Vol. 2 No. 1, June 2022

FUAM Journal of Pure and Applied Science

Available online at
www.fuamjpas.org.ng

An official Publication of
College of Science
Joseph Sarwuan Tarka University,
Makurdi.

FUAM Journal of Pure and Applied Science
© College of Science, Joseph Sarwuan Tarka University, Makurdi
https://fuamjpas.org.ng/
Vol.2. No. 1. June, 2022

Bacteriological Assessment of Hawked Zobo Drink (*Hibiscus sabdariffa*) in Makurdi Metropolis, Benue State

***B.V. Ado, R.A. Odeh and P.T. Aernan**
Department of Microbiology, College of Biological Sciences, Joseph Sarwuan Tarka University Makurdi, Benue State, Nigeria.
*Correspondence E-mail: adobenjamin2014@gmail.com

Received: 16/02/2022 **Accepted**: 24/02/2022 **Published online**: 07/03/2022

Abstract
Zobo is produced from Roselle leaves (*Hibiscus Sabdariffa*) and is consumed by most Nigerians for its thirst-quenching ability, nutritional content, availability and low cost. Thirteen zobo samples were purchased from six locations in Makurdi; namely, Wurukun Market (WM), Wadata Market (WDM), North Bank Market (NBM), Modern Market (MM), Kanshio Area (KA), and University of Agriculture Makurdi (UAM). Samples were assessed by standard microbiological techniques using Nutrient Agar (NA), Eosine Methylene Blue Agar (EMBA) and Salmonella-Shigella Agar (SSA) for Total Viable Counts (TVC). Isolated bacteria were identified using cultural, morphological and biochemical characteristics. Thirty two bacteria comprising nine genera were identified, and their percentage frequencies were; *Staphylococcus* spp. (28.1%), *Escherichia coli* and *Pseudomonas* spp. (18.8%), *Bacillus* spp. and *Klebsiella* spp. (9.4%), *Salmonella* spp. (6.3%), and *Proteus* spp., *Serratia* spp., and *Lactobacillus* spp. (3.1%). TVC on NA were 1.3 x10^5 cfu/mL - 2.0 x10^6 cfu/mL, SSA 3.0 x 10^3 - 2.7 x 10^7cfu/mL and EMBA 8.0 x 10^3 - 1.3 x 10^6 cfu/mL. pH of zobo at room and refrigerated temperatures decreased from 3.40 at zero hours to 2.90(3.10) - 0.90(2.40) at 24 h and 168 h respectively, and with less than 48 h shelf life at room temperature. Most samples recorded TVC above 1x10^4 cfu/mL and coliform counted as unsafe for ready-to-eat food-drinks with the risk of foodborne infection/disease out weighing any benefits of consuming the product. Thus, to promote a wholesome product and safeguard public health relevant authorities should sensitize processors/vendors on good manufacturing practices, and the public on risks of consuming contaminated zobo

Keywords: Zobo, *Escherichia coli*, *Salmonella* spp., *Staphylococcus* spp., *Bacillus* spp.

Introduction

Zobo drink is a non-alcoholic locally processed beverage consumed by many socio-economic, ethnic and religious groups across Nigeria [1]. It is a dark red liquid which tastes like fruit punch and is rich in natural carbohydrates, protein, antioxidants, vitamin A and C, riboflavin, niacin, calcium, iron, magnesium and potassium [1,2], The majority of people consume it because of its nutritive value, health benefits and low prices [2,3]. Some of the health benefits include being diuretic, cholerectic, febrifugal, antihypertensive, anti-helminthic, and antimicrobial [4]. In addition, it reduces blood viscosity and stimulates intestinal peristalsis [3]. Furthermore, consumption of zobo may enhance bone and tooth formation as it is very rich in vitamin C, calcium, magnesium, iron and zinc [3]. Studies show that it has low glycemic index of 33 ± 3 which is suitable for maintaining normal blood sugar and body weight [3, 5]. Thus, it is preferred over carbonated drinks.

Zobo is produced by boiling and filtering the red dried calyces of *Hibiscus sabdariffa* [6]; and is consumed either hot as tea or chilled as refreshment or appetizer at ceremonies [6], or sold in markets, schools, and workplaces or hawked in streets. Since its production is simple and occurs at household level, it is completely unregulated and as such prone to contamination by bacteria [7]. Water is the main component in zobo production and its quality is a major source of contamination [5, 7, 8]. Other sources are poor personal hygiene of processors or retailers, packing materials such as polyethylene bags, recycled plastic bottles, cooling and dilution of product, and addition of sweeteners and other additives [1, 2]. As a ready-to-eat food-drink, zobo is prone to rapid deterioration by bacteria because it lacks post production treatment that could eliminate or at least minimize the bacterial load [1]. Consequently, the greatest challenge to the large-scale production of zobo is its short shelf life of less than 48 hours at room temperature [6, 9]. At ambient temperature, the pH of zobo decreases as bacteria metabolizes the sugars to produce organic acids [9].

Staphylococcus aureus, *E. coli* and *B. cereus* are indicator organisms in accessing the safety of ready-to-eat foods [2]. Bacteria belonging to several genera such as *Bacillus*, *Pseudomonas*, *Streptococcus*, *Staphylococcus*, *Corynebacterium*, *Aeromonas*, *Veillonella*, *Micrococcus*, *Leuconostoc*, *Lactobacillus*, *Escherichia*, *Enterococcus*, and *Proteus* have been isolated from zobo drinks sold in different parts of Nigeria [1, 10, 11, 12, 13]. Most of the bacteria, including *Staphylococcus, Pseudomonas*, *Streptococcus*, *Klebsiella*, *Salmonella*, *Bacillus* spp., *E. coli* are major health concern to man [14, 15]. According to the Soy Food Association of America (SFAA) *Staphylococcus aureus* and *E. coli* should be absent from drinks; otherwise, such are unfit for consumption

[2, 16]. Bacteremia, gastrointestinal tract diseases are caused by some species of *Bacillus*, urinary tract infections by some species of *Proteus*, intestinal disease, diarrhea, meningitis, sepsis by some strains of *E. coli*, enteric fever by *Salmonella*, and staphylococcal food poisoning by *S. aureus* [10].

The poor state of basic public infrastructure such as lack of potable water and inadequate power supply has hindered large-scale production and proper storage of zobo in Nigeria. Thus, contamination and foodborne diseases can be minimized using potable water, sterile packing materials, brief pasteurization of the product after packaging at a temperature ≤80 - ≥100°C, and improving the hygienic status of zobo handlers.

The demand for zobo drink is on the increase in many cities and towns across Nigeria, including Makurdi metropolis. Several studies carried out in different parts of the country; namely, Ibadan, Ogbomoso and Akure, Benin, Aba, and Yenagoa, Kaura-Namoda and Maiduguri amongst others [1, 10, 11, 12, 13], have all isolated microorganisms with potentials to cause foodborne illnesses from zobo drink. However, information on the quality and bacteriological safety of zobo food-drinks sold in Makurdi metropolis is scarce which this study seeks to address. Therefore, the study is to determine the bacteriological safety of zobo drink consumed in our locality, sensitize members of the public towards patronizing the product, and provide information for decision makers to enhance monitoring of zobo drink.

Materials and Methods

Sample collection

Samples of zobo drink hawked in used plastic bottles were purchased from retailers in 500 mL sterile glass bottles with screw-metal caps from six locations in Makurdi; namely, Wurukun Market (WM), Wadata Market (WDM), North Bank Market (NBM), Modern Market (MM), Kanshio Area (KA), and University of Agriculture Makurdi (UAM). Samples were immediately transported in ice packs to Microbiology Laboratory, UAM for microbiological analysis.

Laboratory preparation of zobo

Thirty grams (30 g) of the dried calyces were submerged into a 1000 mL Erlenmeyer flask containing 500 mL of sterile distilled water. It was allowed to stand for 40 minutes at 100°C in a water bath [9]. The calyces were filtered using 2 mm size sieve and the zobo drink was aseptically collected into 250 mL sample bottles [9].

Determination of pH

Ten mL of zobo samples was diluted by 10 mL of distilled water and a standardized pH meter was use to take readings [17].

Preparation of media

Three different media were used to enumerate Total Viable Count (TVC) of the samples. Nutrient Agar (NA) was used for obligate and facultative bacteria, Eosine Methylene Blue Agar (EMBA) for members of the *Enterobacteriaceae* including coliforms, and Salmonella-Shigella Agar (SSA) for *Salmonella* and *Shigella*. All media were prepared according to the manufacturers' instructions.

Inoculation and enumeration of samples

Approximately 1 mL of zobo drink was measured into 9 mL sterile 0.1% peptone water as a diluent to make a 1:10 dilution and the procedure was repeated to obtain a ten-fold serial dilution. One mL of each dilution factor was inoculated on appropriate medium using the pour plate method and incubated at 37°C for 24 - 48 hours [1]. Colonies were counted and expressed as colony-forming units per millilitre (cfu/mL).

Isolation of bacteria

Pure cultures were obtained by streaking of primary cultures on appropriate medium and incubating at 37°C for 24 hours to obtain discrete colonies. Discrete colonies were aseptically transferred onto appropriate slants and incubated at 37°C for 24 hours. Slants were stored at 4°C for further characterization.

Characterization of bacteria isolates

Morphological characterization of isolates was carried out by Gram staining following microscopic examination. All bacteria were cultured on NA prior to biochemical tests (viz. oxidase, indole, catalase, coagulase, citrate, and sugar fermentation test). The resultant characteristics were compared with those of known taxa using Bergey's Manual of Determinative Bacteriology [18, 19].

Oxidase test

A freshly prepared 0.2 g tetra-methyl-phenylene-diamine hydrochloride in 20 mL distilled water was flooded over inoculated plates after 24 hours incubation, and the excess immediately drained off. Colonies with dark purple colour were positive for coliforms [20]

Indole test

An emulsified colony of the test organism was cultured in tryptophan broth and incubated at 37°C for 24hrs, and 0.5 mL Kovac's reagent added to the broth culture. Positive results were characterized by development of a red alcohol layer on top of the reagent within one minute. The test was use to identify *Escherichia coli* [18, 21].

Catalase test

The test was carried out to differentiate *Staphylococcus* spp. from *Streptococcus* spp. and other catalase-positive from catalase-negative bacteria. A pure colony of the test isolate was transferred onto a clean grease-free glass slide. Thereafter, a drop of 3% hydrogen peroxide was added and production of gas bubbles recorded as positive result [20].

Coagulase test

A pure culture was aseptically transferred to a drop of serum on a clean grease-free glass slide, emulsified and observed for clumping. Presence of clumping within 10 seconds indicated a positive test. This test was used to identify *Staphylococcus aureus* [20].

Citrate utilization test

A sterile inoculating needle was use to inoculate Simon's citrate agar slants with the test organism and incubated at 37°C for 24 - 72 hours. The development of a deep-blue colour indicated a positive result and the test was use to identify coliforms [10, 18].

Sugar fermentation test

The sugar ability of isolates to utilize sugars was tested using glucose, lactose, sucrose maltose and fructose. The fermentation broth comprised 0.5 g sodium chloride (NaCl) and 0.0189 mg phenol red in 100 mL of distilled water. Five milliliters (1% solution) of each sugar was separately added into each test tube containing inverted Durham tubes. The test tubes and contents were autoclaved at 121°C for 10 minutes. After cooling, isolates were inoculated and incubated at 37°C up to 72 hours. A colour change from red to yellow was positive for acid production and presence of gas bubbles in Durham tubes positive for gas production. The test was conducted in replicates [22].

Results and Discussion

Zobo is a locally processed ready-to-eat food-drink consumed by many Nigerians. It is preferred for its high thirst quenching ability and nutritional content compared to other drinks such as kunu and soya milk [23]. In this study, a total of thirty two (32) bacteria spread across nine genera were isolated from different samples of zobo. These were: *Staphylococcus* spp., *Escherichia coli*, *Pseudomonas* spp., *Bacillus* spp., *Klebsiella* spp., *Salmonella* spp., *Proteus* spp., *Serattia* spp., and *Lactobacillus* spp. which agreed with some authors [6, 24, 25] (Table 1). Several studies have shown that *Staphylococcus*, *Escherichia*, *Proteus*, *Pseudomonas*, *Salmonella*, *Shigella*, *Enterobacter*, *Klebsiella*, *Serratia*, *Bacillus*, *Streptococcus*, *Lactobacillus*, *Clostridium*, *Corynebacterium*, *Aeromonas* and *Micrococcus* are the most widely isolated bacteria from zobo hawked across Nigeria [1, 2, 10, 19]. And to a large extent, the most predominant isolates are *Staphylococcus*, *Escherichia*, *Proteus*, *Pseudomonas*, *Klebsiella*, *Bacillus*, and *Streptococcus* [10] which agreed with our findings. Since production of zobo is a household trade that is highly unregulated in many localities, in Nigeria [7]; these bacteria gain entry through unhygienic practices, such as, the use of non-potable water for processing, packaging or dispensing of products in polyethylene bags or recycled plastic containers, cooling and diluting of product, adding of sweeteners and flavours, and hawking [1, 2].

Table 1: Bacteriological diversity and distribution found in purchased zobo samples

Location	Bacteria isolate	Number (%)
MM	*E. coli*	2(6.30)
MM	*Proteus* spp.	1(3.10)
MM	*Klebsiella* spp.	1(3.10)
MM	*Pseudomonas* spp.	1(3.10)
MM	*Staphylococcus* spp.	1(3.10)
NBM	*Staphylococcus* spp.	3(9.40)
NBM	*Klebsiella* spp.	1(3.10)
NBM	*E. coli*	1(3.10)
KA	*Staphylococcus* spp.	1(3.10)
KA	*Bacillus spp.*	1(3.10)
KA	*Salmonella* spp.	1(3.10)
WUM	*Pseudomonas*	2(6.30)
WUM	*Bacillus* spp.	2(6.30)
UAM	*E. coli*	2(6.30)
UAM	*Pseudomonas* spp.	2(6.30)
UAM	*Staphylococcus* spp.	1(3.10)
UAM	*Salmonella* spp.	1(3.10)
WDM	*E. coli*	1(3.10)
WDM	*Klebsiella* spp.	1(3.10)
WDM	*Pseudomonas* spp.	1(3.10)
WDM	*Staphylococcus* spp.	2(6.30)
CTR	*Serratia* spp.	1(3.10)
CTR	*Staphylococcus* spp.	1(3.10)
CTR	*Lactobacillus* spp.	1(3.10)
Total		**32(100)**

Key: MM = Modern Market, NBM = North Bank Market, WDM = Wadata Market, WM= Wurukum Market, KA = Kanshio Area, UAM = UniAgric Makurdi, CTR = Control.

In the study, *Staphylococcus* spp. had the highest number 9(28.1%), followed by *Escherichia coli* and *Pseudomonas* spp. each with 6(18.8%), *Bacillus* spp. and *Klebsiella* spp. each with 3(9.4%), and *Salmonella* spp. with 2(6.3%); while *Proteus* spp. *Serratia* spp. and *Lactobacillus* spp. each had 1(3.1%). However, in another study *Bacillus cereus* and *Staphylococcus aureus* were the most frequently isolated with frequencies of 80 % and 50% respectively; while *Escherichia coli*, *Corynebacterium* spp. and *Clostridium* spp. each had 10% [2]. Similarly, another study reported percentage frequencies of 21.6% and 16.2% for *Bacillus* spp. and *Staphylococcus aureus* respectively [13]. The high prevalence of *Staphylococcus* spp. in the study indicated poor personal hygiene of processors or vendors, improper storage, use of low quality raw materials and unsanitary environment [1]; thus, a potential risk of *Staphylococcal* food poisoning. Whereas, presence of *E. coli* and *Salmonella* spp. clearly showed faecal contamination and the risk of enteric fevers on acute or chronic basis [1]. This is because many gastro intestinal illness of assumed unknown aetiology arise from consuming drinks contaminated with microorganisms [1]. Similarly, the high prevalence of *Bacillus* spp. in the samples is of public health concern. It is established that *B. cereus* produces diarrhea-type food poisoning which progressively leads to liver failure or even death. This is due to the production of pH and heat-stable toxins in contaminated drinks [2].

Table 2 shows Total Viable Count (TVC) on different media. Total Viable Count on Nutrient Agar ranged from 1.0×10^5 - 2.0×10^6 cfu/mL, while on EMBA and SSA the TVC ranged from 8.0×10^3 - 1.3×10^6 cfu/mL and 3.0×10^3 - 2.7×10^7 cfu/mL. All counts on NA were above the stipulated limit of 1.0×10^4 cfu/mL by NAFDAC for ready-to-eat foods respectively [9, 26]. Except for few samples, TVC on EMBA and SSA were above the stipulated limits which indicated high contamination and rendered the product unfit for consumption. More so, the product quality violated the recommended limit of zero coliform for ready-to-eat foods [10].

Several studies have reported similar microbial counts in zobo drink consumed across Nigeria. In Kano, a total viable count of < 30 - 1.23×10^4 cfu/mL was reported while total viable count of 0.3 - 4.4×10^6 cfu/mL, and total coliform count of 0.1 - 6.5×10^5 cfu/mL were obtained in Aba. Similarly, total viable counts of 5.20 - 7.70 cfu/mL, total coliform of $\times 10^4$ cfu/mL were obtained in Jos. Also, total viable count of 3.0×10^2 - 1.0×10^5 cfu/mL were recorded in Awka, while 1.2×10^2 - 1.2×10^6 cfu/mL was obtained in Osun [1, 2, 6, 19]. These findings corroborate the result of our study. The slight variations may be due to handling, quality of materials used for processing and the hygienic status of processors and vendors.

Furthermore, zobo is rich in natural carbohydrates, protein, vitamins and minerals such as calcium, magnesium, potassium, zinc, iron and phophorus [2, 5, 6]. Therefore, bacteria quickly deteriorate the product at room temperature resulting in low nutrient content, off-flavours, odours, appearance or texture. This causes it to serve as a vehicle for infection or foodborne diseases when unrefrigerated [1, 6]. The high coliform counts and the isolation of *E coli* and *Salmonella* spp. is of public health concern. Reportedly, *E. coli* is responsible for prevalence of diarrhea, fever, nausea, and cramps in children and adult exposed to contaminated drinks [27]. Of equal importance is the presence of *Staphylococcus* spp. and *Baccillus* spp. which causes food poisoning in contaminated drinks, and with inoculum above 10^4 cfu/mL capable of producing infection in humans [2].

Table 2: Total viable count (cfu/mL) obtained from zobo samples on different media

Sample Location	NA	SSA	EMBA
MM	4.0×10^5	3.8×10^5	1.3×10^6
NBM	3.0×10^5	1.3×10^4	8.7×10^5
KA	1.8×10^6	1.5×10^4	8.5×10^5
WM	1.3×10^5	3.0×10^3	8.0×10^3
UAM	1.9×10^6	2.5×10^6	2.8×10^5
WDM	2.0×10^6	2.7×10^7	5.0×10^5
CTR	1.0×10^5	-	-

Key: NA = Nutrient Agar, SSA = *Salmonella* - *Shigella* Agar, EMBA = Eosine Methylene Blue Agar.
MM = Modern Market, NBM = North Bank Market, WDM = Wadata Market, WM= Wurukum Market, KA = Kanshio Area, UAM = UniAgric Makurdi, CTRL = Control.

Table 3 presents the cultural and morphological characteristics of bacterial isolates on different media. *E. coli* showed round green metallic sheen colonies and *Kblesiella* spp. presented pink circular colonies both on EMBA. *Staphylococcus* spp. showed round golden yellow colonies on NA and *Salmonella* spp. presented round black colonies on SSA. *Staphylococcus aureus*, *E. coli* and *B. cereus* are indicator organisms in accessing the safety of ready-to-eat foods. According to the Soy Food Association of America (SFAA) *Staphylococcus aureus* and *E. coli* should be absent from drinks; otherwise, such are unfit for consumption [2, 16]. This raises public health concerns because unsuspecting members of the community, including schoolchildren consume contaminated beverages. *Staphylococcus aureus* and *E. coli* are notable producers of heat-stable enterotoxins which increase with proliferation of the bacteria [2]. Therefore, consumption of the contaminated beverages could result in food poisoning which would over shadow the nutritional benefits of consuming the product. The presence of *Lactobacillus* spp. in the samples indicated that the zobo could probably undergo fermentation when kept for a long

time. The morphological and biochemical characterization of bacteria isolated from samples of zobo drink is shown in Table 4. Plate I presents some micrographs of the isolated bacteria showing cell structure and Grams reactions. Contrary to the findings of other studies [1, 13], Gram negative bacteria were the predominant isolates obtained from zobo samples in this study.

Table 3: Cultural characteristics of bacteria isolated from zobo drink on different media

Media	Shape of colony	Colour	Elevation/Consistency	Bacteria isolate
EMBA	Round	Greenish metallic sheen	Convex and mucoid	*Escherichia coli*
EMBA	Round	Colourless glistening Flat colony		*Proteus* spp.
EMBA	Circular	Pink	Convex and mucoid	*Klebsiella* spp.
EMBA	Round	Colourless	Convex	*Pseudomonas* spp.
SSA	Round	Black	Flat	*Salmonella* spp.
NA	Round	Golden yellow	Raised and moist	*Staphylococcus* spp.
NA	Irregular	Grey-white	Flat and swarming	*Bacillus* spp.
NA	Irregular	Reddish	Swarming	*Serratia* spp.
NA	Irregular	Creamy	Flat and swarming	*Lactobacillus* spp.

Key: EMBA = Eosine Methylene Blue Agar, SSA = Salmonella - Shigella Agar, NA = Nutrient Agar

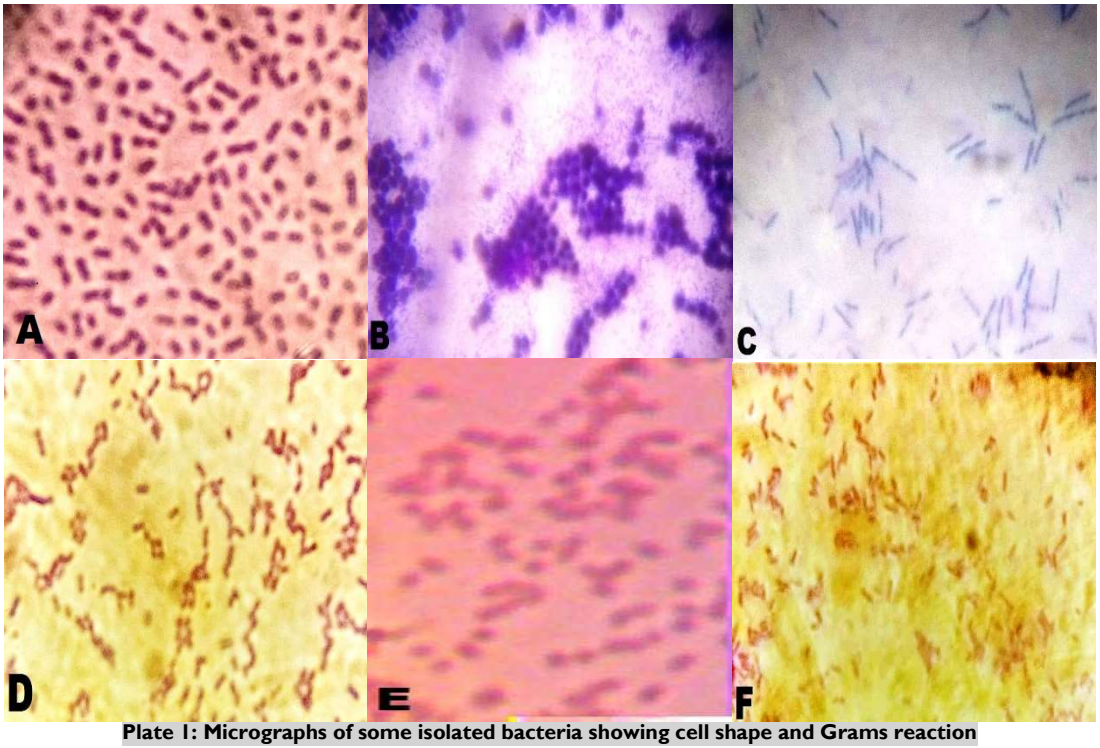

Plate 1: Micrographs of some isolated bacteria showing cell shape and Grams reaction
(**A**) *Pseudomonas* spp., (**B**) *Staphylococcus* spp., (**C**) *Bacillus* spp., (**D**) *Proteus* spp. (**E**) *E. coli*, (**F**) *Klebsiella* spp.

Table 4: Morphological and biochemical characterization of bacteria isolated from samples of zobo drink

Code	Isolate	Cell shape	Gram reaction	Cat.	Oxi.	Coa.	Cit.	Ind.	H_2S production	Lac.	Glu.	Suc.	Fru.	Mal.
MM_1	*E. coli*	Rod	-	+	-	-	-	+	-	AG	AG	AG	AG	A
MM_2	*Proteus* spp.	Rod	-	+	-	-	+	+	-	-	AG	-	-	-
MM_3	*Klebsiella* ssp.	Rod	-	+	-	-	+	-	-	AG	AG	AG	-	AG
MM_4	*Peudomonas* spp.	Rod	-	+	+	-	+	-	-	-	A	-	A	-
MM_5	*E. coli*	Rod	-	+	-	-	-	+	-	AG	AG	AG	AG	-
MM_6	*Staphylococcus* spp.	Cocci	+	+	-	+	+	-	-	A	A	A	A	A
NB_1	*Staphylococcus* spp.	Cocci	+	+	-	+	-	-	-	A	A	-	A	A
NB_2	*Klebsiella* spp.	Rod	-	+	-	-	+	-	-	AG	AG	AG	-	AG
NB_3	*Staphylococcus* spp.	Cocci	+	+	-	+	+	-	-	A	A	-	A	A
NB_4	*Staphylococcus* spp.	Cocci	+	+	-	-	+	-	-	A	A	-	A	A
NB_5	*E. coli*	Rod	-	+	-	-	-	+	-	AG	AG	AG	AG	A
KA_1	*Staphylococcus* spp.	Cocci	+	+	-	+	+	-	-	A	A	-	A	A
KA_2	*Bacillus* spp.	Rod	+	+	-	-	+	-	-	A	A	-	A	A
KA_3	*Salmonella* spp.	Rod	-	+	-	-	-	-	+	-	A	-	-	A
WU_1	*Pseudomonas* spp.	Rod	-	+	+	-	+	-	-	-	A	-	A	-
WU_2	*Pseudomonas* spp.	Rod	-	+	+	-	+	-	-	-	A	-	A	-
WU_3	*Bacillus* spp.	Rod	+	+	-	-	+	-	-	A	A	-	A	A
WU_4	*Bacillus* spp.	Rod	+	+	+	-	+	-	-	-	A	A	-	-
UA_1	*E. coli*	Rod	-	+	-	-	-	+	-	AG	AG	AG	AG	A
UA_2	*Pseudomonas* spp.	Rod	-	+	+	-	+	-	-	-	A	-	A	-
UA_3	*Pseudomonas* spp.	Rod	-	+	+	-	+	-	-	-	A	-	A	-
UA_4	*Staphylococcus* spp.	Cocci	+	+	-	-	+	-	-	A	A	-	A	A
UA_5	*Salmonella* spp.	Rod	-	+	-	-	-	-	+	-	A	-	-	A
UA_6	*E. coli*	Rod	-	+	-	-	-	+	-	AG	AG	AG	AG	A
WD_1	*E. coli*	Rod	-	+	-	-	-	+	-	AG	AG	AG	AG	A
WD_2	*Klebsiella* spp.	Rod	-	+	-	-	+	-	-	AG	AG	AG	-	AG
WD_3	*Pseudomonas* spp.	Rod	-	+	+	-	+	-	-	-	A	-	A	-
WD_4	*Staphylococcus* spp.	Cocci	+	+	-	-	+	-	-	A	A	-	A	A
WD_5	*Staphylococcus* spp.	Cocci	+	+	-	+	-	-	-	A	A	A	A	A
CTR_1	*Serratia* spp.	Rod	-	+	-	-	+	-	-	-	A	A	A	-
CTR_2	*Lactobacillus* spp.	Rod	+	-	-	-	-	-	-	A	A	A	A	A
CTR_3	*Staphylococcus* spp.	Cocci	+	+	-	-	+	-	-	A	A	-	A	-

Key: Cat.=Catalase, Oxi.=Oxidase, Coa.=Coagulase, Cit.=Citrate, Ind.=Indole, Lac.=lactose, Glu.=Glucose, Suc.=Sucrose, Fru.=Fructose, Mal.=Maltose (+)=Positive Test, (-)=Negative Test, AG=Acid and Gas production, A=Acid production.

The greatest challenge to the large-scale production of zobo drink is its short shelf-life. Zobo drink has a shelf-life of less than two days when stored at room temperature. During storage, bacteria rapidly utilize the available nutrients for growth and production of various metabolites; including organic acids, which results in deterioration or spoilage of the food-drink [1, 9]. Consequently, the pH of zobo drinks decreases with increase in bacterial load and metabolic activities at room temperatures [9, 23]. In the study, the average pH of the zobo samples was 3.4. However, Adesokan *et al.* [28] in his study reported a pH ranged of 3.94 - 7.67 while another study reported a pH range of 2.9 - 4.3 but with a mean pH value of 3.04 which agreed with our study [1].

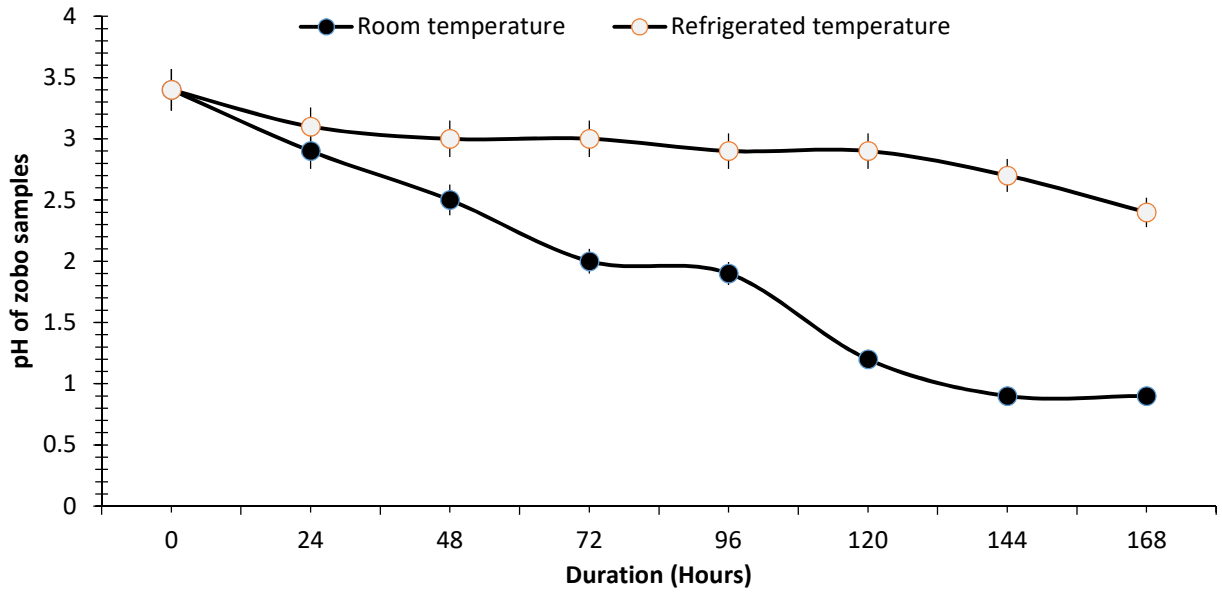

Figure 1: pH variations of zobo stored at room and refrigerated temperatures

Figure 1 presents the pH of zobo stored at room and refrigerated temperatures for 0 - 168 h. The result showed a drastic decrease in pH of zobo samples at room temperatures from pH 3.4 - 2.9 after 24 h, and 2.5 - 0.9 after 48 h and 168 h respectively. This corroborates another study which showed that the pH of zobo decreases when stored at ambient temperature due to increased metabolic activities of proliferating bacteria [9]. The refrigerated zobo witnessed a slight decrease in pH from pH 3.4 - 3.1 after 24 h, 3.0 - 2.4 after 48 h and 168 h respectively. However, due to incessant public power failures and the high cost of operating generator sets, storage of zobo by refrigeration is uneconomical and not a present day reality in Nigeria. More so, another study suggested that zobo deteriorates even at refrigerated temperatures [10].

Conclusion

The study established that the bacteria load and coliform count were above the stipulated limits, indicating high levels of contamination. Furthermore, the isolation and prevalence of *Staphylococcus* spp., *E.* coli, *Klebsiella* spp., *Bacillus* spp., and *Salmonella* spp. indicated the poor quality of the product and posed a risk to public health, which negates any benefits of consuming the product. Therefore, to promote the cottage industry and wholesome zobo in addition to public safety-relevant authorities should sensitize processors and vendors on good manufacturing practices, and the public on the risks of consuming contaminated zobo.

Declaration of conflicting interest

The authors declared no potential conflicts of interest.

References

[1]. Raimi, O.R. 2013. Bacteriology Quality of Zobo Drinks Consumed in Some Parts of Osun State, Nigeria. *Journal of Applied Science and Environmental Management* 17 (1):113 - 117.

[2]. Anagu, L. Okolocha, E. Ikegbunam, M. Ugwu, M. Oli, A. and Esimone, C. 2015. Potential Spread of Pathogens by Consumption of Locally Produced Zobo and Soya Milk Drinks in Awka Metropolis, Nigeria. *British Microbiology Research Journal* 5(5): 424 - 431.

[3]. Ibitoye1, F.O. Oyetayo, A.M. Aribisala, O.K. and Giwa, O.E. 2017. Investigation of Bacteria Associated with the Spoilage of Zobo Drink Fortified with Scent Leaf and Ginger. *Archives of Current Research International* 9(1): 1 - 7.

[4]. Suya, M. and Dele, B.I. 2014. Antibiogram of food-borne pathogens secluded from ready foods and Zobo Drinks Sold in Nigeria. *Frontiers of Agriculture and Food Technology* 2 (2):172 - 174.

[5]. Ekanem, J.O. Daniel, U.J. Akpan, B.C. and Akpan, E.A. 2018. Microbiological Assessment and Proximate Composition of Sorrel (Zobo) Drinks Sold in Ikot Ekpene Metropolis, Akwa Ibom State, Nigeria. *Journal of Research in Forestry, Wildlife & Environment* 10(4): 92 - 99.

[6]. Ezeigbo, O.R. Uhiara, S. Nwodu, J.A. and Ekaiko, M.U. 2015. Bacteriological Assessment of Hawked Sorrel Drink (Zobo Drink) in Aba, South-East Nigeria. *British Microbiology Research Journal* 5(2): 146 - 151.

[7]. Umar, M. Mohammed, I.B. Abdulkarim, I.M. Yusuf, G. Yaya, A.A. and Leo, G. 2016. Comparative Studies on the Prevalence of *Salmonella* Species in Two Homemade Fermented Beverages (Zobo and Kunun-Zaki) Sold At Samaru, Zaria, Kaduna, Nigeria. *International Journal of Scientific and Research Publication* 6 (3): 428 - 435.

[8]. Okeke, I.N. Lanikanra, A. and Czeczulin, J. 2000. Heterogenous Virulence of Enter Aggregative *E. coli* Isolated from Children in South Western Nigeria. *Journal of Infectious Diseases* 181(1):252 - 260.

[9]. Seiyaboh, E.I. Oku, I.Y. and Odogbo, O.M. 2013. Bacteriological Spoilage of Zobo (A Nigerian Drink Prepared from the Calyces of *Hibiscus Sabdariffa* L. (Malvaceae). *The International Journal of Engineering and Science* 2(11): 46 - 51.

[10]. Izah, S.C. Orutugu, L.A. and Kigigha, L.T. 2015. A Review of the Quality Assessment of Zobo Drink Consumed in Nigeria. *ASIO Journal of Microbiology, Food Science and Biotechnological Innovations* 1(1): 34 - 43.

[11]. Adeniji, P.O. 2017. Nutritional, Sensory and Microbiological Quality Assessment of fortified Zobo Drink: A Home-Prepared Traditional Nigerian Beverage. *Journal of Nutrition Food and Science* 7(5): 627. doi: 10.4172/2155-9600.1000627.

[12]. Omeremu, D.M. Enoch, A.S. and Azuonwu, O. 2019. Bacteriological Quality Assessment of Zobo Drink Sold in Bayelsa State Nigeria. *Journal of Applied Life Sciences International* 20(1): 1 - 8.

[13]. Udensi, C. G. Nwankpa, U. D. Amanze, E. K. Nwokafor, C. V. Udekwu, C. E. and Ndubuisi, C. W. 2020. Microbiological Analysis of Zobo Drink Preserved with Scent Leaves (*Ocimum gratissimum*) *South Asian Journal of Research in Microbiology* 8(2): 1-10.

[14]. Addis, M. and Sisay, D. 2015. A Review on Major Food Borne Bacterial Illnesses. *Journal of Tropical Diseases* 3(4): 176. doi:10.4176/2329891X.1000176.

[15]. Elikwu, C.J. Shobowale, E.O. Nwadike, V.U. Tayo, B. Okangba, C.C. Shonekan, O.A. Omeonu, A.C. Faluyi, B. Ile, P. Adelodun, A. Popoola, A. and Mubele, M. 2015. Antimicrobial Susceptibility Patterns of Enterobacteriaceae Isolated from Stool Samples at a Semi-urban Teaching Hospital. *American Journal of Biomedical and Life Sciences* 3(6): 127 - 130.

[16]. SoyFood Association of America, Voluntary Standards for the Composition and Labeling of Soymilk in the United States. 1996;14.
Available:www.soyfoods.org/../smstandards.pdf.

[17]. Onuoha, O.G. Haruna, U.S. Yelmi, B.M. Samuel, E. Uhiara, N.S. and Ngwu, P.C. 2014. Storage Study on Colour Retention in Zobo Concentrates by Increasing Concentration of Ginger (*Zingiber officinale*). *African Journal of Food Science* 8(6): 292 - 296.

[18].Cheesbrough M. 2006. Biochemical test to identify bacteria: District Laboratory Practices in Tropical Countries, 2nd Edition, *Cambridge University Press, UK,* 1 - 454.

[19]. Kigigha, L.T. Samson, G.A.S. Izah, C and Aseibai, E.R. 2018. Microbial Assessment of Zobo Drink Sold in Some Locations in Yenagoa Metropolis, Nigeria. *EC Nutrition* 13(7): 470 -476.

[20]. Bristone, C. Mariyam, K. Ogori, A.F. Badau, M.H. and Joeguluba, O. 2018. Microbial Quality Evaluation of Zobo Drink Sold in University of Maiduguri. *Food Science Nutrition and Technology* 3(1): 000137.

[21]. Ayandele, A.A. 2015. Microbiological Analyses of Hawked Kunun and Zobo Drinks within LAUTECH Campus, Ogbomoso, Oyo State, Nigeria. *IOSR Journal of Environmental Science, Toxicology and Food Technology* 9(10): 52 - 56.

[22]. Reiner K. 2016. Carbohydrate Fermentation Protocol. *American Society for Microbiology* 1 – 10.Asm.org/ASM/media/Protocol-images/Carbohydrate-Fermentation-Protocol.pdf? Accessed 3/3/2022.

[23]. Gbarakoro, S.L. and Miikue-Yobe, T. 2019. Comparative Analysis of the Physico-Chemical Parameters and Mineral Contents of Selected Home-Made Juices Sold in Port Harcourt, Nigeria. *International Journal of Chemistry and Chemical Processes* 5(1): 30 -41.

[24]. Nwachukwu E, Onovo, O.M, Ezeama, C.F. 2007. Effect of Lime Juice on the Bacterial Quality of Zobo Drinks Locally Produced in Nigeria. *Research Journal of Microbiology* 2:787-791.

[25]. Adebayo-Tayo B.C. and Samuel, U.A. 2009. Microbial Quality and Proximate Composition of Dried *Hibiscus*

sabdariffa clyxes in Uyo, Eastern Nigeria. *Malaysian Journal of Microbiology* 5(1):13 - 18.

[26]. NAFDAC 2004. National Agency for Food and Drug Agency. Beverage and Water quality standard for consumption. Annual Report. In: WHO (2004). Sanitation and Health programme. Managing water in the home.

[27]. Nweze, E. A. 2010. Aetiology of diarrhea and virulence properties of diarrhoeagenic *E. coli* among patients and healthy subjects in Southeast Nigeria. *Journal of Health Population and Nutrition* 28(3): 245 - 252.

[28]. Adesokan, I.A. Abiola, O.P. Adigun, M.O. and Anifowose, O.A. 2013. Analysis of Quality Attributes of *Hibiscus sabdariffa* (Zobo) Drinks Blended with Aqueous Extract of ginger and garlic. *African Journal of Food Science* 7(7): 174-177.

Cite this article

Ado B.V., Odeh R.A. and P.T. Aernan (2022). Bacteriological Assessment of Hawked Zobo Drink (*Hibiscus sabdariffa*) in Makurdi Metropolis, Benue State. FUAM Journal of Pure and Applied Science, **2**(**1**):40-48