

Microbial Quality Evaluation of *Zobo* Drink Sold in University of Maiduguri

Bristone C^{1*}, Mariyam K¹, Ogori AF², Badau MH¹ and Joeguluba O³

¹Department of Food Science and Technology University of Maiduguri, Maiduguri, Nigeria

²Department of Home sciences, Federal University Gashua, Gashua, Nigeria

³Department of Agricultural Education Federal College of Education, Kontagora, Nigeria

***Corresponding author:** Bristone C, Department of Food Science and Technology University of Maiduguri, Maiduguri, Nigeria, Tel: +2347036423913; E-mail: bristonecharles@yahoo.com

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Abstract

Determination of the microbial quality of *Zobo* drink was carried out to ascertain its health risk due to increase of consumers' interest. The total aerobic bacteria plate count, coliform count, moulds count, yeasts count, and staphylococcus count of *Zobo* drink sold within the University of Maiduguri Campus were investigated. The method used was collection and analysis of samples from four cardinal locations (North East, North West, South East and South West) of the University of Maiduguri. A total of 12 samples, three from each cardinal point were analyzed for the above named organisms by culturing on selective media (corn meal, mannitol salt, nutrient, macConkey and potatoes dextrose agar). Results obtained shows that the total bacteria plate count ranged from 6.9×10^3 to 9.6×10^3 CFU/ml, total coliform count ranged from 3.7×10^3 to 6.0×10^3 CFU/ml, total moulds count ranged from 1.3×10^3 to 2.3×10^3 CFU/ml, total yeasts count ranged from 1.6×10^3 to 2.4×10^3 CFU/ml and total staphylococcus count ranged from 1.0×10^3 to 1.4×10^3 CFU/ml. The type of microorganisms identified based on their morphological and biochemical characteristics are *E. coli*, *Candida albicans*, *Staphylococcus*, *Klebsiella*, *Shigella* and *Salmonella sp.* Their percentage occurrences from the different samples were also observed. This study revealed facts about possible exposure of consumers of commercial *Zobo* to health hazard. So, a good hygienic practice during its processing is recommended to eliminate contaminants. The use of modern technology for its processing would also reduce this health risk.

Keywords: *Zobo*; Drink; Microbial counts; Evaluation; Quality

Introduction

Zobo is a red non-alcoholic local beverage made from different varieties of dried petal, succulent aqueous acid extracts of Roselle calyx [1]. The beverage has a sour taste and often sweetened. *Zobo* is a name derived from "Zoborodo". It is a local Hausa name as reported by Ayandele [2]. *Hibiscus sabdariffa* has other names such as "Gongura" in Hindi, "Krajeab" in Thailand, "Bissap" in Senegal, "Sorrel" in Caribbean [3]. This non-alcoholic drink is quite popular especially in Northern Nigeria [4].

The increase in religious and health campaign against alcoholic beverages in Nigeria and the subsequent decrease in the consumption of alcoholic beverages in certain areas have made *Zobo* to be a great potential local alternative to imported drinks such as red wine. *Zobo* is found to be rich in vitamin, carbohydrate, protein, calcium, and iron, mineral and other antioxidants. Besides these, it has been used in folk medicine as diuretic, mild laxative treatment for cardiac and nerve diseases and also management of cancer. *Zobo* is also reported to be a good traditional medicine for the treatment of several diseases like hypertension and urinary tract infection [5].

Zobo calyx of Roselle plant has different varieties. The dried matured calyx of the flower of Hibiscus plant may be red, bright red or brown which can yield different colours of *Zobo* drinks [5].

Zobo calyx of *Hibiscus sabdariffa* plant is an annual herb that is widely cultivated in India and Africa [6]. There is an increase in demand of *Zobo* calyx for the production of *Zobo* drink due to its low price and health benefits. The economic recession in Nigeria has made *Zobo* an acceptable choice too many. This has led to its consumption by millions of people from different socio-economic class [7]. The chilled drinks of *Zobo* from shops are being used as a source of refreshment in different gathering. This drink is usually consumed by many groups of people because of its non-alcoholic content and refreshing taste [3].

Ayandele [2] reported that spices usually added being an agricultural commodity may contain high level of microbial load. A large number of lactic acid bacteria, coliform, mould and yeast in *Zobo* drinks have been reported. These microorganisms have implication for food spoilage and possible infection as they utilize the carbohydrate content of *Zobo* drink for fermentation process to produce undesirable compounds. Also

according to the research carried out by Amusa, et al. [6] indicated that the consumption of this local drink is of public health significance. The local drink may also serve as vehicle for zoonotic and food-borne diseases or possible transmission of pathogens such as Staphylococcosis, Salmonellosis, Brucellosis, Tuberculosis, *Escherichia coli* as reported by Amusa, et al. [6] and Ayandele [2].

It is expected that consumption of *Zobo* drink is of more health benefits. However, the recent increase in food infections and poisoning around the world affected the derivatives of these benefits [7]. In spite of nutritional challenges of *Zobo* drinks, it is also often contaminated with diverse group of microorganisms which could be harmful to the individual who consumed it. Packaging materials may also serve as a source of contamination if not well sterilized. School premises are not exception of this problem. Similarly, it is of public health concern in universities where tools for health examination are available but sometimes being neglected. So, institution such as University of Maiduguri where standard hygiene is supposed to be checked and maintained has made this research significant.

The consumption of local drink is of public health concern and so there is a need to evaluate particularly the microbiological quality of *Zobo* sold within the University of Maiduguri to ascertain its level of contamination.

Materials and Method

Study area

This research was conducted within University of Maiduguri. The food samples (*Zobo* beverage) are sold within the University of Maiduguri campus, Borno state. All chemicals and reagents that were used for this study were obtained from the Department of Food Science and Technology, University of Maiduguri. The samples were collected from the four cardinal point of the University of Maiduguri. Each of the three samples collected from the four cardinal points were analysed and the average taken as described by Sylvester, et al. [8].

Description of Food

Zobo is a non-alcoholic beverage, it is a reddish liquid drink that have a sour test. It is obtained from the calyces of *Hibiscus sabalariffa* and has a shelf life of about 2-3 days under room temperature storage condition as reported by Bello, et al. [9].



Figure 1: Zobo drink

Sample and sampling

Three samples each was purchased from the four cardinal point of the University of Maiduguri, representing different sources and manufacturing processes. It was aseptically transferred into a sterile container. The samples were placed in an iced cooler and immediately transferred to laboratory for microbiological analysis as describe by Ayandele [2].

Sterilization of material

All materials that were used in the course of this project such as glassware's were properly washed with detergent and water to remove dirty and contaminations and dried properly. The wash glassware's was sterilized in a portable laboratory autoclave at a temperature of 121°C for 15 minutes as described by Bukar, et al [10]. All media used was also sterilized in the autoclave at a temperature of 121°C for 15minutes Nwachukwu and Osuocha [11].

Preparations of culture media

Nutrient agar (NA): Nutrient Agar was prepared by dissolving 28 g of nutrient agar powder in 1000 ml of distilled water in a clean flask. The mouth of the flask was plugged with non-absorbent cotton wool wrapped with aluminum foil paper that was extended up to the neck of the flask as described by Sylvester, et al. [8]. The flask was placed on a bunsen flame and allows to boil and mix completely. It was sterilized in an autoclave at 121°C for 15 minutes and allowed to cool to 45°C and aseptically dispensed into Petri dishes. Nutrient agar was used for the total bacterial aerobic plate count [12].

Macron key Agar (MA): This agar was prepared by dissolving bile salt, Then 48.5 g of the powder was dissolved in 1000 ml of distilled water. The pH was adjusted to 7.8. It was autoclave at 121°C for 15 minutes

and allowed to cool to a temperature of 45 - 50°C before pouring into plates. This was used to determine coliforms as described by Cheesbrough [13]. This is a selective and differential media designed to isolate and differentiate organism based on their ability to ferment lactose as described by Sebastia, et al. [14].

Corn meal agar (CMA): Corn meal agar was used to isolate yeast and it's prepared by dissolving 17 grams of corn meal powder in a 1000 ml of distilled water. The mixture was heated gently to dissolve the medium completely. 1 % of polysorbate was added and sterilized in autoclave at 121°C for 15 minutes. It was cool at room temperature before pouring into petri dish containing 1 ml of the sample as described by Zumbes et al. [1].

Potatoes dextrose agar (PDA): The medium PDA was prepared by using 39 grams of potatoes dextrose agar powder. It was dissolved in 1000 ml distilled water. It was heated to boiling, in order to get mixed completely. Then sterilized in an autoclave at 121°C for 15minutes, this particular media will be used to this particular media [15].

Mannitol salt agar (MSA): The medium was prepared by dissolving 108 grams of mannitol salt agar in 1000 ml of distilled water, after which it was allowed to stand for 10 minutes, swirled to dissolve properly. The mixture was sterilized in an autoclave at 121°C for 15minutes and allowed to cool to a temperature of 45°C before pouring into the appropriate petri dish as described by Fowoyo [16]. Mannitol salt agar was used to determine and enumerate the bacteria *Staphylococcus*.

Microbiological Analysis

Serial dilution: Distilled water was used for serial dilution, 9 ml each was pipette into a sterile screw cap test tube, the distilled water in the test tube was autoclaved at 121°C for 15 minutes and allowed to cool temperature of 45°C before serial dilution. In each case of dilution, 1 ml of Zobo drink was pipette into 9 ml tube. The process continues until dilution up to 10^3 of the dilution factor was made inoculated into three well labelled petric dish as described by Chidi, et al [17].

Plating of culture and incubation: Pour plate method was used for plating the samples, 1 ml of the diluents of 10^3 was transferred to the petric dish, this was done in triplicate and well labeled (10^3 , 10^3 , 10^3). This procedure was done for each of the samples taken from the four cardinal point. The agar was poured into the petric dish containing 1 ml of the sample, slightly shaken and allowed to solidify and transferred into the incubator at 37°C for

24 hours exception of yeast and mould which extended to 48 hours for easy identification according to the study carried out by Sanni and Adesulu [18].

Viable cell count: The representative petric dish incubated was visualized under a colony counting machine and was used to count the total bacterial count (labtech, india) and result was expressed as colony forming unit per millilitre (CFU/ml) at the end of the count as reported by Olayemi, et al. [19].

Identification of microbial isolate: Identification of the microbial isolate was performed using classical methods based on their morphological and biochemical characteristic with reference to systematic manual of bacteriology described by Cheesbrough [13].

Gram staining technique: Gram staining reaction has the wide application that is capable of distinguishing virtually all bacteria into one of two large group — gram positive or gram negative as describe Dr Hans Christian Gram (1884). Smear of each isolate was made on the slide and heat fixed. Primary stain (crystal violet) was added in drops. Lugols iodine was added for 45 seconds decolorized with acetone and washed with water. It was then air dried examined at X100 under oil immersion as described by Bello, et al. [9]. Positive gram staining appears purple and negative grams staining appeared pink.

Motility: The medium used for motility test (agar with concentration of 0.5%) was inoculated with test organism. A stab of each inoculate was made at the centre of each tube. The tube at 37°C was incubated for 24 hours. A diffused growth at the place of inoculation was considered as positive and restricted growth was considered as negative [20].

Catalase test: Catalase test was carried out using a drop of hydrogen peroxide. 2 ml of 3% hydrogen peroxide (H₂O₂) was placed in a clean test tube. A sterile wire loop was used to pick a colony of the test organism and mixed with 2 ml of 3% hydrogen peroxide (H₂O₂) in the test tube and observed for the production of gas bubbles which indicates a positive reaction. This test was used to identify *Staphylococcus Aureus* [21].

Coagulase test: The use of blood plasma is being introduced in coagulase test. A loop full of human plasma was added to culture isolate on a slide. Positive isolate gave agglutination reagent with plasma. Test was also carried out at 37°C for 24 hours' positive tubes showed

coagulation of the plasma in the tube. This test was used to identify *Staphylococcus Aureus* [21].

Citrate utilization: The ability of an organism to utilize citrate as the only carbon and energy source for growth and ammonium salt as the sole source of nitrogen. Slants of citrate agar were made and the isolates were streaked on the surface of the slant. Positive result gave blue coloration and negative result retained the original colour. The test was used to identify the coli forms as described by Sylvester, et al. [8].

Urease Test: Bacteria, particularly those growing naturally in an environment exposed to urine, may decompose urea by means of the enzymes urease. This ability was tested for using Christensen medium with heavy inoculation of the isolates was made and the agar slants in tube was observed after 24 hours and then incubated further for hours. This test was used to identify coli forms as reported by Musa and Hamm (2013) [22].

Oxidase test: A few drops of kova's reagent were added to piece of filter paper on a petri dish. The bacteria isolates were then smeared on the filter paper with a glass rod. The paper was observed. Positive result gave a dark purple color while negative result showed no color change. This test was used to identify coliforms. As reported by James, et al. [21].

Indole test: This was done by colorimetric reaction with P-Dimethyl-amino-benzaldehyde (Kovac reagent) to determine the ability of isolate to decomposed the amino acid, tryptophane into indole, Peptone Broth was prepared and dispensed in test tube and were inoculated with the isolate and incubated at 37°C for 4 days. 0.5 ml of Kovac reagent was added to each and was tube shaking gently. Positive results were characterized with red alcohol layer; the negative result gives a yellow colour at the junction of the medium. The test used for identification of *Escherichia coli* as described by Ayandele [2].

Methyl red: This was used to detect the production of sufficient acid during fermentation of glucose which indicated by change in colour of the methyl red indicator. Isolates were inoculated into tube of previously prepared glucose peptone and incubated at 37°C for 2 days. Then 5 drops of methyl red solution were added to each tube and colour change was observed. Positive result gave yellow with the indicator as reported by Raima [23].

Voges – proskauer: Tubes of glucose phosphate peptone water were inoculated and incubated at 37°C for 2 days. 1

ml of 40% KOH and 3 ml of 5% solution of 2-naphrol in absolute ethanol was added to each tube. A positive result gives crimson colour in 30 minutes [12].

Isolation and Identification of yeast (*Candida albicans*): The presence of *Candida albicans* was distinguished from the other yeast by the germ tube test using corn meal agar as described by Sanni and Adesulu (18). A straight wire was used to pick of a colony from the surface of potatoes dextrose agar, a deep cut was made on the corn meal agar. A sterile flamed cover slip was plates over the side at inoculum and the plate incubated at 37°C for 24 hours. The cover slip was examined for streaks under microscope using the low power objective, the presence of mycelium bearing ball-like cluster of budding cell and thick walled chlamyospores is characteristic of *Candida aibicans albicans* Briade, et al. [24].

Statcal Analysis: All data were analysed using Microsoft excel program as reported by Zumes, et al. [1]

Results and Discussion

(Table 1) showed the mean total of microbial count of *Zobo* drink on different culture media from the four cardinal points within the University of Maiduguri. The total bacteria plate count ranged from 6.9×10^3 to 9.6×10^3 CFU/ml, total coliform count ranged from 3.7×10^3 to 6.0×10^3 CFU/ml, total moulds count ranged from 1.3×10^3 to 2.3×10^3 CFU/ml, total yeasts count ranged from 1.6×10^3 to 2.4×10^3 CFU/ml and total staphylococcus count ranged from 1.0×10^3 to 1.4×10^3 CFU/ml. Each of these result represents an average value which corresponds with the result obtained reported by Egber, et al. [4].

Sample	TBC	TCC	TSTC	TMC	TYC
South East (Commercial Area)					
A	7.1×10^3	5.1×10^3	1.2×10^3	1.6×10^3	1.7×10^3
B	7.0×10^3	4.3×10^3	1.0×10^3	1.7×10^3	1.8×10^3
C	8.6×10^3	5.3×10^3	1.3×10^3	1.6×10^3	1.3×10^3
South West (Acada Area)					
D	7.1×10^3	4.0×10^3	1.0×10^3	2.1×10^3	1.6×10^3
E	7.1×10^3	5.0×10^3	1.2×10^3	1.8×10^3	2.3×10^3
F	8.1×10^3	4.7×10^3	1.1×10^3	2.4×10^3	2.0×10^3
North East (Complex Area)					
G	8.2×10^3	3.7×10^3	1.4×10^3	2.0×10^3	2.0×10^3
H	6.9×10^3	4.6×10^3	1.2×10^3	1.6×10^3	1.8×10^3
I	9.6×10^3	6.0×10^3	1.2×10^3	2.0×10^3	2.3×10^3
North West (Unimaid Quarters)					
J	9.4×10^3	4.7×10^3	1.3×10^3	2.0×10^3	2.2×10^3
K	9.1×10^3	4.0×10^3	1.2×10^3	1.8×10^3	1.7×10^3
L	7.4×10^3	5.8×10^3	1.1×10^3	1.7×10^3	1.6×10^3

Table1: Microbial count (CFU/ml) of *Zobo* drinks obtained from the University of Maiduguri

Each value is a mean of triplicate determination

Note: TBC = Total bacterial count, TCC = Total colifoms count, TSTC = Total staphylococcus count, TMC = total mould count, TYC = Total yeast count

The samples with highest microbial count based on category are: sample I from the North West with total aerobic bacteria count of 9.6×10^3 CFU/ml, followed by sample I from North East with total coliform count of 6.0×10^3 CFU/ml, next to this one was sample F (South West) with 2.4×10^3 CFU/ml of total moulds count, followed by sample E (South West) and I (North East) both with 2.3×10^3 CFU/ml total yeasts count, and lastly sample G from North East with 1.4×10^3 CFU/ml of total staphylococcus count (Table 1). All samples indicated significant level of

contamination. Their presence may be due to both pre and post contamination. Although all the samples were contaminated with varying levels of microbial counts that can be classified as unsatisfactory [1]. It is possible that the occurrence of these pathogens occurred during processing, which was reported as the major source of contamination of locally made drinks by Fowoyo [16]. Necessary precautions might have been neglected and as such contamination could be inevitable as reported by Musa and Hamza [22].

(Table 2 and 3) shows the isolation of *Staphylococcus aureus* (an enterotoxins producer). This organism was reported to be responsible for staphylococcal food poisoning, which may also cause similar effect in *Zobo* drink. From this study, samples of *Zobo* have revealed that *Zobo* drink can be a potential source of bio toxins which may cause health issues such as either being acute or chronic [19]. The presence of *Staphylococcus aureus* in *Zobo* drink is a pointer to largely poor hygiene, improper storage facilities and use of low quality raw material [25-43]. The isolation of yeast from these drinks may be linked to contamination (Table 2 and 3). The percentage

occurrence of *E. coli* was observed to be present in all the samples of *Zobo* drink. *E. coli* is an important member of the coli form group, which its presence could render beverage unsuitable for consumption. *Staphylococcus aureus* was also isolated from the samples. It is a normal flora of the skin, nose, and throat Musa and Hamza [22]. The presence of the following organisms which were identified could cause diseases like fever (*salmonella*), shigellosis (*shigella*), *E. coli* causes food poisoning and *Staphylococcus aureus* staphylococcal food infection and intoxication.

Cultural characteristic	Gram reaction	Motility Test	Coagulase	Catalase	Methyl red test	Indole test	Citrate test	Oxidase test	Voges-Proskauer	Urease test	Isolate
Smooth oily lactose fermenter	-Rod	+	-	+	+	+	-	-	-	-	<i>E.Coli</i>
Smooth cream colony	+ cocci	-	+	+	+	+	-	-	-	-	<i>Staphylococcus</i>
Grey white irregular colony	-Rod	+	-	+	-	-	-	-	+	+	<i>Klebsiella</i>
Pale colour non lactose fermenting colonies	-Rod	-	+	+	+	-	+	-	-	-	<i>Shigella spp</i>
Smooth cream colony	+ cocci	-	+	+	+	+	-	-	-	-	<i>Staphylococcus spp</i>
Large grey white mucoid colonies	-Rod	+	-	+	+	-	+	+	+	-	<i>Salmonella spp</i>

Table 2: Cultural, morphological and biochemical characteristics of bacterial isolate from *Zobo* drink

Note: + = positive , - = negative

Isolate	Sample location				
	South East	South West	North East	North West	% Occurance
<i>E.coli</i>	+	+	+	+	50
<i>Salmonella</i>	+	-	+	-	25.5
<i>S. aureus</i>	+	+	+	+	50
<i>Shigella</i>	-	-	+	-	12.5
<i>Candida albicans</i>	-	-	+	+	25
<i>Shigella</i>	+	+	-	+	37.5

Table 3: Percentage occurrence of microbial isolates in *Zobo* drinks

Note: - = Negative, + = Positive

Occurrences of these microorganisms are largely due to their presence in nature. Their association with foods such as the commercial *Zobo* may be as a result of poor hygiene or poor sanitary condition as reported by Raima [23]. The isolation of coliform bacteria in all the *Zobo* samples exceeds the recommended limit of zero

coliform/ml in drinks. These coliforms are potential hazard for human especially during food consumption [23]. Coliforms, whose natural habitat is the intestinal tract of man and animal, revealed possible association of these faecal indicators into the commercially procured *Zobo*. Their presence may also indicate the presence of

faecal or contamination by sewage introduced into the *Zobo* via the use of contaminated water or from the unsanitary environment during processing [2].

Conclusion

It was revealed in this study the type of microorganisms encountered. These organisms include coliforms, yeasts, moulds, *E. coli*, *Salmonella*, *Shigella* and *Staphylococcus aureus*. These are contaminants and are microbial associates of commercially sold *Zobo* drink. Their presence and levels are indicators of public health risk.

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