RESEARCH NOTE

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Microbiology and quality assessment of '*burukutu*' a Nigerian fermented alcoholic beverage

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Abstract

Burukutu is an indigenous alcoholic beverage made in Nigeria from guinea corn. Microbial quality of the brewed burukutu was investigated using standard spread plate method. Ten (10) samples of commercially prepared burukutu were aseptically collected in 500 mL sterile bottles in triplicate from ten randomly selected brew houses in Army barracks and Angwan kaje, Minna, Niger state, Nigeria. The total viable counts, coliform counts and fungal counts ranged from 6.7×10^{3} cfu/mL – 7.9×10^{7} cfu/mL, 1.6×10^{6}cfu/mL – 2.6×10^{6} cfu/mL and 2.3×10^{4}cfu/mL – 1.54×10^{5} cfu/mL respectively for all locations sampled. The microorganisms isolated from commercially produced burukutu are Esherichia coli, Staphylococcus aureus, Bacillus subtillis, Aspergillus niger, Aspergillus flavus and species of Enterobacter, Klebsiella, Saccharomyces, Streptococcus, and Fusarium. Staphylococcus aureus, Bacillus subtillis, E. coli, Aspergillus flavus and Saccharomyces species were isolated from burukutu prepared in the laboratory. The proximate analysis revealed that, pH values ranged from 3.0-3.9, temperature ranged from 27.20 - 29.00, titratable acidity ranged from 0.14-0.16, alcoholic content ranged from 1.8-3.6%, dry matter content ranged from 3.6-8.0%, ash content ranged from 0.16-0.36% and crude protein content ranged from 3.18-3.29%. While the mineral analysis revealed that, magnesium content of the samples ranged from 119.30–1073.61ppm. Laboratory prepared burukutu was found to contain the highest calcium (3532.10ppm) and the lowest (917.28ppm) was found in burukutu from Angwan Kaje. The iron content of the samples ranged from 76.00-113.20ppm. The data obtained from proximate and mineral analysis were subjected to a One-Way Analysis of Variance (ANOVA) which showed that there were no significant differences ($p>0.05$) in levels of crude protein, ash content, temperature, magnesium and iron. While there were significant differences ($p<0.05$) in levels of pH, total titratable acidity, dry matter content, alcohol content and calcium analyzed. Consumption of burukutu may pose a public health hazard because of reported abilities of some fungi isolated to produce mycotoxins. There is therefore need for training local brewers on basic hygiene and measures to reduce risk of contamination by microorganisms.

Key words: Alcoholic beverage; Burukutu; Microorganisms; Mineral analysis; Proximate analysis; Quality assessment
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Inroduction

Burukutu is an indigenous alcoholic beverage made from guinea corn (*Sorghum vulgare* and *Sorghum bicolor)*. Sorghum is one of the cereals cultivated in the tropical region of Africa and is about the largest cultivated crop in the northern Guinea savanna areas of Nigeria (Kolawole *et. al.*, 2007).

It has a vinegar-like flavour, a pleasant sour taste, a reddish brown colour and consistency of a thin gruel. Burukutu is very popular among low and average income groups in the middle belt region of Nigeria. It is consumed in the Northern Guinea savanna region of Nigeria, Republic of Benin and in Ghana (Norman *et. al.*, 1999 and Kolawole *et al.*, 2007).

Traditional methods of production are non-standard in terms of raw materials, equipment, finished products quality and handling (Wonang and Opoefe, 1999). Burukutu is usually prepared in filthy environment and served in unsterilized calabashes. The food handlers and most often non-potable water used in burukutu production could also be vehicles for transmission of pathogens and food borne diseases. The process of production of burukutu involves malting, mashing, fermentation and maturation (Ekundayo, 1969).

Common organisms responsible for fermentation of foods are acid-forming bacteria such as lactic acid bacteria (LAB) such as *Lactobacillus, Lactococcus, Leuconostoc, Enterococcus, Streptococcus, Aerococcus*and *Pediococcus* known as obligate fermenters, flavorful organisms (aromatic compound microorganisms) and *Propionibacteriums* species (Bukola and Abiodun, 2008; Chelule *et. al.*, 2010; Agarry *et. al.*, 2010). The genera *Lactobacillus, Leuconostoc, Pediococcus* and *Streptococcus* are the main species that play a key role in the

safety and acceptability of the products of carbohydrates in tropical climate (Nwachukwu *et al.*, 2010). Most pathogenic microorganisms found in food cannot survive the low pH, hence, Lactic acid fermentation of food has been found to reduce the risk of having pathogenic microorganisms grow in the food (Abdel *et. al.*, 2009).

Efforts have been geared towards studying the physical and chemical characteristics of burukutu; as such there seem to be dearth of information on the microbiota been documented and particularly the proximate and mineral analysis proving that a well-developed understanding of these is needed. Therefore, this research represents one of the few studies in this area. The diverse microbial communities are known to play a crucial role in the fermentation of burukutu and the health hazard of some pathogenic microbes that could cause human diseases if present. Thus, the study of microbiological characteristics of burukutu lays a foundation to promote better understanding of the microorganisms associated with this fermented alcoholic beverage. The study was designed to determine the microbial quality of commercially prepared burukutu sold in Minna, Niger state, Nigeria as compared to laboratory brewed burukutu.

Materials and methods

Sample collection and preservation

Ten (10) samples of commercially prepared burukutu were aseptically collected in 500 mL sterile bottles in triplicate from ten randomly selected brew houses in two locations (Army barracks mami market and Angwan kaje) in Minna, Niger state, Nigeria. The fresh samples were kept in an ice box while transporting to the Department of Microbiology, Federal University of Technology, Minna laboratory and preserved at 4°C until further experiment in order to prevent the samples from undergoing biodegradation due to microbial action (APHA, 2005). Sample was brought out from the refrigerator and left at room temperature before use.

Laboratory production of burukutu

Burukutu was prepared according to the method described by Mbajiuk a*et al.* (2010) Figure 1.

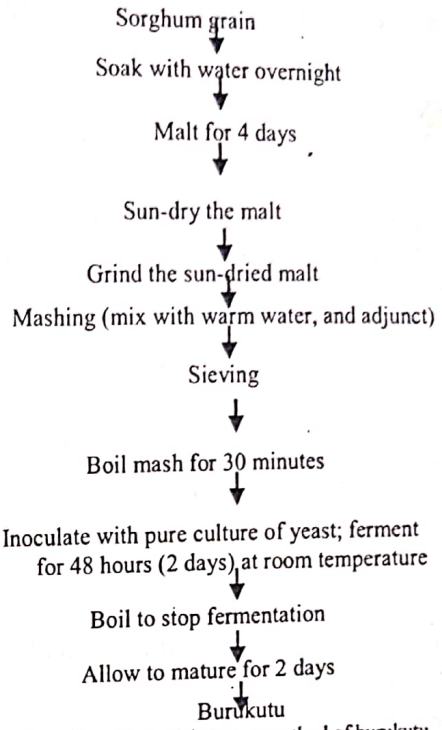

Figure 1: Flow chart for the laboratory method of burukutu production from sorghum grain Source: Mbajiuka *et al.* (2010).

Microbial analysis

Media preparation

The media used in the study were Sabouraud dextrose agar (SDA), nutrient agar (NA), MacConkey agar (MAC).

Total bacterial count

Appropriate serially diluted burukutu samples were aseptically transferred in triplicate onto nutrient agar plates. The plates were incubated at 37°C for 24-48 hours. Colonies which developed on the plates were counted using the colony counter (Model 6399/Stuart Scientific Co. Ltd. Great Britain) and expressed as colony forming units per millimeter (cfu/mL) of samples. The colonies differing in size, shape and colour were selected from the different plates on the nutrient agar and sub-cultured repeatedly to obtain pure isolates. The pure isolates were maintained on agar slants for further characterization and identification.

Mycological analysis

Appropriate serially diluted burukutu samples were inoculated onto Sabouraud dextrose agar (SDA) to identify the fungal isolates. The plates were incubated at 28°C for 48-72 hours.

Characterization and identification of microbial isolates

Bacterial isolates

The bacterial isolates were characterized based on colonial morphology, cultural characteristics, Gram's reaction and biochemical tests as described by Cheesebrough (2003); and Oyeleke and Manga (2008). The bacterial isolates were identified by comparing their characteristics with those of identified species using the schemes of Cowan and Steel (1985), Buchanan and Gibbons (1974), Holt *et al.* (1994).The biochemical tests carried out on the bacterial isolate were catalase test, carbohydrate fermentation test, citrate utilization test, coagulase test, indole test, methyl red test, starch hydrolysis and Voges-prokauer test.

Fungal isolates

Fungal identification was carried out using mycological atlas (Alexopolous and Mims, 1979). The fungi isolated were characterized based on the colour of the aerial and substrate hyphae, type of hyphae, shape and kind of asexual spore, sporangiophore and conidiophores and the characteristic of spore head.

Proximate analysis

pH

The pH was analyzed by using the pH meter (Crison micro pH 2000 model). It was switched on and allowed to stand for 5 minutes. The electrode was then dipped into 10 mL of the burukutu sample for some time until a steady reading was obtained. The pH reading was recorded for each sample (Onyeagba, 2004).

Titratable acidity (TTA)

Five milliliters (5 mL) of the sample was titrated to a pink color with 0.1M NaOH, using 3 drops of 1% phenolphthalein as indicator. The TTA values were noted for each sample.

Alcohol content

Samples were distilled using a glass distillation apparatus to recover the alcohol-water mixture. While an alcohol meter was used to determine the percentage alcohol content of the distillate obtained.

Dry matter content

Five grams of each of the samples were weighed into a pre weighed petri dish and dried in an oven at 100°C for 24 hours. The dried samples were then weighed after cooling in a desiccator (Association of Analytical Chemists (AOAC), 1990).

Ash content

Ten grams of each of the samples were weighed into a small dry crucible of known weight and charred on a low furnace. The charred material was ashed in a muffle furnace at 550°C for 2 hours. The ashed material was removed from the furnace and cooled. It was then kept in a dessicator and weighed (Association of Analytical chemists (AOAC), 1990).

Crude protein

The samples were digested with concentrated H_2SO_4, concentrated NaOH (40%), K_2SO_4 and $CuSO_4$. Five milliliters (5 mL) of the digest was placed into a micro-kjeldahl distillation apparatus and excess concentrated NaOH was added to make the solution strongly alkaline. Ammonia was distilled into 5 mL of boric acid indicator in a titrating flask. Above 45 mL of the distillate was then collected. Titration was done with 0.01 M HCL (Gregory, 2005).

Mineral analysis

Analysis of the samples was carried out using standard methods to determine the calcium, magnesium and iron content (Association of Analytical Chemists (AOAC), 1990).

Statistical analysis

Data were analyzed using statistical package for social science (SPSS) version 16 and presented as means ± standard error of mean. Comparisons between mean were done using Analysis of Variance (ANOVA) and Least Significant Different (LSD). Values of $P<0.05$ were considered as statistically significant as described by Mahajan (1997).

Results

The study revealed that microbial counts in burukutu from different locations in Minna ranged from 6.7×10^7cfu/mL – 7.9×10^7cfu/mL, 1.6×10^6cfu/mL – 2.6×10^6cfu/mL and 2.3×10^4cfu/mL – 1.54×10^5cfu/mL for total viable counts, coliform counts and fungal counts respectively (Table 1). There was no

significant difference in the microbial counts in burukutu samples analysed.

Table 1. Total viable count for bacteria, coliform and fungi isolates

Location	Total Viable Counts (cfu/mL)	Coliform count (cfu/mL)	Fungal count (cfu/mL)
Labpratory	$6.7x\ 10^{7}$	$1.6x10^{6a}$	$2.3x10^{4a}$
Mami Market	$6.7\times\ 10^{7a}$	$2.0\times\ 10^{6a}$	$9.3\times\ 10^{4a}$
Angwan Kaje	$7.9\times\ 10^{7a}$	$2.6\times\ 10^{6a}$	1.54×10^{5a}

Values on the same column with different superscript (a, b, c) are significantly different ($p<0.05$), while those with the same superscript (a, b, c) are not significantly different ($p>0.05$).

The results from the microbial analysis shows that the samples harbours different microorganisms which include *Escherichia coli, Staphylococcus aureus, Bacillus subtillis, Aspergillus niger, Aspergillus flavus* and species *of Enterobacter, Klebsiella, Saccharomyces, Streptococcus,* and *Fusarium* (Table 2).The frequency of occurrence of the microbial isolates revealed that *Bacillus subtillis* (15.4%) had the highest frequency of occurrence while *Klebsiella* sp (2.6%) had the least frequency for bacteria isolates. *Saccharomyces* sp (42.8 %) had the highest frequency of occurrence while *Fusarium* sp (1.3%) had the least frequency for fungal isolates (Table 2).

Table 2. Frequency of occurrence of microbial isolates in burukutu samples from different locations.

Isolates	Number of isolates from different locations			Total number isolated	Percentage (%)
	A	B	C		
S. aureus	2	4	3	9	11.5
E. coli	0	3	4	7	9.0
Streptococcus sp.	0	2	2	4	5.1
B. subtilis	3	5	4	12	15.4
Enterobacter sp.	0	1	3	4	5.1
Klebsiella sp.	0	0	2	2	2.6
A. niger	0	1	3	4	5.1
A. flavus	1	1	0	2	2.6
Fusarium sp.	0	0	1	1	1.3
Saccharomyces sp.	8	11	14	33	42.8
Total	14	28	36	78	100

Key A = Laboratory B = Mami Market C = Angwan Kaje

The results of the mineral analysis of burukutu from different locations are presented in Table 3.It was revealed that laboratory prepared burukutu had the highest calcium content (3532.10 ppm) while the lowest (917.28ppm) calcium content was obtained from Angwan Kaje. Burukutu from different locations had magnesium and iron at varying levels (Table 3).

Table 3. Mineral analysis of the 'burukutu beverage,

Location	Elements (PPM)		
	Calcium	Magnesium	Iron
Laboratory brewed burukutu	3532.10^{a}	119.30^{a}	76.00^{a}
Burukutu from Mami market	1305.36^{b}	1073.61^{a}	113.20^{a}
Burukutu from Angwan Kaje	917.28^{b}	622.68^{a}	95.60^{a}

Values on the same column with different superscript (a, b, c) are significantly different ($p<0.05$) while those with the same superscript (a, b, c) are not significantly different ($p>0.05$).

The proximate analysis results from the study showed that pH values ranged from 3.0-3.9, temperature ranged from 27.20 - 29.00, titratable acidity ranged from 0.14-0.16, alcoholic content ranged from 1.8-3.6%, dry matter content ranged from 3.6-8.0%, ash content ranged from 0.16-0.36% and crude protein content ranged from 3.18-3.29%. There was no significant difference in the crude protein content, ash content and temperature (Table 4).

Table 4. Proximate analysis of the sampled 'burukut' drink.

Location	pH	Temperature	Total titrable content	Dry matter (%)	Ash Content (%)	Alcohol Content (%)	Crude Protein Content (%)
Laboratory brewed burukutu	3.86a	28.50a	0.16a	6.10ab	0.21a	1.80b	3.29a
Burukutu from mami market	3.11b	27.20a	0.14c	8.00a	0.36a	2.92ab	3.18a
Burukutu from Angwan Kaje	3.04b	29.00a	0.15b	3.60b	0.16a	3.60a	3.23a

Values on the same column with different superscript (a, b, c) are significantly different ($p<0.05$) while those with the same superscript (a, b, c) are not significantly different ($p>0.05$).

Discussion

The microorganisms isolated from commercially prepared burukutu are *Escherichia coli, Staphylococcus aureus, Bacillus subtillis, Streptococcus* species, *Enterobacter* species, *Klebsiella* species *Aspergillus niger, Aspergillus flavus, Saccharomyce* species *and Fusarium* species while *Staphylococcus aureus, Bacillus subtillis, E. coli, Aspergillus flavus and Saccharomyces* species were isolated from laboratory prepared burukutu. This corroborated with the work of Kolawole *et al.* (2007) who reported similar microorganisms isolated from burukutu and pito samples in Ilorin, Nigeria as *Staphylococcus aureus, Escherichia coli, Bacillus subtilis, Streptococcus* species, *Proteus* species, *Rhizopusstolonifer, Aspergillus flavus, Aspergillus niger, Saccharomyces cerevisiae and Mucor* species.

Yeast species dominated the samples from all locations which could be as a result of the acidic pH that favoured fungi growth. *Saccharomyces* sp. *a*nd other fungi isolated are associated with fermentation of burukutu. According to Mohammed *et. al.*(1999), *Saccharomyces cerevisiae* and other yeasts are responsible for the alcoholic fermentation and also contribute to the flavour and acceptability of the product in combination with *Streptococcus* sp. *Bacillus subtilis* was the bacteria with the highest frequency and it's a Gram positive organism found in the soil and its presence in the burukutu could be from the sorghum grain, followed by *Staphylococcus aureus* whose presence is due to the fact that they inhabit the skin of human from where its being transferred into the burukutu samples while *Escherichia coli* which is a coliform bacteria and its presence in the sample may be due to the fact that water used in the preparation of the burukutu was contaminated with faecal matter. *Streptococcus* sp., *Enterobacter* sp. and *Klebsiella* sp. has the lowest frequency of occurrence. The presence of *E. coli*, *Enterobacter* sp. and *Klebsiella* sp. are of public health concern as they are members of coliform group capable of causing gastroenteritis and urinary tract infections. The presence of *Aspergillus* species are of nutritional and epidemiological concern according to International Commission on Microbiological Specifications for Food (ICMSF), (1996) as they are capable of producing mycotoxins.

Results from the proximate analysis revealed that the pH values ranged from 3.0-3.9 which concurred with the pH range of 1.8-3.9 reported for burukutu by Kolawole, *et al.* (2007). The titratable acidity ranged from 0.14-0.16, the alcoholic content ranged from 1.8-3.6%, dry matter content ranged from 3.6-8.0%, ash content ranged from 0.16-0.36% and crude protein content ranged from 3.18-3.29%. The difference in the values of the proximate analysis for the samples may be due to the quality of the grains used and the processing method.

Mineral analysis results revealed that the magnesium content of the samples ranged from 119.30–1073.61ppm. Laboratory prepared burukutu was found to contain the highest calcium (3532.10ppm) and the lowest (917.28ppm) was found in burukutu from Angwan Kaje. The iron content of the samples ranged from 76.00 - 113.20ppm. Our study thus exhibited comparable results with published data (Okafor, 1990; Igyor *et. al.*, 2006; Kolawole *et al.*, 2007; Nwachukwuel *et. al.*, 2010). Mineral elements are essential for regulating and building the body cells and aid in fighting depression.

Conclusion

The study revealed the existence of some pathogenic microorganisms in burukutu that are threat to public health and to the people consuming it. Regulatory authorities should intervene by setting-up standards for the production techniques as well as health status of personnel. Good hygienic practice should be ensured during its production. Routine microbiological analysis of burukutu should be carried out during the production process.

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