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Microorganisms Associated with the Production of *Burukutu* (An Alcoholic Beverage) in Kebbi State, Nigeria

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Abstract

Burukutu, typically produced from the grains of guinea corn, is a popular indigenous alcoholic beverage of a vinegar-like flavour consumed in some parts of Africa. Quite a number of microbes play roles in the production of *Burukutu*. The aim of the present study was to determine different microorganisms involved in the three stages of *Burukutu* production. *Burukutu* samples were collected (in triplicate) from three different vendors at Army Barrack Mami Market, Kebbi State, Nigeria. In each case, samples were collected at the three stages (soaking, souring and maturing) and analysed using standard procedures. Results showed that total viable count ranged from 2.8×10^6 cfu/ml to 6.0×10^2 cfu/ml, *Enterobacteriaceae* count ranged from 2.0×10^3 cfu/ml to 1.8×10^2 cfu/ml and fungal count (mould and yeast) ranged from 5.0×10^4 cfu/ml to 1.5×10^2 cfu/ml. While significant number of potential pathogens such as *Escherichia coli and Staphylococcus aureus* were isolated, *Lactobacillus* spp, *Lactococcus* spp, *Actobacter* spp, *Saccharomyces cerevisiae* and *Candida albicans* were found to be predominant. The results also showed that, most of the microorganisms involved at the various stages of production originated from the normal flora of sorghum grains, with the exception of the maturing stage. At the final maturing stage *Acetobacter spp. Lactobacilli* spp as well as fungi which include predominantly *Saccharomyces cerevisiae,* and *Candida albicans*. The microorganisms isolated have the potential to not only cause spoilage of *Burukutu* but can also be used as starter culture for the production of fermented beverages.

Keywords: Microorganisms, *Burukutu*, Alcoholic beverage, Fermentation, Food microbiology

1. Introduction

Burukutu is a popular indigenous alcoholic beverage of a vinegar-like flavour, consumed in the Northern Guinea savannah region of Nigeria, Republic of Benin and in Ghana [1]. Its basic characteristics include slightly sour taste due to the presence of acids including lactic acid, a pH of 3.3 to 3.5 and opaque colour because of suspended solids and yeast [1]. It contains vitamins, iron, manganese, magnesium, phosphorus, and calcium as well as about 26.7 g of starch and 5.9 g of protein per litre. It is mainly produced from the grains of guinea corn [2].

Typically, *Burukutu* is produced by soaking sorghum grains in water throughout the night, after which steeping water is removed, spread on a surface, usually a mat, covered using a layer of plant leaves and allowed to germinates [3]. During germination, water is added daily for typically one week, after which it is dried and ground into powder. To the ground malt, starch of raw grain and/or sweet potato and hot water is added [3, 4]. The blend is usually allowed to sour for 48 h, before it is boiled for about 4 h and kept for another 48 h to mature. The final product is usually a dense beverage (about 3-8% alcoholic) called *Burukutu*. However, some of the endogenous sorghum microorganisms are pathogenic or may produce toxic substances, such as mycotoxins, but pasteurization of freshly brewed *Burukutu* sample at 60 ^oC for 30 min delayed its spoilage for up to two weeks [5,6].

No doubt microorganisms play a significant role in the production of fermented products such as *Burukutu*. Presence of different types of microbes

in fermented foods such as alcoholic beverages has been linked to autochthonous microorganisms of raw materials, preparation surface or sometimes introduced as starter organisms [7]. Microorganisms mainly associated with fermentation of cereals for the production of alcoholic beverages include non-filamentous fungi such as *Candida* spp and lactic acid bacteria including *Lactobacillus reuteri, Lactobacillus plantalum, Lactococcus lactis,* and *Lactobacillus fermentum* [8-10]. In the traditional setting, production of alcoholic beverages relies on introduction of earlier starter culture, thoughts the introduction of already produced fermented product. These starter cultures are interestingly available in commercial quantity, which when used enhance overall quality and quantity of processed fermented products [11]. Additionally, the use of these commercial starter cultures improves the whole process of fermentation. Typically, local production of fermented foods and beverages is carried out in an unhygienic setting that allows a number of microbes to produce the intended end product. Unfortunately, this exposes the final product to not only product contamination, but deterioration [12, 13] occasioned by poor uncontrolled production practice. Therefore, this study aimed at determining the microbial community involved at the three stages of *Burukutu* production with a view to using them to develop starter culture for a standardised production of *Burukutu* as well as establishing the level of contamination of the final product. This is likely to result in enhanced production of *Burukutu*, resulting in the production of products with improved quality.

2. Materials and Methods

2.1 Study Area

The study was carried out in Kebbi State, located in the north-western part of Nigeria. Kebbi State is situated between latitudes 10° 8′ N – 13° 15′ N, and longitudes 3° 30′ E – 6° 02′ E. The State is bordered by Sokoto and Zamfara States to the East, Niger State to the South, Benin Republic to the West and the Niger Republic to the North. The population of the State was 3,238,628 in 2006 (NPC, 2006) and projected to be 3,952,766 in 2012. The State occupies an area of about 36,229 square kilometres [14].

2.2 Sample Collection

Samples during the three stages of processing of *Burukutu* were obtained from Mami Market, Army barrack Birnin Kebbi, Kebbi State, Nigeria. Three batches of *Burukutu* samples were collected from three different *Burukutu* producers in triplicate. The samples were put in sterilized bottles and transported to the Department of Microbiology Laboratory, Federal University Birnin-Kebbi for microbial analysis [15].

2.3 Sample processing

For solid sample (soaked grains), 90 ml of sterilized distilled water was added into 10 g of the ground grains and mixed thoroughly to attain homogenous mixture. Using micropipette, 1 ml of the diluents is transferred into 9 ml of sterilized distilled water in a test tube of 10^{-1}, then 1 ml was also collected from 10^{-1} test tube and transferred to 10^{-2} test tube and this continued to test tube 10^{-6} [16]. For the liquid sample (matured *Burukutu*), 90ml of sterilized distilled water was added into 10ml of the sample. Using micropipette, 1m of the diluents was transferred into 9ml of sterilized distilled water in a test tube 10^{-1}, then 1 ml was also collected from 10^{-1} test tube and transferred to 10^{-2} test tube and this continued to test tube 10^{-6}, then 1ml was inoculated [16].

2.4 Enumeration and Isolation of Bacteria, Moulds, Yeasts and Members of Enterobacteriaceae

At each stage of *Burukutu* production, the pour plating technique was employed using Nutrient Agar (NA) for bacteria, Potato Dextrose Agar (PDA) for moulds, Malt Extract Agar (MEA) (supplemented with streptomycin sulphate) for yeasts, Eosin Methylene Blue (EMB) agar for the members of *Enterobacteriaceae*. Plates for bacteria were incubated at 37 °C for 24 h and for fungi at 27 °C for 7 days. Colonies and spore forming units formed on the media were counted and sub-cultured. Bacterial isolates were examined using microscopy, Gram staining, sugar fermentation test, biochemical tests, such as urease test, catalase test, citrate utilization test and indole test according to the methods of Beveridge [17] and Cheesbrough [18]. Fungal isolates were identified based on their macromorphological and micromorphological features using light microscope and standard mycological manuals by Barnett and Hunter [20], Alexopoulus *et al.* [21] and Kidd *et al.* [22].

2.5 Statistical Analysis

All samples were collected in triplicates. The statistically significant difference in the bacterial and fungal counts among three processing stages was determined by one-way ANOVA test. Data were analysed using SPSS Version 20 and P value of <0.05, was considered statistically significant.

3. Results and Discussion

3.1 Microbial Load of Burukutu

The microbial load of *Burukutu* analysed as shown in Table 3.1 revealed that total viable count ranged from 2.8×10^6 cfu/ml to 6.0×10^2 cfu/ml, *Enterobacteriaceae* count ranged from 2.0×10^3 cfu/ml to 1.8×10^2 cfu/ml and fungal count (mould yeast) ranged from 5.0×10^4 cfu/ml to 1.5×10^2 cfu/ml. Overall, reduced microbial load was observed during maturing stage and insignificant increase was observed during souring. Furthermore, the concentration of microorganisms was statistically significant ($(p\leq0.05)$ in some cases.

Table 3.1: Microbial Load (Log Cfu/Ml) of *Burukutu* At Various Stages of Production

Sampling location	Microbial count parameter	Soaking stage	Souring stage	Maturing stage
A	Total viable count	$2.1\times10^5\pm0.31^a$	$2.8\times10^6\pm0.12^a$	$5.0\times10^3\pm0.33^b$
	Enterobacteriaceae	$5\times10^2\pm0.11$	$2.0\times10^3\pm0.09$	$3.0\times10^2\pm0.04$
	Mould and yeast	$8.1\times10^2\pm0.22^a$	$7.0\times10^2\pm0.18^a$	$5.0\times10^4\pm0.70^b$
B	Total viable count	$2.8\times10^5\pm0.14$	$3.0\times10^5\pm0.1$	$5.5\times10^2\pm0.10$
	Enterobacteriaceae	$9.1\times10^2\pm0.01$	$2.1\times10^2\pm0.10$	$1.8\times10^2\pm0.13$
	Mould and yeast	$2.2\times10^2\pm1.2^a$	$6.3\times10^2\pm0.21^a$	$8.2\times10^3\pm0.17^b$
C	Total viable count	$7.4\times10^4\pm0.02^a$	$5.7\times10^5\pm0.11^a$	$6.0\times10^2\pm0.30^b$
	Enterobacteriaceae	$2.2\times10^2\pm0.15$	$5.5\times10^2\pm0.31$	$1.6\times10^2\pm0.21$
	Mould and yeast	$1.5\times10^2\pm0.21$	$6.3\times10^2\pm0.13$	$8.2\times10^2\pm0.32$

Note: Values are presented in mean ± standard deviation (SD), values with different superscript in the same row are significantly different ($p\leq0.05$). A, B and C represent the three vendors.

The relatively high microbial load observed during soaking cannot be unconnected with the increased microbial activity, as the microbes were able to actively utilise the available substrate in water. Presence of water in substrate increases the ability of microorganism to access and utilise substrates. This is in line with the findings of Oriola *et al.* [12] as well the findings of Générose *et al.* [23], who documented that increase in bacterial load at the initial stages of local beer production before fermentation was a result of their dominancy due to favourable condition and nutrient availability. On the other hand, increased alcoholic content and accumulation of organic acids in the medium might have been responsible for low concentration of bacteria and high fungal load during maturing. A similar finding has been reported by Babatunde and Oladejo [24] and Teshome [25], who in their separate studies reported that lactic acid bacteria produced many organic acids, such as lactic, acetic and propionic acids produced during fermentation as end products which provide an acidic environment unfavourable for the growth of many pathogenic and spoilage microorganisms.

3.2 Microflora of Burukutu

The microflora of the *Burukutu* during soaking, souring and maturing are presented in Tables 3.2 and 3.3**.** Eight (8) bacterial genera including *Escherichia coli*, *Staphylococcus aureus*, *Acetobacter* spp, *Bacillus cereus*, *Pseudomonas aerogenosa*, *Streptococcus* spp, *Lactococcus* spp and *Lactobacilli* spp were isolated in samples collected at various stages of Burukutu production from the production sites (Table 3.2). Whereas the seven (7) fungal species belonging to seven genera including fermenting yeast and mould, were isolated (Table 3.3).

Table 3.2: Frequency of Occurrence of Bacteria Isolated from *Burukutu* Sold in Mami Market, Army Barrack, Birnin Kebbi

Organism	Number of isolates			Frequency of occurrence (%)
	Soaking stage	Souring stage	Maturing stage	
Escherichia coli	6	1	1	8 (11.94)
Staphylococcus aureus	4	4	0	8 (11.94)
Acetobacter spp	1	2	2	5 (7.46)
Bacillus cereus	4	3	1	8 (11.94)
Pseudomonas aerogenosa	1	2	0	3 (4.78)
Streptococcus spp	2	2	2	6 (8.96)
Lactococcus spp	3	2	8	13 (19.40)
Lactobacilli spp	2	2	12	16 (23.88)
Total	23	18	26	67 (100)

Table 3.3: Frequency of Occurrence of Fungi Isolated from *Burukutu* Sold in Mami Market, Army Barrack, Birnin Kebbi

Organism	Number of isolates			Frequency of occurrence (%)
	Soaking stage	Souring stage	Maturing stage	
Aspergillus flavus	4	3	1	4 (12.50)
Aspergillus niger	2	2	1	5 (15.63)
Rhizopus stolonifera	2	2	1	5 (15.63)
Saccharomyces cerevisiae	0	0	3	3 (9.38)
Chaetomium oryzae	1	2	0	3 (9.38)
Penicillium citrinum	1	0	0	1 (3.13)
Candida albicans	1	1	5	7 (21.88)
Total	12	10	11	32 (100)

At soaking stage, all the bacteria and fungi isolated in the present study were present except *P. citrinum* and *S. cerevisiae*. Interesting common environment pollutants such as *A. flavus, A. niger* and bacteria such as *E. coli*, *S. aureus* and *B. cereus* were isolated. These organisms might have been introduced from the plants, utensils and vessels and poor sanitary conditions under which *Burukutu* is produced could also bring about microbial contamination, including contamination with potentially pathogenic microbes. This is in agreement with assertion of Baylis *et al*. [26] Ogunbanwo *et al.* [27] who reported that presence of bacteria such as *Enterobacteriaceae* shows that a failure occurred during processing and their absence indicates that proper hygienic conditions were maintained during the food manufacturing process. Bacteria such as *E. coli, S. aureus* are common on fermenting plant materials and have also been found in the natural fermentation of cereal products; thus, their high load obtained before fermentation in the present work could be due to their possible presence on the sorghum grains from the farm where they were harvested. Furthermore, local alcoholic drinks are typically produced in a dirty environment which not only increases the risk of product contamination, but also, deterioration of final product [12, 13].

During souring, a slight decrease in number of bacteria isolates was recorded, whereas fungal isolates remain relatively the same. This can be linked to increased acidity in the medium which is known to influence the activities of microorganisms. Hassan *et al.* [28] observed that decrease in pH had considerable effect on growth of bacteria negatively during fermentation, and enhanced fungal growth.

At the final stage, *buruku* fermentation process was predominated by *Lactobacilli*, *Lactococcus*, *Acetobacter* and fungal species include *S. cerevisiae* and *C, albicans*. This corroborates the findings of other scholars who studied similar fermented food and documented that yeast species and *Lactobacillus* were the commonest microbes participating in the production of fermented foods [29, 30]. The disappearance of some microorganisms such as *E. coli* and *S. aureus* during the final stage of the production of the

Burukutu may be linked to the increased acidity and the low pH of the fermenting malted grains. Proliferation of lactic acid bacteria in many foods create an unfavourable condition for many bacteria by rapidly increasing acidity in the medium to approximately 3.5, making other microbes unable to grow [31, 32]. Additionally, *Lactobacilli* have the ability to release antimicrobial agents such as hydrogen peroxide which can kill or inhibit the growth of other organisms [33]. This can be attributed to the relative *Enterobacteriaceae* decline during maturing stage.

Presence of lactic acid bacteria and yeast in the final stage may be indicative of their role in the production of *Burukutu*. The mutual association between yeasts and lactic acid bacteria had been noted in several cereal foods [34, 35]. Fermentation of carbohydrates into organic acid by lactic acid bacteria has been reported by Mithun *et al.* [36] and Bhardwaj *et al.* [37]. Acidic medium favoured yeast growth which underlines yeasts multiplication observed in the present study. The presence of moulds at the initial stage of *Burukutu* production and the subsequent elimination at the souring was also reported previously by Omemu *et al.* [34].

4. Conclusion

The present study has provided useful information on the types of microbial communities involved in three stages of *Burukutu* production. Total viable count ranged from 2.8×10^6 cfu/ml to 6.0×10^2 cfu/ml, *Enterobacteriaceae* count ranged from 2.0×10^3 cfu/ml to 1.8×10^2 cfu/ml and fungal count (mould yeast) ranged from 5.0×10^4 cfu/ml to 1.5×10^2 cfu/ml. while significant number of potential pathogens such as *E. coli and S. aureus* were isolated, *Lactobacillus* spp, *Lactococcus* spp, *Actobacter* spp, *Saccharomyces cerevisiae* and *Candida albicans* were found to be predominant. The information from this work would assist in the production of consistent quality of *Burukutu* by using them in selection for starter culture.

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