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Microbiological analysis of Burukutu beverage produced in southern part of Nigeria

Chikodili G. Anaukwu, Franklin C. Nwagwu, Onyedika I. Okafor, Chinyere C. Ezemba, Chinedu C. Orji, Kingsley C. Agu and Etim J. Archibong

Department of Applied Microbiology and Brewing, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria

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ABSTRACT

Burukutu samples were randomly purchased from different shops at Elele market, Army Barracks, Alimini, River state and Obinze market,Army Barracks, Owerri,Imo state, Nigeria. A total of 30 samples were aseptically collected and compositely screened for total aerobic plate count, total coliform count, total fecal coliform count and total fungal count using Nutrient agar (NA), MacConkey(MCA), Eosin methylene blue (EMB) and Sabouraud Dextrose Agar (SDA) respectivelyby pour plating technique. The microorganisms isolated belong to the genera Escherichia, Staphylococcus, Bacillus, Lactobacillus, Streptococcus, Aspergillus, Rhizopus and Saccharomyces. The Total aerobic plate count of the samples from Elele and Obinze market were 6.20±0.26 cfu/ml and 5.85±0.26 cf u/ml respectively. Total fecal coliform count of the samples from Elele market was 5.10±0.10 cfu/ml, while that from Obinze market was 5.06±0.23cfu/ml. The total colifo rm count and total fungal count of the samples from Elele market were 5.80±0.08 and 6.20±0.22cfu/ml, while sa mples fromObinze market recorded 5.50±0.17 and 6.08±0.08cfu/ml.Staphylococcus aureus was the highe st occurring bacteria at 27.27% while Sacccharomycescerevisiae was the highest occurring fungi at 40.91%. Statistical analysis showed that therewas no significant difference in the microbial counts of the samples collected from Elele market and Obinze market at p-value > 0.05.

Keywords: Burukutu, Total coliform count, Total fecal coliform count, Total fungal count, Total aerobic plate count.

INTRODUCTION

Burukutu is a popular alcoholic beverage of vinegar-like flavor, consumed mainly in the northern region of Nigeria, in the republic of Benin, and in Ghana. It is produced mainly from thegrains of guinea corn of the species *Sorghum vulgare*and *Sorghum bicolor*[1].Preparation of burukutu involves steeping, germination, fermentation and maturation as described by Edundayo[2]. The resulting product is a cloudy alcoholic beverage called burukutu.Burukutu contains almost all essential amino acids in required proportion except cystine and tryptophan which are being completely destroyed by heat during boiling[3].Microorganisms associated with the fermentation include bacteria, yeast and occasionally moulds. A number of factors determine the type of microorganisms present in any burukutu; the most important of these are the location of the sorghum plant, the composition of the grain, and the stage of fermentation, equipment and personnel[4]. The genera of bacteria found in burukutu include

Lactobacillus, *Leuconostoc*, and lactic acid bacteria. The yeast found in burukutuare mainly *Saccharomyces* and *Candida*[5].*Lactobacillusfermentum*, *Lactobacilluscellobiosis*, *Lactobacillusbulgaris*, acetic acid bacteria and *Leuconostocmeseteroides* are bacteria associated with sorghum seed and burukutu [6].*Saccharomycescerevisiae*, *Saccharomyces chavelieria,Candidakrusei* and *Candidaguilliermondii*, are fungi associated with burukutu and sorghum seed[7].

Burukutu has a short shelf-life of 1 – 8 days. The short shelf-life may be due to the low lactic acid content, low alcohol content, high concentration of vitamins and fermentable sugars and the presence of lipoxidation products [8].

Elele market and Obinze market is located inside army barracks in Alimini, River state and Owerri, Imo state, Nigeria. The burukutu is produced and sold by people living inside the barracks. The aim of this work is to assess the microbiological qualities of burukutubeverage sold in EleleAlimini market, River state and Obinze market, Imo state, Nigeria.

MATERIALS AND METHODS

Sample collection:
The burukutu beverage samples were obtained from Obinze market Owerri,Imo state and Elele market Alimini, River state, Nigeria. A total of thirty samples of burukutu were purchased from various vendors in the markets, and were aseptically transported to the laboratory in ice parked cooler. They were analyzed immediately on reaching the laboratory.

Chemical Reagents: The microbiological media used were products of Oxoid and DIFCO Laboratories, England. Theyinclude nutrient agar used for the estimation of total aerobic plate count,MacConkey agar for enumeration of total coliform count, eosin methylene blue agar for the enumeration of total fecal countand sabouraud dextrose agar used for the enumeration of fungal organisms.

Enumeration of Microorganisms
The method described by Eze*et al*.[8] was used. Samples ofburukutu were serially diluted in ten folds. Total aerobic plate count(TAPC), total coliform count(TCC),total fecal count(TFC^1) and total fungal count(TFC^2) were determined using pour plate technique. Then the molten nutrient agar, MacConkey, sabouraud dextrose agar and eosin methylene blue agar were poured into the Petri dishes containing 1ml of 10^{-6} dilution for the isolation of the total aerobic bacteria,coliform,fungi,andfecal coliformrespectively. They wereswirled to mix and colony counts were taken after incubating the plates at 30˚C for 48h and preserved by subculturing the bacterial isolates onto nutrient agar and sabouraud dextrose agar slants for the bacterial and fungal isolates respectively. Each sample was analyzed in triplicate.

Characterization and Identification of Isolates
Bacteria isolates were identified by their cell morphology, Gram stain reaction and various biochemical tests. Biochemical tests performed include motility test, oxidase test, catalase test, citrate utilization test, indole test, methyl red test, VogesProskauer test, coagulase, starch hydrolysis, sugar(glucose, sucrose,maltose) fermentation test. The tests were performed according tothe methods of [9, 10].Microbial identification wasperformed using the keys provided in the *BergeysManual of Determinative Bacteriology* [11].

Fungal isolates were examined macroscopically andmicroscopically using Lactophenol blue cotton test. They were identified using fungal atlas [9].

RESULTS AND DISCUSSION

Bacterial organisms isolated from burukutu samples from Elele market and Obinze market belong to the genera *Staphylococcus,Bacillus*, *Lactobacillus*, and *Streptococcus*, while fungal organisms include*Aspergillus species*, *Rhizopus species* and *Sacccharomyces cerevisiae*.The mean counts of the microorganisms isolated from the burukutu samples are shown in Table1. The TAPC, TCC, $TFC^1$and TFC^2 for samples from Elele market are$6.00\pm0.26Log_{10}$, $5.80\pm0.08Log_{10}$, $5.10\pm0.10Log_{10}$, and $6.10\pm0.22Log_{10}$ respectively, while the TAPC, TCC, $TFC^1$and TFC^2 for samples from Obinze market are $5.85\pm0.26Log_{10}$, $5.50\pm0.1.7Log_{10}$, $5.06\pm0.23Log_{10}$, and

6.08±0.08Log_{10}. Statistical analysis showed that therewas no significant difference in the microbial counts of the samples collected from Elele market and Obinze market at p-value > 0.05. Lynn *et al*.[12](2014), in their work on isolation of some pathogens in burukutu, a local drink, sold in Sengere village, Girie Local Government, Adamawa state, observed a total aerobic bacteria count ranging from 1.11 x 10^4-4.00 x10^4 in nutrient agar medium, and indicated that some of the microorganisms isolated are pathogenic.

The high microbial counts obtained from burukutu samples in this study can be attributed to a number of factors,one of which is the display of the sorghum grains on the market table without any form of packaging , thus allowing bacteria and other microorganisms from air to settle on them. Eze*et al*.[8], recorded high microbial count in their study on microbiological and nutritional qualities of burukutu sold in mammy market Abakpa, Enugu State, Nigeriaand noted that the high microbial count can be attributed to large number of people that visit the marketresulting in increased microbial numbers.

Table 1: The mean count microorganisms isolated from burukutu sold at EleleAlimini market, Rivers State and Obinze market Owerri, Imo State.[Log_{10} (X±SD) Cfu/ml i.e. Logarithm Value of the Mean± Standard Deviation]

Samples	TAPC	CC	TFC^1	TFC^2
Elele	6.20±0.26	5.80±0.08	5.10±0.10	6.20±0.22
Obinze	5.85±0.26	5.50±0.17	5.06±0.23	6.08±0.08

KEY: TAPC – Total aerobic plate count; TCC– Total coliform count, TFC^1- Total fecal coliform count; TFC^2– Total fungal Count

Table 2: Percentage occurrence of bacteria isolated from burukutu sold at Elele, Alimini and Obinze, Owerri.

Bacterial isolates	Frequency of occurrence in Elele market	Frequency occurrence in Obinze market	Total number of isolates	Percentage occurrence (%)
Escherichia coli	11(55%)	9(45%)	20	25.97
Staphylococcus aureus	11(52.3%)	10(47.6%)	21	27.27
Bacillus species	6(54.5%)	5(45.4%)	11	14.29
Lactobacillus species	6(54.5%)	5(45.4%)	11	14.29
Streptococcus species	8(57.1%)	6(42.9%)	14	18.18
Total			77	100

Table 3: Frequency of occurrence of fungi isolated from burukutu sold at Elele, Alimini and Obinze, Owerri.

Fungal isolates	Frequency of occurrence in Elele market	Frequency of occurrence in Obinze market	Total number of isolates	Percentage occurrence(%)
Aspergillus species	11(52.4%)	10(47.6%)	21	31.82
Rhizopus species	8(44.4%)	10(55.6%)	18	27.27
Saccharomyces cerevisiae	13(48.1%)	14(51.9%)	27	40.91
Total			66	100

The percentage occurrence of bacteria isolated from the two markets as shown in Figure 1 indicates that *Streptococcus* sp. occured highest at 57.1% in Elele market and lowest at 42.9% in Obinze market. The microbial load of the bacterial isolates was observed to be relatively highest in Elele market. Figure 2 shows the percentage occurrence of fungi isolated from the two markets. *Saccharomycescerevisiae* and*Rhizopus species* occurred highest at frequency of 51.9% and 55.6% in Obinze market while *Aspergillus* species occurred highest at 47.6% in Elele market.

Table 2 shows that *Staphylococcus aureus* was the highest occurring bacteria while *Saccharomycescerevisiae* was the highest occurring fungi (Table 3).

E. coli is an important member of the coliform group[8]. It is a fecal coliform and part of the normal flora of the intestine of human and vertebrates. Its presence in burukutu poses a health threat and care should be taken during preparation of burukutu to ensure zero presence of *E. coli.*

The presence and highest occurence of *S. aureus* in the samples may be attributed to handling duringproduction [7]. *Staphylococcus aureus* is a normal flora on human skin, and can easily contaminate the burukutu during production if aseptic conditions are not adhered to.

Saccharomyces cerevisiae and some of other fungi isolated are associated with fermentation. *Saccharomyces cerevisiae* was isolated as the yeast species responsible for the alcoholic fermentation of burukutu.Achi [13], also isolated *Saccharomyces cerevisiae* as the dominant yeast in burukutu production.

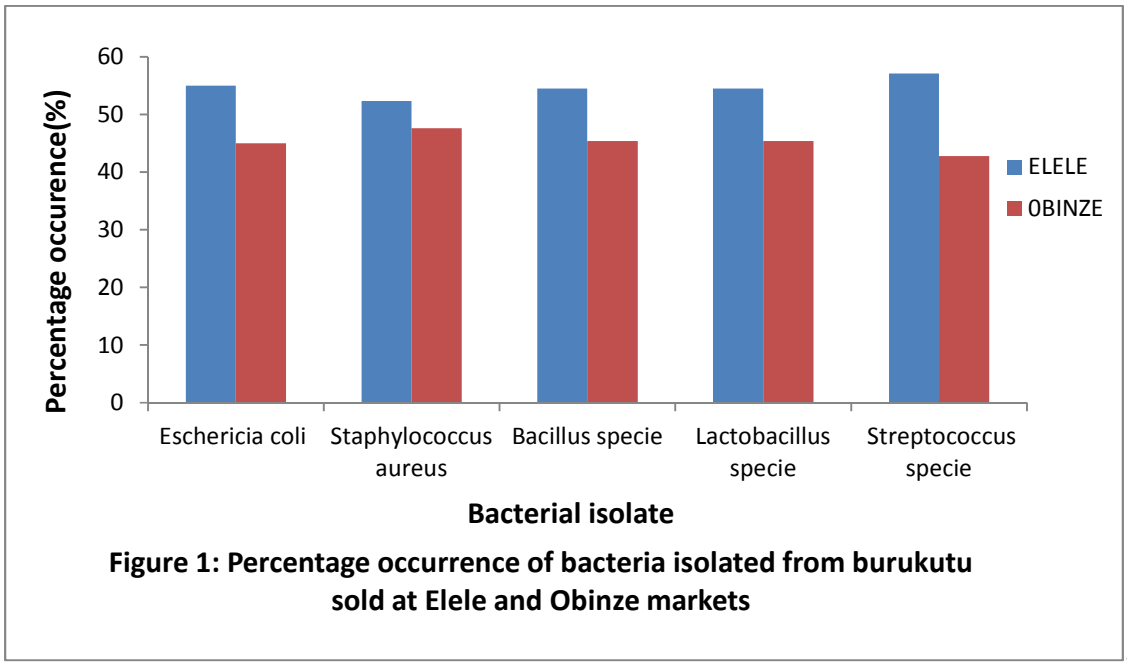

Figure 1: Percentage occurrence of bacteria isolated from burukutu sold at Elele and Obinze markets

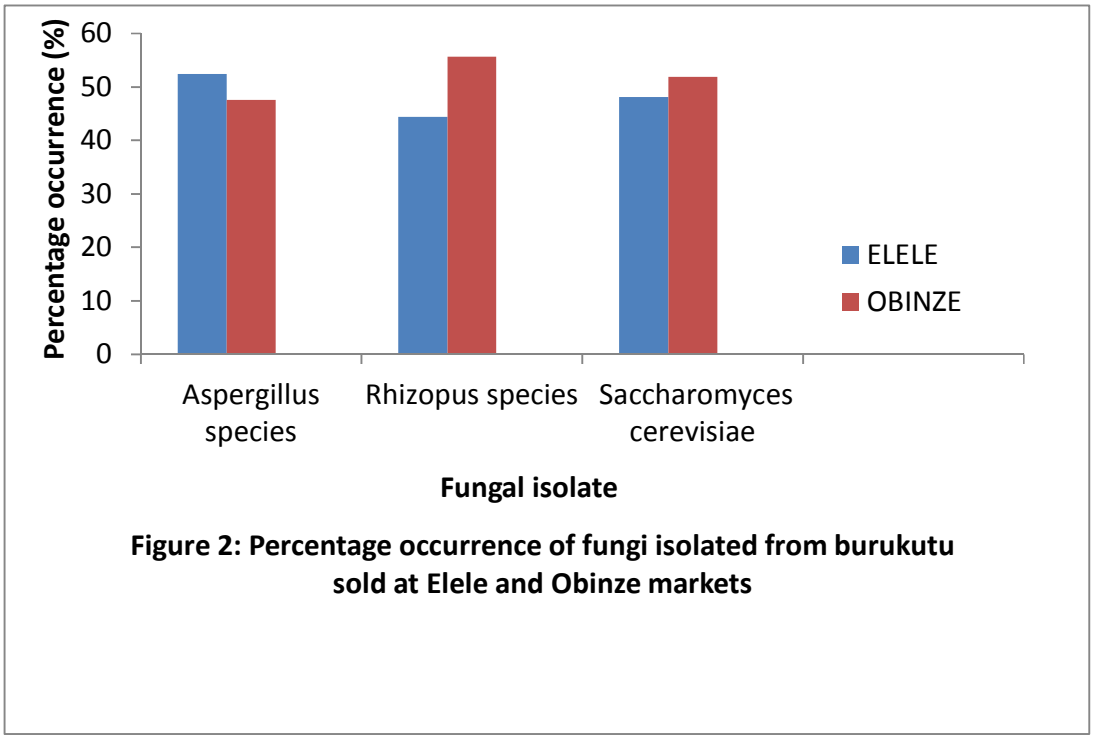

Figure 2: Percentage occurrence of fungi isolated from burukutu sold at Elele and Obinze markets

CONCLUSION

The results obtained from this study indicates that burukutu beverages contain microbial populations, therefore microbiological safety of fermented alcoholic drinks must be ensured by observing proper hygienic conditions during the preparation or processing of the drinks. Handlers of the drinks must be of good health and must obey all rules of personal hygiene, they must be free of any disease and undergo regular routine medical checkup.

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