al*., 2002). Starch content ranged in between 0.74 g/100 g (MeR1) and 1.38 g/100 g (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

Table 4. Proximate composition of the microbial starter cakes and 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/NaR2	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/AsR1	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/AsR2	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/AsR3	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/AsR4	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/AsR5	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/ArR1	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

Table 5. 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/100 g) ± SD	Starch (g/100 g) ±SD	Amylose (g/100 g) ± SD	TPC (mg/100 g) ±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}	13.4 0±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

Table 6. 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±0.81^{bc}	0.0^{a}	0.0^{a}	4.30±0.21^{d}
AsS1/AsR1	6.28±0.75^{ab}	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±1.14^{abc}	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}

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 5. The sample NaR1 had the highest content of polyphenols (10.06 mg/100 g) followed by AsR3 (5.05 mg/100 g) and AsR4 (4.71 mg/100 g) 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 (Das *et al*., 2012). High content of polyphenols and antioxidant activity was also observed by Zujko and Witkowska (2014) in different type of wines and beer and concluded that the antioxidant potential of the foods tested was related to the total polyphenol contents.

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 6. 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.* (2002). 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 (2004) 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 (Mayo *et al*., 2010). 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.* (2007) 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 (2004), 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.* (2002) 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 (Sriphochanart and Skolpap, 2010) and most of the LAB are also probiotic in nature (Ljungh and Wadström, 2006), which adds to the uniqueness of this type of beer.

Amylolytic yeasts and moulds accomplish starch hydrolysis and fermentation in a wide range of traditional alcoholic foods and beverages (Steinkraus, 1996). 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 (Tamang and Thapa, 2006). Shrestha *et al.* (2002) 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 (Thapa and Tamang, 2004; Tamang and Thapa, 2006).

Jeyaram *et al.* (2008) 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 *et al*. (2012). 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 *Staphylococcus species* is not of much concern as most of them are harmless and reside normally on the skin and mucous membranes of organisms (Ryan, 2004). 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 (Mayo *et al.*, 2010).

Conclusion

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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