Physicochemical and Bacteriological Assessment of Traditional Sorghum Beers in Jimeta, Nigeria

ABSTRACT

Sorghum beer popularly known as “burukutu” in Nigeria is a traditional beverage whose consumption has been passed down through generations. This beer, consumed while actively fermenting, harbors a diverse array of microorganisms. This study aims to evaluate the bacteriological and physicochemical characteristics of traditional sorghum beers sold in Jimeta, Adamawa State, Nigeria. Beer samples were obtained from eight traditional beer joints within the Jimeta metropolis, Adamawa State, Nigeria. The physicochemical analysis of the samples revealed titratable acidity ranging from 0.91% to 2.65%, pH values from 4.20 to 4.50, total reducing sugars from 10.1 mg/mL to 15.0 mg/mL, ethanol content from 3.12% to 4.69%, and total solids from 6.50 mg/mL to 7.60 mg/mL. The total bacterial count, determined using aerobic plate count, ranged from 8.0×10^2 to 1.0×10^{10} CFU/mL. Pure cultures from each sample were identified through cultural characteristics, Gram staining, motility, and biochemical tests including citrate utilization, catalase, indole, urease, methyl red, Voges-Proskauer and coagulase. Total of 44 isolates were identified, with *Staphylococcus aureus* and *Streptococcus* sp. being the most prevalent (8 isolates), followed by *Escherichia coli* and *Pseudomonas aeruginosa* (6 isolates), *Klebsiella pneumoniae* and *Acinetobacter* sp. (4 isolates), and *Bacillus subtilis*, *Bacillus cereus*, *Serratia* sp. and *Proteus mirabilis* (2 isolates). The presence of these potential pathogens raises great concerns about the safety of these beers. Improved sanitary practices during production, handling and serving are necessary to ensure consumer safety.

Keywords: Sorghum beer; microorganisms; physico-chemical analysis; bacterial contamination; sanitary practices.

1. INTRODUCTION

Brewing and consumption of sorghum beer has been a common practice among different ethnicities across Africa since time immemorial. African sorghum beer is produced mainly from the grains of guinea corn of the species *Sorghum vulgare* and *Sorghum bicolor* [1]. Although the most widely used grains in its production are members of the sorghum family, a number of the brewers do use maize and millet for the brewing process [2].

Ancestral reports from many African ethnic groups, indicated that the beer is widely consumed as means of refreshment in various festivals and ceremonies such as marriage, praying for rain, communication with ancestors, birth, handing-over of dowry, circumcision and burial ceremonies [3]. The manufacturing process of African traditional sorghum beer essentially involves malting, drying, milling, souring, boiling, mashing and alcoholic fermentation, but variations may occur depending on the geographic localization [4]. The resulting product is a cloudy alcoholic beverage. The beer has different names across

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Africa some of which include burukutu and pito in Nigeria [1], ikigake or amarwa in Rwanda, Merrisa in Sudan, Amegba or Bili-Bili of Cameroun [5], doro in Zimbabwe [6], dolo in Burkinafaso [7] and tchoukoutou in Benin and Togo [8] among others.

African sorghum beers have been reported to possess a number of nutritional qualities including, calories, B-groups of vitamins such as thiamine, folic acids, riboflavin, nicotinic acids, and essential amino acids such as lysine [4]. Similarly, it has also been reported to contain almost all essential amino acids in required proportion except cystine and tryptophan which are being completely destroyed by heat during boiling [9].
In addition to its nutritional qualities, Ogbonna et al. [10] accessed some important parameters with regards to the functionality and healthiness of Burukutu brewed with four different cereals (sorghum, millet, maize and composite materials of the three in equal ratios). The study revealed that the beer is healthy as it contains some functional phytochemical ingredients that can prevent the onset of certain chronic degenerative diseases including hypertension. They also added that, on the basis of its nutrient composition, burukutu, therefore is a superior traditional beverage that should be conferred some form of high social status.

However, despite its wide recognition as a healthy and nutrient rich beer, increasing globalization coupled with the introduction of Western beers, traditional sorghum beer has become less attractive because of its poor hygienic quality and organoleptic variations. Microorganisms play a crucial role in traditional sorghum beer fermentation, with factors such as equipment, and personnel influencing microbial presence and activity. The beer is mostly consumed while it is actively fermenting and in some places, it is usually taken unpasteurized, this places the consumers at risk of contracting infection with these organisms especially some potentially hazardous bacteria [11]. This study is aimed at determining the bacteriological and physicochemical properties of sorghum traditional beer (Burukutu) sold in Jimeta Adamawa State, Nigeria.

2. MATERIALS AND METHODS

Sample collection: Beer samples were purchased from eight traditional beer joints within Jimeta Metropolis, Adamawa State. Samples were collected in sterile plastic bottles as described by Anaukwu et al. [1]. All samples were stacked in an ice parked cooler and were immediately transported to laboratory for processing.

Determination of physico-chemical properties of the beer samples: The pH, titrable acidity, reducing sugars, ethanol and total solids of the local beer samples were determined as suggested by Lyumugabe et al. [5].

Measurement of pH: The pH of the samples was determined by analyzing 20 mL of each sample using a laboratory pH meter until a stable reading was obtained.

Total acidity: The total acidity of the samples was determined by titrating each sample against 0.1M sodium hydroxide (NaOH) using phenolphthalein as an indicator, as described by Konfor et al. [12].

Determination of total reducing sugars: Total reducing sugars were determined using the 3,5-dinitrosalicylic acid (DNSA) assay with a glucose DNSA standard curve at an absorbance of 510 nm, following the method described by Lam et al. [13].

Total solids: Total solids were determined by evaporating 5 mL of each sample in an evaporating dish of known weight at 105˚C for 1 h in a laboratory oven. The difference in weight was computed as total solid per mL of the sample.

Determination of ethanol concentration: The concentration of ethanol was determined by a spectrophotometric method utilizing the dichromate oxidation reaction, as described by Sriariyanun et al. [14]. Two milliliters of each sample were centrifuged at 5000 rpm for 5 min to obtain a clear supernatant. One milliliter of the sample was mixed with 1 mL of tri-n-butyl phosphate (TBP) and vortexed vigorously for one min. The mixture was centrifuged at 34200 ×g for 5 min. Two liquid phases were observed, and 500 µL from the clear and transparent upper layer (TBP layer) was dispensed into a tube containing 500 µL of dichromate reagent ($K_2Cr_2O_2$ dissolved in 5M H_2SO_4). The mixture was vortexed vigorously for 1 min and incubated at room temperature for 10 min until a green coloration was observed. Similarly, different concentrations of ethanol ranging from 1% to

10% (v/v) were prepared and subjected to the dichromate oxidation reaction alongside the samples. One hundred microliters of the oxidation products were diluted in 900 µL of sterile distilled water, and the absorbance was measured at 595 nm. A standard curve was generated, which was used to determine the ethanol concentration in the samples in percentage volume by volume (% v/v).

Sample preparation, culture and enumeration of bacteria: Upon arrival to the laboratory prior to bacteriological analysis, each sample was subjected to 10 fold serial dilution using autoclaved distilled water as diluent as suggested by Yusuf et al. [11]. Microbial enumeration was carried out using pour plate technique by inoculating 10^{-3} dilution of each sample in nutrient agar plates as described by Anaukwu et al. [1]. Each sample was also inoculated on MacConkey agar. The plates were incubated at 37˚C for 24 h.

Total aerobic plate counts (TAPC) were determined from each plate for each sample and the results were presented in colony forming units per milliliter (cfu/mL) using the following equation:

$$\text{Microbial count (cfu/ml)} = \frac{n}{v \times df}$$

where

n: is the number of microbial colonies counted on the plate.
v: the sample volume fetched to the petri dish (0.5 mL).
df: the dilution factor used (10^{-3}).

Isolation, characterization and identification of bacteria: After 24 h of incubation, pure culture of morphologically distinct bacterial colony was sub-cultured on freshly prepared nutrient agar using streak plate method. All the isolates were further subjected to Gram staining and biochemical tests for identification: citrate utilization, catalase, indole, urease, methyl red, Voges-Proskauer and coagulase as described by Swaminathan et al. [15].

3. RESULTS

Physico-chemical properties of the beer samples: The titrable acidity of the samples ranges from 0.91% to 2.65%, pH, 4.20 to 4.50, total reducing sugars ranged from 10.1 mg/mL to 15.0 mg/mL, ethanol raged from 3.12% to 4.69% and lastly, total solids ranged from 6.50 mg/mL to 7.60 mg/mL (Table 1).

Table 1. Physico-chemical analysis of beer samples

S/N	Parameters	Samples							
		A	B	C	D	E	F	G	H
1	Titrable acidity (%)	2.65	1.50	1.47	1.51	2.45	1.55	0.91	2.60
2	pH	4.30	4.35	4.40	4.37	4.45	4.25	4.20	4.50
3	Total reducing sugars (mg/ mL)	14.2	13.5	11.2	10.1	15.0	13.2	12.8	14.0
4	Ethanol (%v/v)	3.12	4.51	3.67	4.51	4.55	4.59	4.69	3.70
5	Total solid (mg/ mL)	7.60	6.50	6.90	7.45	7.15	7.30	7.51	7.59

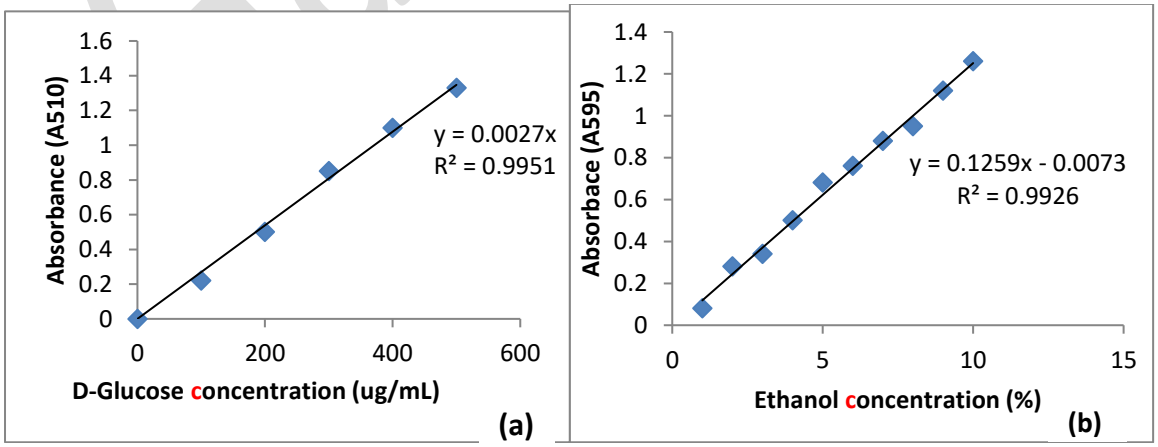

Fig. 1. (a) Standard curve for determination of total reducing sugars using 3,5-Dinitrosalicylic acid (DNSA) assay. (b) Standard curve for determination of ethanol concentration using dichromatic oxidation reaction

Table 2. Total aerobic plate count (TAPC)

S/N	Samples	TPC (cfu/ mL)
1	A	1.52×10^9
2	B	8.00×10^2
3	C	1.36×10^{10}
4	D	2.80×10^9
5	E	1.00×10^8
6	F	1.20×10^9
7	G	2.00×10^8
8	H	1.75×10^9

Key: TPC: Total plate count, CFU/ mL: Colony forming units per milliliter

Total aerobic plate count (TAPC): Aerobic plate count indicated a substantial bacterial growth in all the beer samples, with sample "C" having the highest bacterial count (1.36×10^{10}) CFU/ml, followed by samples D, H, A, F, G and E. The sample with the lowest bacterial count was sample "B" with (8.00×10^2) CFU/ml. A substantial coliform counts were recorded across all the samples with the exception of sample "C" (Table 2).

Bacterial isolates identified from each sample: A total of 44 bacterial isolates were obtained from all the samples, belonging to 10 different bacterial species. The most frequently identified species were *Staphylococcus aureus* and *Streptococcus* sp., each with 8 isolates. This was followed by *Escherichia coli* and *Pseudomonas aeruginosa*, each with 6 isolates. *Klebsiella pneumoniae* and *Acinetobacter* sp. were identified in 4 isolates each. The species with the lowest occurrence were *Bacillus subtilis*, *Bacillus cereus*, *Serratia* sp., and *Proteus mirabilis*, each with 2 isolates (Table 3).

Table 3. Cultural, microscopic and biochemical identification of the isolates

S/N	Cultural Xtics	Microscopy		Biochemical characteristics								Isolates identified	Frequency
		Gst	Shape	Cit	Cat	Mot	Ind	Ure	MR	VP	Cog		
1	Circular, entire, raised, smooth	+	Cocci	+	+	-	-	+	+	+	+	*Staphylococcus aureus*	8
2	Circular, entire, pink, smooth	-	Rods	-	+	+	+	-	+	-		*Escherichia coli*	6
3	Rough, opaque, irregular edges	+	Rods	+	+	+	-	-	-	+		*Bacillus subtilis*	2
4	Circular, entire, convex	+	Cocci	+	-	-	-	-	+	-		*Streptococcus spp*	8
5	Circular, entire margin	-	Rods	+	+	+	-	+	-	-		*Acetobacter spp*	4
6	Pink, mucoid, circular colonies.	-	Rods	+	+	-	-	+	-	+		*Klebsiella pneumoniae*	4
7	Rough, opaque, irregular edges	+	Rods	+	+	+	-	+	-	+		*Bacillus cereus*	2
8	Large Opaque colonies, irregular margins	-	Rods	+	+	+	-	-	-	-		*Pseudomonas aeruginosa*	6
9	Circular Entire margin	-	Rods	+	+	+	-	+	-	+		*Serratia spp*	2
10	White, swarming colonies	-	Rods	+	+	+	-	+	+	-		*Proteus mirabilis*	2
						Total							44

Key: Xtics: Characteristics, Gst: Gram stain, Cit: Citrate, Cat: Catalase, Mot: Motility, Ind: Indole, Ure: Urease, MR: Methyl Red, VP: Vorges-Proskauer, Cog: Coagulase, +: Positive, -: Negative

Table 4. Distribution of Bacterial species among the samples

S/N	Samples	Gram positives	Gram negatives	Total
1	A	*B. cereus* *S. aureus* *Streptococcus* sp.	*E. coli*	4
2	B	*B. subtilis,* *S. aureus,* *Streptococcus* sp.	*P. aeruginosa* *Serratia* sp. *Acetobacter* sp.	6
3	C	*Streptococcus* sp. *S. aureus*	*P. aeruginosa* *Acetobacter* sp.	4
4	D	*S. aureus* *Streptococcus* sp.	*E. coli* *P. aeruginosa*	4
5	E	*S. aureus* *Streptococcus* sp.	*E. coli,* *K. pneumoniae* *P. aeruginosa* *P. mirabilis*	6
6	F	*B. cereus,* *S. aureus* *Streptococcus* sp.	*E. coli* *Acetobacter* sp. *K. pneumoniae* *P. aeruginosa* *P. mirabilis*	8
7	G	*S. aureus* *Streptococcus* sp.	*E. coli,* *K. pneumoniae* *Serratia* sp. *Acetobacter* sp.	6
8	H	*B. subtilis* *S. aureus* *Streptococcus* sp.	*E. coli* *K. pneumoniae* *P. aeruginosa*	6
	Total	20(45.5%)	24(54.5%)	**44**

Distribution of bacterial species across the beer samples: Assessment of the distribution of the bacterial species across the samples indicated that sample "F" has the highest number, with 8 different species, followed by samples B, G, E and H, with 6 different bacterial species each, followed by A,C and D, with 4 different species each. Out of the 44 bacterial isolates, Gram positive bacteria have slightly lesser prevalence with 20 (45.5%) than the Gram-negatives, with 24 (54.5%) (Table 4).

4. DISCUSSION

Traditional sorghum beers are of cultural significance and are often deeply rooted in local culture and community, providing a sense of identity and social connection. They are among the most cherished beverages in different ethnicities Africa. The interplay between the physicochemical properties and the microbiological profiles of these beers could offer a deeper understanding of their flavour and safety.

In this study, local sorghum beer (burukutu) samples were collected from eight commercial joints in Jimeta, Nigeria for analysis. The physico-chemical properties of the samples, including titrable acidity, pH, total reducing sugars, ethanol and total solids, were assessed. The results suggested uniformity in these parameters across the samples. This lack of variation may be attributed to the consistency in raw materials and production processes among the beer producers [4]. The titratable acidity of beer is important as it helps dictate the overall acidity of the beer and also affects its taste, flavour and overall character. In this study, the titratable acidity of the beer samples ranged from 0.91% to 2.65%, with pH values ranging from 2.0 to 4.50. Similar results were reported by Attchelouwa et al. [16], who found the titratable acidity of Ivorian sorghum beer to be approximately 0.9%, with a pH range of 3.2 to 3.4. Mashau et al. [17] also accessed the physicochemical and microbiological properties of South African traditional sorghum beer (Mahewu) and also reported a titrable acidity range of 0.63% to 1.17%. Reducing sugars are

primary sources of energy for the cells especially glucose which is the brain's primary fuel. Traditional sorghum beers unlike modern beers are known to be rich in reducing sugars which also add to their nutritional values. The total reducing sugars of the beer samples studied were found to range from 10.1 mg/mL to 15.0 mg/mL. Similarly, results from a similar study by Adeleke and Abiodun [18] reported the reducing sugar amount in pito (a traditional sorghum beer) sold in Osun state, Nigeria to be 1.2% (12.0 mg/mL). Sawadogo-Lingani also reported that the African traditional sorghum beers contain reducing sugars at concentrations ranging from 0.05% to 0.53% (0.5 mg/mL to 5.3 mg/mL). For a beverage to be considered beer, it must contain alcohol, specifically ethanol, which is the most abundant and primarily responsible for the intoxicating effects of beer. In this study, the ethanol concentrations of the beer samples ranged from 3.12% to 4.69%, which is sufficient to cause intoxication, although the effects may vary depending on individual factors such as body weight, gender, metabolism, and drinking experience. Similarly, Ire et al. [19] assessed the physicochemical parameters of burukutu in Port Harcourt, Nigeria, and reported an ethanol content of 4.7% after 48 h of fermentation. In a study conducted by Stephen et al. [20], the total alcohol content of sorghum traditional beer was reported to be 10.29%, which is significantly higher than the levels obtained in this study. This discrepancy could be due to differences in brewing conditions, fermentation time, and other factors such as the fermentation biomass, which can vary significantly among brewers. Lastly, total solids ranged of the beer samples were assessed and it was found to range from 6.50 mg/mL to 7.60 mg/mL. Traditional sorghum beers re known to contain high amount of total solids compared to modern beers. This could be due to the brewing methods, involving less filtration and clarification compared to modern techniques, which can result in higher turbidity often caused by suspended particles, such as proteins, yeast, carbohydrates, and other organic materials, and consequently higher total solids in the beer. Bacterial contamination of foods and locally produced beverages has been a public health concern, particularly the presence of enteric bacteria. In this study, microbiological assessments of the beer samples revealed considerable bacterial growth, including the enteric types, in all the samples analyzed. This finding aligns with Anaukwu et al. [1], who reported that traditional sorghum beers (burukutu) sold in southern Nigeria was heavily contaminated with bacteria, including enteric types. Eze et al. [21] also recorded significant bacterial counts in burukutu sold in Enugu State, Nigeria.

A total of 44 different bacterial isolates were identified, encompassing both Gram positive and Gram negative bacteria. Gram negative bacteria were the most prevalent, with 24 isolates (54.5%), compared to Gram positive bacteria, which had 20 isolates (45.5%).The most frequently identified species were *Staphylococcus aureus* and *Streptococcus* spp., each with 8 isolates (18.2%). A similar result was also reported by Anaukwu et al. [1] that *Staphylococcus aureus* and *Streptococcus* spp. were the most common bacteria in burukutu samples. They suggested that the presence of *S. aureus* might be due to handling during production, as it is a normal flora on human skin and can easily contaminate the beer during processing if aseptic conditions are not maintained. Similarly, some earlier studies have reported significant numbers of *Streptococcus* spp. in burukutu [11,22]. These bacteria are normal flora of the throat and buccal cavity and can easily contaminate food and beverages if proper preventive measures are not followed.

Other bacterial isolates included *Escherichia coli* and *Pseudomonas aeruginosa* (each with 6 isolates, with each accounting for 13.6%), *Acetobacter* sp. and *Klebsiella pneumoniae* (each with 4 isolates, accounting for 9%), and *Bacillus subtilis*, *Bacillus cereus*, *Serratia* spp. and *Proteus mirabilis* (each with 2 isolates, accounting for 4.5%). Similar findings were reported by Umar et al. [23], who also identified *B. cereus*, *E. coli*, *K. pneumoniae* and *B. subtilis*. Some of these bacteria are potential pathogens, and their presence, even in small numbers, could render the beverage unsuitable for human consumption.

E. coli is an important member of the coliform group and has long been used as an indicator of fecal contamination in food sources or the environment [24]. Its presence in six (6) of the samples analyzed indicates possible contamination of the beer with bacteria of fecal origin and therefore places the consumers at risk of contracting infection with some pathogenic enteric bacteria and hence unsafe for consumption. Anaukwu et al. [1] also reported that the presence of *E. coli* in burukutu poses a health threat, and care should be taken during preparation to minimize beer contamination.

Pseudomonas aeruginosa and *Klebsiella pneumoniae* are well-known human pathogens. *K. pneumoniae* can cause infections in different parts of the body, with respiratory tract infections being the most common [25]. A study conducted as conducted by Oluwole et al. [26] also highlighted *Klebsiella spp* among the microbial contaminant commonly associated with fermented traditional wine made from palm trees.*Pseudomonas aeruginosa* is a Gram negative opportunistic pathogen that causes various infections, including skin and soft tissue infections, ulcerative keratitis, otitis externa, bloodstream infections, pneumonias, and urinary tract infections [27]. *Klebsiella pneumoniae* is also known as an important agent of food spoilage, secreting lipases and proteases that cause off-odors and forming biofilm on surfaces [28]. Its presence in some of the beer samples is, therefore, concerning. Li et al. [29] stated that *P. aeruginosa* has been an underestimated food pathogen in various food groups including milk, meat, water and also vegetables. They also added that the bacterium is a notorious pathogen owing to its possession of various virulence factors, biofilm forming capability as well as antimicrobial resistance making it a rising concern among consumers and food supervision department. *Acetobacter* spp. are known to be important contaminants in many traditional alcoholic drinks. They are aerobic and are generally considered non-pathogenic for humans. Consumption of beers containing *Acetobacter* and *Lactobacillus* could be linked to different health benefits as they contribute to good microbial balance inside the human gut [30]. The *Acetobacter* convert ethanol to acetic acid which is also known as vinegar. Acetic acid has a significant impact on beer flavor, imparting a sharp sourness and vinegary notes [30,31].

Serratia spp. and *P. mirabilis* are important human commensals but can also play pathogenic roles in some cases.Oluwole et al. [26] also reported the presence *Serratia* spp. as one of microbial contaminant of traditional alcoholic beverage made from palm trees. *Bacillus subtilis* is recognized as a safe and reliable human probiotic, associated with bioactivities such as vitamin production and immune stimulation [32]. It also plays a role in fermentation, having a shorter cycle than *S. cerevisiae* [33]. Conversely, *Bacillus cereus* is known to cause food poisoning and poses a significant health threat when present in food or beverages [34].

5. CONCLUSION

Traditional sorghum beers hold significant cultural value and play an essential role in the social and communal life of various African ethnicities. The beer samples analysed in this study, revealed uniform physicochemical properties, suggesting consistent production methods among local brewers. The findings indicated the presence of titratable acidity, reducing sugars, ethanol, and total solids within expected ranges, aligning with previous studies. However, the microbiological assessment highlighted significant bacterial contamination, including potential pathogens such as *E. coli* and *Pseudomonas aeruginosa*, raising concerns about the safety of these beverages. This result therefore, calls for the need for improved sanitary practices during production and handling of local beers.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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