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# Study on the physicochemical, microbial and sensory characteristics of alcoholic beverage produced by indigenous method

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**Abstract**

An alcoholic beverage is a drink containing ethyl alcohol produced by distilling ethanol by means of fermenting grain, fruit, or vegetables which in low doses causes euphoria, reduced anxiety, and sociability. The purpose of the study was to prepare alcoholic beverage and evaluate its physicochemical, microbial and sensory quality. In this study, alcoholic beverages were prepared from the mixed fruits using approximately 300 g of each fruits through the indigenous modified method. The prepared four samples in respect of different types of fruits and beaten rice were sample $S_1$ (Grape, Apple, Banana, Pineapple, Plum), $S_2$ (Grape, Pineapple, Orange, Apple), $S_3$ (Jackfruit, Watermelon, Tomato), $S_4$ (Beaten rice). The alcoholic beverage was filtered, cooled, bottled and stored in refrigerator at 40C and analyzed physicochemical, microbial and sensory characteristics of four samples ($S_1$, $S_2$, $S_3$, $S_4$) of the beverage. From the analysis, it observed that the beverage produced by indigenous modified method showed optimum level of protein (2.18-3.93%), moderate pH (3.5-4.5), alcohol (5.88-12.04%) and acidity (0.54-1.08%). The results obtained from microbiological analysis showed that the standard plate counts were less than 30 colonies and the fungi were absent in all the four samples. Among four samples, the $S_2$ was the best product in respect of microbial, nutritional and sensory evaluation.



## Introduction

A beverage is a liquid which is specifically prepared for human consumption. In addition to satisfying a basic human need, beverages form part of the culture of human society. In others words, any one of various liquids for drinking, usually excluding water which is used as a drink for the purpose of relieving thirst and introducing fluid to the body, nourishing the body, and stimulating or soothing the individual (Arnold and John, 2005; Data *et al.*, 2012). Food beverages can be classified into two major classes namely, the alcoholic and the non-alcoholic beverages. Alcoholic beverages include wines of different sources, spirit e.g. Brandy, Whisky, Beer (Gama and Shemington, 1977). A non-alcoholic beverage includes carbonated and non-carbonated beverages. The carbonated beverages are made in the presence of carbon compound especially carbon dioxide. Examples include soda water, cocacola, ginger ale, tonic water, pepsi-cola etc. None carbonated beverages are those that are merely juice from fruits drink and nectars, vegetable juice, water chocolate drinks, coffee, tea, black currant etc. ( Adebayo *et al*., 2010; Chowdhury *et al*., 2011). The natural chemical balance of grapes lets them ferment without the addition of sugars, acids, enzymes, or other nutrients. Alcoholic beverages which are distilled after fermentation, fermented from non-cereal sources such as grapes, plums, cherries, or apples. Alcoholic beverage are the third most popular drink overall after water and tea (Nelson and Max, 2005).

Locally made beverage is a very popular among the Bangladeshi people consumed by the lower socioeconomic classes. This beverage is one of the complex mixtures which contain macro-molecules such as protein, carbohydrate and lipids. The major important component which are used in the preparation are grape, banana, apple, pineapple, plum, orange, jackfruit, tomato, watermelon and beaten rice. During the preparation, the ingredients needed are molasses, borax ($Na_2B_4O_7.10H_2O$), ammonium chloride $NH_4Cl$. The most abundant constituent of

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these beverage is water and it act as the medium in which all other constituents are dissolved and contain only traces amount of inorganic substances. There were no scientific studies found regarding physicochemical, microbial and sensorial quality of those beverages. The aim of the present study was to prepare alcoholic beverage and evaluate its quality in term of physicochemical, microbial and sensory analysis.

## Materials and Methods

This study was conducted at the Department of Food Engineering and Tea Technology, Shahjalal University of Science and Technology, Sylhet and Department of Microbiology and Hygiene, Sylhet Agricultural University, Sylhet. The flow diagram of preparation of alcoholic beverage is given below.

*Production of alcoholic beverage*

The prepared four samples in respect of different types of fruits and beaten rice were sample $S_1$ (Grape, Apple, Banana, Pineapple, Plum), $S_2$ (Grape, Pineapple, Orange, Apple), $S_3$ (Jackfruit, Watermelon, Tomato), $S_4$ (Beaten rice). The beverage was prepared using 300 g of each fruits. The fruits were cutting into pieces and filling into pot and water was mixed with fruits (1: 2) and filling into pot. Others ingredients such as molasses (2500 g), borax ($Na_2B_4O_7 \cdot 10H_2O$) 100 g, Ammonium chloride NH4Cl (nushadir salt) 100 g etc. were added into pot and fermentation was allowed for 3-4 weeks. After fermentation the product, beverage was obtained through distillation process. When distillation was completed, the beverage was filtered, cooled, bottled and stored in refrigerator at $4^0$C.

*Determination of pH and acidity*

The pH and acidity was determined according to the method of AOAC (1990). For acidity determination, 18 ml of the prepared beverage was measured and shaken with 200 ml of $CO_2$ free water in a conical flask and placed in a water bath at 40ºC for one hour. It was filtered and 100ml of the clear filtrate was titrated with 0.05M of NaOH solution using Phenolphalein as an indicator.

*Determination of total solid*

The total solid was determined according to the method of AOAC (1990). Five gram of beverage was weighed into a flat-bottomed metal dish (or Small beaker) and placed on boiling water for about 30 minutes until the liquid evaporated leaving the solid. It was then transferred into an oven maintained at


Figure 1. Flow diagram of preparation of Beverage produced by indigenous method

100ºC for 2½ h. It was then transferred to desiccators, cooled and weighed. It was heated in the oven again for 1 hour, cooled and weighed.

*Determination of protein content*

The protein content was determined according to the method of AOAC (1990). Ten ml of the prepared beverage was added to 0.05 ml of 0.5% phenolphthalein indicator. It was mixed and allowed to stand for a few minutes and neutralized with 0.1M NaOH to the standard pink colour. 2 ml of formalin was added, mixed and allowed to stand for few minutes. The new acidity produced was titrated with 0.1M NaOH to the same pink colour. Then 2 ml of the formalin +10 ml of $H_2O$ were titrated separately with 0.1M NaOH as blank.

*Determination of ash content*

The ash content was determined according to the method of AOAC (1990). The crucible dish was cleaned, dried ignited, cooled and weighed and 24.4 g of the beverage was weighed accurately and directly in the dish. The substance was dried on a boiling water bath and the charred over a bursen flame or hot plate in fume cupboard until no more soot was given out. Then, it was then ashed with a muffle furnace at 500ºC to obtain final weight.

*Determination of moisture content*

The moisture content was determined according to the method of AOAC (1990). This method is based on loss on dry at an oven temperature at 105ºC. Besides water the loss will include other matter volatile at 105ºC. Five gram of the beverage was weighed into a pre-weighted flat dish and dried at an oven temperature of 105ºC for 3 h. It was allowed to cool in an airtight desiccator and reweighed. It was heated in the oven again for half an hour, cooled and weighed. The process was repeated until constant weight was obtained.

*Determination of alcohol content*

The redox titration method was used for the determination of alcohol content according to the method of AOAC (1990). Ten mL aliquot of wine was taken into a 250.0 mL volumetric flask and make up to the mark with water and transferred 20.0 mL of this diluted sample to a conical flask. The titration was done using potassium dichromate solution.

*Microbiological analysis*

The microbial analysis were carried out by the spread plate method using Plate count agar, Sabouraud dextrose agar and nutrient agar, lactose broth for total aerobic bacteria and total yeast count respectively. Colony counts were done after the appropriate period of incubation. The standard plate count was done by the Spread plate technique (APHA, 1998). In which plate colony count are 30- 300 should be included for count and others should be discarded. In total fungal count technique, the sample was approximately diluted (0.5 ml) transferred to a SDA plate. Then the sample was distributed evenly over the surface by a special spreading technique. After colonies were grown, they were counted and the numbers of microbes in the original sample were calculated. In most probable number technique, counting the number of tubes showing a positive result and comparing with standard chart, a statistical estimate of the most probable number (MPN) of bacteria were made. This is an estimate of lactose fermenting bacteria. In Gram Staining technique, the fixed smear was treated with ammonium oxalate crystal violet solution for 30 seconds. This was gently rinsed off and iodine solution was applied for 30 seconds. This was drained off and 95% ethanol was then applied for 20 seconds as a decolorizing agent. Finally a counter stain, safranine was then added for 10 seconds. The result was recorded as gram positive or negative.

*Sensory evaluation*

Sensory attributes (such as colour/appearance, flavour, texture, and overall acceptability) were evaluated using a 9 point hedonic scale (where 1-9 represent extremely poor to excellent respectively) by 30 panelists (gender: 8 men: 8 women; age group: 20-40) selected from teachers, students, staff and faculty of several departments (Reza *et al*., 2013). Samples were served in clean transparent glasses. Questionnaires and water for mouth rinsing between each tasting were provided. Prior to evaluation, a session was held to familiarize panelists with the product. Panelists were asked to read through the questionnaires and the meaning of each attribute (colour/appearance, flavour, texture, and overall acceptability) was explained to the panelists to avoid any misinterpretation (Meilgaard *et al*., 1991; Kilcast and Subramanian, 2000).

*Statistical analysis*

All samples were analysed in triplicates and results averaged. Statistical analysis was assessed by using SPSS 18.0 Software (SPSS Inc., Chicago, USA). The significant difference between mean values were determined by independent t-test at significance level of $p<0.05$. Furthermore, significant differences between the mean values were determined by using the analysis of variance (ANOVA) and Duncan's multiple range test was conducted at a significance level of $p<0.05$.

## Results and Discussion

*Physicochemical characteristics of beverage*

The Physicochemical parameters determined are shown in Table 1. The pH of four samples beverage found in the range of 3.5-4.5. The pH of $S_1$, $S_2$, $S_3$, and $S_4$ were 3.8, 4.0, 4.1 and 4.4 (Table 1) respectively. The sample S4 was the highest pH 4.4 and sample $S_1$ was the lowest pH 3.8. This showed that sample $S_1$ was more acidic than others 3 samples $S_2$, $S_3$, and $S_4$ respectively. Igyor *et al*. (2006) reported that the pH value of traditional Nigerian beer (Burukutu) ranged from 3.36-4.86. The normal pH of bottom fermentation beers at the end of fermentation was 4.2-4.4, rarely 4.0 or less (DeClerck, 1957).

The percentages of protein in beverage found 3.35% for $S_1$, 2. 96% for $S_2$, 3.93 % for $S_3$ and 2.18% for $S_4$ (Table 1). The protein content of the beverage made it to be more nutritious than any of the alcoholic beverage. The $S_3$ was the highest protein content (3.93%) and $S_4$ was the lowest protein content (2.18%). This result however differed from the work of Adebayo *et al.* (2010) who observed that protein content of kunu beverage. This was similar to the earlier findings where the protein content of cashew

Table 1. Physicochemical characteristics of Beverage produced by indigenous method

| Sample name | pH | Protein % | Acidity % | Moisture content % | Alcohol % |
|---|---|---|---|---|---|
| S-1 | 3.8±0.02[a] | 3.35±0.12[c] | 1.08±0.02[c] | 83.53±0.36[a] | 12.04±0.11[d] |
| S-2 | 4.0±0.01[a] | 2.96±0.03[b] | 0.8±0.01[b] | 85.37±0.89[b] | 10.86±0.17[c] |
| S-3 | 4.1±0.02[a] | 3.93±0.06[d] | 0.72±0.06[b] | 85.42±0.52[b] | 9.93±0.06[b] |
| S-4 | 4.4±0.03[b] | 2.18±0.04[a] | 0.54±0.03[a] | 91.4±0.72[c] | 5.88±0.07[a] |

*[a-d] Mean values within the same column with different letters differs statistically ($p \leq 0.05$).

Table 2. Microbiological analysis of Beverage produced by indigenous method

| Sample name | SPC CFU/ml | FC CFU/ml | E. coli count MPN/ml | Colony in NA |
|---|---|---|---|---|
| $S_1$ | < 30 colony | Fungus absent | 0.00 | No colony found |
| $S_2$ | < 30 colony | Fungus absent | 0.00 | No colony found |
| $S_3$ | < 30 colony | Fungus absent | 0.00 | No colony found |
| $S_4$ | <30 colony | Fungus absent | 0.00 | No colony found |

apple juice observed (Olaoye *et al.*, 2007).

The acidity of beverage found 1.08, 0.81, 0.72 and 0. 54 for $S_1$, $S_2$, $S_3$ and $S_4$ respectively. The acidity in the beverage was calculated as potassium dihydrogenphosphate ($KH_2PO_4$) and then converted into percentage. Thus results revealed that the beverage of $S_1$ had the highest acidity (1.08%) followed by $S_2$ (0.81%) and S3 (0.72 %) and then S4 (0.544 %) was the lowest value. These results were similar to the work of Adebayo *et al.* (2010) who observed that acidity of kunu beverage. This result however differed from the work of Adeleke and Abiodun (2010) and they observed that the acidity of local beverage in Osun State, Nigeria. Some brewers claimed that the acidity of alcoholic beverage ranged from 0.12- 0.17 percentage but acidity of alcoholic beverage depends on the character of the product.

In this study, the moisture content of beverage found to be 83.53% for $S_1$, 85.37% for $S_2$, 85.42% for $S_3$ and 91.4% for $S_4$ (Table 1). These results were similar to the findings of Chowdhury and Ray (2007) and they studied the moisture content on jamun wine. These results were also similar to the findings of Adebayo *et al*. (2010) and they observed the moisture content on kunu beverage. The $S_4$ was the highest moisture content (91.4%) and $S_1$ was lowest moisture content (83.53%). Adebayo *et al*. (2010) found that the percentage of moisture in kunu beverage was 94% for all samples. Chowdhury and Ray (2007) studied that the moisture content of jamun wine was 83.2%.

The alcohol content of beverage found 12.04%, 10.86%, 9.93% and 5.88% for $S_1$, $S_2$, $S_3$ and $S_4$ (Table 1) respectively. Thus results revealed that beverage of $S_1$ was the highest alcohol content (12.04%) followed by $S_2$ (10.86%) and S3 (9.93%) and then $S_4$ (5.88%) was the lowest alcohol content. This result however differed from the work of Adeleke and Abiodun (2010) and they observed the alcohol content Burukutu of local beverage in Osun State, Nigeria. These results were similar to the findings of Adeleke *et al.* (2010) who observed that alcohol content of ogogoro beverage. The percentages of alcohol in alcoholic beverage were found 4% for beer, 12% for wine, and 40% for hard liquor. Patricia *et al*. (2001) reported that the percentages of alcohol in alcoholic beverage were found in the range of 3.2-4.0% for beer, 3.2-7.0% for malt liquor, 7.1-14.0% for table wines, 8.0-14% for sparkling wines, 14.0-24% fortified wines, 40 – 95 % for hard liquor. The percentages of alcohol in alcoholic beverage were found in the range of 4-8% for beer, 10-22% for wine, 40-50% for Brandy, 40-55% for whisky and Rum.

*Microbiological analysis*

The analysis of microbiological characteristics of the beverage is shown in Table 2. The results obtained from microbiological analysis showed the standard plate count, fungal count, and most probable number count (Table 2). The standard plate counts of prepared beverages were less than 30 colonies. The fungi found absent in almost all the four samples. In all four samples, the presence of coliform was about 0.00/ml. In nutrient agar, no colony is found in the beverage samples. All the four samples were Gram-negative. The result of this work was differed to the report of Adeyemi and Umar (1994) who worked

Table 3. Microbiological analysis of Gram staining of Beverage

| Name of sample | Shape | Colour | Result |
|---|---|---|---|
| $S_1$ | Rod shape | Pink colour | Gram-negative |
| $S_2$ | Rod shape | Pink colour | Gram-negative |
| $S_3$ | Rod shape | Pink colour | Gram-negative |
| $S_4$ | Rod shape | Pink colour | Gram-negative |

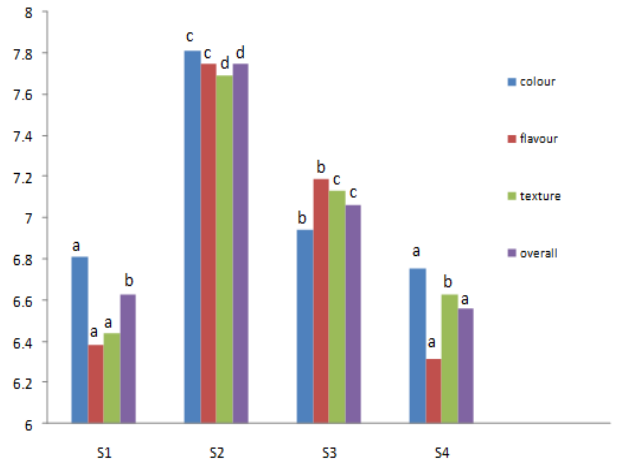


Figure 2. A bar diagram on the score of sensory characteristics of Beverage produced by indigenous method

on micro-organisms associated with the production of "kunu" using two different cereals (sorghum and millet) isolated eight bacteria and six fungal species. It was also differed with the observation of Akinrele (1970) who worked on the fermentation studies of maize to produce a traditional African starch cake "Eko". He isolated various species of bacteria, fungi and yeasts. From the above table (Table 3) we observed that all the samples $S_1$, $S_2$, $S_3$, and $S_4$ are rod shape and pink colour. When cells are appear to pink to red colour then the Bacterial cells are Gram-negative. The results revealed that $S_1$, $S_2$, $S_3$, and $S_4$ are Gram-negative.

*Sensory evaluation of beverage*

The Sensory evaluation of the Beverages was carried out for consumer acceptance and preference using 30 panelists. The sensory properties were done based on colour, flavour, texture and overall acceptability using 9 point Hedonic scale where 1 represents extremely dislike" and 9 "extremely like" respectively. Some differences are observed in the frequency distribution of the hedonic values (Figure 2). The panel scores of $S_2$ were the highest (7.81, 7.75, 7.69 and 7.75) mean value with respect to colour, flavour, texture and overall acceptability respectively and the panel scores of $S_4$ were the lowest (7.75, 6.31, and 6.56) mean values in case colour, flavour and overall acceptability respectively but on the basis of texture, the panel scores of $S_4$ were the highest (6.63) mean values in comparison to $S_1$ sample. This meant that the samples $S_2$ was acceptable in all cases in terms of colour, flavour, texture and overall acceptability. This result however differed from the work of Adebayo *et al.* (2010) who observed that the colour, flavour, texture and overall acceptability of kunu beverage. This result was similar to the findings of Joelia *et al.* (2007) who studied the sensory characteristics of cashew apple juice with respect of colour, flavour, and overall acceptability. Chowdhury and Ray (2007) reported that Preliminary sensory evaluation analysis jamun wine somewhat inferred (except colour/appearance) to commercial grape wine ($p< 0.05$) but the attributes like aroma, taste, after taste and colour/appearance were scored at about 3.0 (like much) However, the panelists rated flavour scores between 2.0- 3.0 (like moderately- like much) probably because of high tannin content in jamun wine which imparted somewhat an astringent flavour. Nevertheless, the jamun wine was acceptable to all the panelists. Adebayo *et al*. (2010) studied that sensory attributes of "kunu" samples with respected to taste, odour, colour, texture, flavour and general acceptability.

## Conclusion

Based on the results, we can conclude that the prepared beverage is a very nutritious drink that can supply nutrient requirement for human consumption. Beverage is one of the complex mixtures which contain Macro-molecules such as protein, carbohydrate, vitamin, calories etc. Also from the analysis, it was observed that Beverage produced by indigenous method gives the highest nourishment to the body and they are more nutritive value and good source of energy because of their high amount of protein, moderate pH and acidity. The beverages can be further developed to be marketed as healthy drink with fruit juices, fruit concentrates, protein concentrates. Future work shall focus on exploring packaging options for the developed products, market research, in order to juice consumer insight, to understand the marketability of the developed products, identifying suitable varieties of sweet for commercial production and exploring the use of fruit syrup as a sugar alternative in different food product categories.

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