Original Research Article

Bacteriological Quality Assessment of Nigerian Indigenous Beverages Consumed in Calabar, Southern Nigeria

ABSTRACT

Aim: Locally prepared drinks and beverages are gradually replacing the conventional sugary carbonated soft drinks, particularly because of the health effects associated with some of the components in these drinks. This study was aimed at evaluating the bacteriological quality of locally produced drinks/beverages (kunu, zobo and tiger-nut drinks), produced and sold in Calabar metropolis. **Methodology**: Samples (tiger-nut, zobo and kunu drink) were collected from two major markets (Watt and Marian) within the study area and evaluated using standard bacteriological techniques. **Results**: The mean total bacterial counts ranged from 2.4 x 10^5CFU/mL (from Watt market) to 4.56 x 10^5CFU/mL (from Marian market). The mean total coliform counts ranged from 5.0 x 10^5CFU/mL (from Marian market) to 4.01 x 10^5CFU/mL (from Watt market). In general, the result revealed that zobo drinks were highly contaminated with counts ranging from 4.7 x 10^5 - 4.56 x 10^5CFU/mL while the least counts were obtained from kunu drinks, (2.4 x 10^5 - 3.42 x 10^5CFU/mL). Bacteria genera isolated from the samples were presumptively identified as species of *Pseudomonas, Staphylococcus, Bacillus*, *Enterobacter*, *Klebsiella*, *Lactobacillus*, *Micrococcus*, *Streptococcus* and *Proteus*. *Staphylococcus* spp had the highest percentage occurrence (22.2%), while *Klebsiella* and *Streptococcus* spp were the least prevalent (2.8%) respectively. **Conclusion**: This study revealed that the microbiological quality of the analyzed samples was above the acceptable standards and unfit for human consumption. There a need to ensure that these drinks are prepared under strict hygienic conditions to achieve near sterile products (kept free from potentially hazardous bacterial pathogens that could pose some public health risks after the consumption of some of these drinks).

Key Words: Bacteriological; Assessment; Beverages; Kunu, Zobo, Tiger-nut, Calabar.

HIGHLIGHTS:

- Contributions of locally made beverages to food security mostly in developing countries like Nigeria cannot be over-emphasize.
- Despite the nutritive value of locally made drink, the presence of microorganisms can render its unsafe for consumption.
- Policy development (based on geography, climate, potential contaminants or pathogens and their disease patterns) and establishment of regulatory frameworks and its implementation are the essential and foremost steps in food safety system.

1.0 INTRODUCTION

Beverage is any food usually consumed in a liquid form and may include tea, juices, soft and alcoholic drinks. In West Africa, including Nigeria, there is a growing disinterest in the consumption of conventional carbonated drinks as a result of the perceived health implications of excess sugars. Therefore, locally prepared beverage drinks are taking preeminence (Kigigha *et al*., 2018; Oku *et al*., 2018). As opined by Agbo and Tahir (2018), some of the locally prepared beverages that have gained worldwide acceptance, but which are indigenous to Nigeria include; zobo, tiger-nut and kunu drinks. They are gradually taking the consumption vacuum left by consumers of carbonated drinks (Oku *et al*., 2018). Aside their thirst-quenching properties, locally made non-alcoholic beverages are also good sources of nutrients such as protein, carbohydrate, vitamin, calcium, iron and antioxidants (Badua *et et al*., 2018; Omeremu *et al*., 2019).

Zobo drink is prepared by either steeping or boiling the calyx of the sorrel plant *Hibiscus sabdariffa* in portable water and usually sweetened with sugar and served chilled to consumers (Mohammed *et al*., 2017). According to Adanlawo and Ajibade, two varieties of *H. sabdariffa* exist in Nigeria, the red/brown and green variety (Kigigha *et al*., 2018). Zobo in Nigeria have different names according to different ethnicities; it is called 'Isapa' by Yoruba, 'zobo' by Hausa and 'Aukan' by Igbo speaking tribe (Mohammed & Ismail, 2014). The microbiological status of zobo drink depends highly on the production protocol and this varies among individuals and community (Foline *et al*., 2011), resulting in high microbial density seldom exceeds tolerant limits for ready-to eat food (Izah *et al*., 2016).

Tiger-nut beverage is a non-alcoholic drink obtained from tiger-nut tuber, which is consumed widely in Africa. Tiger-nut (*Cyperus esculentus*) belongs to the family of *Cyperaceae* (Badua *et al*., 2018). In Nigeria, tiger-nut is known as 'Aya' in Hausa, 'Ofio' in Yoruba and 'Akiausa' in Igbo. Three varieties (black, brown and yellow) are cultivated in the country and among these, only two varieties; (yellow and brown) are readily available in the markets. The yellow variety is preferred to all other varieties because of its inherent properties like its bigger size and attractive colour. Tiger-nut beverages are popularly known in the northern part of Nigeria as 'Kunuaya' (Musa & Hamza, 2013). Its local production involves washing the tiger-nuts thoroughly in order to remove soil and dirt. Once they are washed, the nuts are then soaked for about 4 - 8 hours, after which they can be ground along with coconut, date fruit or pineapple into a mash. During the process, some cold water is added in a ratio of 3 liters of water for unit kilogram of tiger-nuts; and the mixture is left to mix. When the appropriate time is spent, it is pressed and sieved and then known quantity of sugar or honey can be added depending on volume obtained. The mixture is again filtered to get the pure filtrate. Once this is done, it is often served cold (Musa & Hamza, 2013).

Kunu is a popular cereal based non-alcoholic beverage prepared with either of the following substrates; millet (*Pennisetum typhoideum*), maize (*Zea mays*), or sorghum (*sorghum bicolor*), but millet is the most preferable substrate. Spices such as ginger, black pepper, red pepper, cloves and sugar are commonly added as flavor and taste improvers (Sule *et al*., 2016). As opined by Asuquo *et al*. (2017), traditional production of kunu is still on primitive technology and as such production protocol is not standardized. Generally, the process of preparation involves wet milling of cereal grains with spices, wet sieving and partial gelatinization of the curry, sugar addition, bottling and sales.

Studies have shown that a large number of lactic acid bacteria, coliforms, moulds, and yeast are involved in spoilage of fruit juices which in turn cause food poisoning to consumers (Ayandele *et al* 2015; Sule *et al*., 2016), while local beverage drinks may serve as vehicle of foodborne diseases (Asuquo *et al*., 2017). The quality of locally made beverages is strictly maintained in developed countries under some laws and regulations but in many developing and under developed countries including Nigeria, the manufacturer is not concern about the microbiological safety and hygiene of beverages (Batool *et al*., 2013**).** Calabar being a center of tourist resorts in Nigeria, there is always a great demand for kunu, zobo and tiger-nut drinks particularly during summers. Therefore, it is necessary to verify the safety of consuming this food drinks. Hence, this research was conducted to evaluate the "kunu, zobo and tiger-nut" drinks sold in Calabar Metropolis, for the presence of bacterial contaminants.

2.0 MATERIALS & METHODS

2.1 Sampling Technique

A total of twenty-four (24) locally made beverages were purchased randomly from two different markets in Calabar metropolis including Watt market and Marian market. The samples which include tiger-nut drink; zobo drink and kunu drink packaged in plastic bottle were purchase from vendors. Twelve (12) samples from each market (i.e. 4 each of kunu, zobo and tiger-nut). The samples were transported to the laboratory for analysis within 1 hour of collection.

2.2 Enumeration of Bacteria Count/Isolation

The bacteria load of the samples was determined following the method described by Ogodo *et al.* (2018). Briefly, serial dilutions of the various samples were made up to 10^{-7} with sterile distilled water. About 0.5ml of dilution 10^{-5} was evenly spread on Nutrient agar, MacConkey agar and Mannitol salt agar and incubated at 37^oC for 24 hours. Plates were screened for discrete colonies after incubation period and the actual numbers of bacteria (total bacteria count) were estimated as colony forming unit per ml (cfu/ml) of the sample. Total coliform count (TCC) and total staphylococcal count (TSC) were performed in similar manner using MacConkey agar and Mannitol Salt agar (MSA) mediums respectively. Distinct colonies were taken and sub-cultured in fresh sterile medium and incubated at 37^oC for 24 hours to obtain pure cultures. Pure bacterial isolates were stored on nutrient agar slant prepared in Bijou bottles and kept for further studies.

2.3 Characterization and Identification of Bacterial Isolates

Bacterial isolates were characterized and identified by observation of colonial and morphological characteristics, Gram reaction and biochemical tests. The various biochemical test used for identification were the citrate utilization, catalase, methyl red, vorges-proskauer, coagulase, triple sugar iron, oxidase, motility, indole and carbohydrates fermentation.

3.0 RESULTS

3.1 Bacterial Load Obtained from Samples

The bacterial loads of various locally made drinks and beverages tested in the study are shown in Tables 1. The result revealed that total aerobic bacterial count from kunu ranged from 2.4 x 10^5CFU/mL - 3.42 x 10^5CFU/mL. Total coliform count varied from 5.4 x 10^5CFU/mL - 2.42 x 10^5CFU/mL. The staphylococcal count ranged from 5.9 x 10^5CFU/mL - 2.42 x 10^5CFU/mL (Table 1). From zobo drink, the total bacterial counts, total coliform and staphylococcal counts ranged from 1.12 x 10^5CFU/mL to 4.56 x 10^5CFU/mL; 7.4 x 10^5CFU/mL to 4.01 x 10^5CFU/mL (Table 1) and 4.7 x 10^5CFU/mL (Table 1) to 4.22 x 10^5CFU/mL (Table 1) respectively. Aerobic bacterial count from tiger-nut drink, ranged from 8.2 x 10^5CFU/mL to 4.32 x 10^5CFU/mL, mean coliform count ranged from 5.0 x 10^5CFU/mL to 2.4 x 10^5CFU/mL while mean staphylococcal count ranged from 7.1 x 10^5CFU/mL to 2.0 x 10^5CFU/mL (Table 1).

Generally, the total bacterial counts, total coliform and Staphylococcal counts in samples obtained from Watt market ranged from 2.4 x 10^5CFU/mL - 4.12 x 10^5CFU/mL, 5.4 x 10^5CFU/mL - 4.01 x 10^5CFU/mL and 8.0 x 10^5CFU/mL - 3.00 x 10^5CFU/mL respectively (Table 1). Similarly, the total bacterial count, total coliform and total Staphylococcal counts in samples purchase from Marian market ranged from 8.2 x 10^5CFU/mL to 4.56 x 10^5CFU/mL, 5.0 x 10^5CFU/mL to 3.11 x 10^5CFU/mL and 4.7 x 10^5CFU/mL to 4.22 x 10^5CFU/mL (Table 1).

3.2 Physiochemical Characterization and Identification of Isolates

The physiochemical characteristics of bacterial isolates from the samples are shown in Table 2. Out of 36 isolates, the microscopic examination revealed that 17(47.2%) were Gram-negative while 19(52.8%) were Gram-positive (Table 2). The isolates that were presumptively identified include species of *Enterobacter (*in zobo and tiger-nuts), *Serratia* and *Klebsiella* (in tiger-nut only), *Pseudomonas* and *Staphylococcus* (in all (100%) of the samples evaluated), *Lactobacillus* (in kunu and zobo), *Bacillus* spp occurred in zobo only while *Micrococcus* and *Proteus* (in kunu and tiger-nuts only) (Table 3).

Table 1: Mean Total Aerobic Bacterial Counts Obtained from the Samples

Location	Sample	Mean Total Bacterial Count (CFU/mL)	Mean Total Coliform Count (CFU/mL)	Mean Total Staphylococcal Count (CFU/mL)
Watt market	Kunu	2.14×10^5 (2.4×10^5-3.24×10^5)	1.29×10^5 (5.4×10^5-2.42×10^5)	1.25×10^5 (8.0×10^5-2.26×10^5)
	Zobo	2.43×10^5 (1.66×10^5-3.21×10^5)	1.99×10^5 (9.1×10^5-4.01×10^5)	1.64×10^5 (9.6×10^5-3.00×10^5)
	Tiger-nut	2.59×10^5 (1.42×10^5-4.12×10^5)	1.25×10^5 (9.6×10^5-1.32×10^5)	1.25×10^5 (8.3×10^5-2.01×10^5)
Marian market	Kunu	2.29×10^5 (9.8×10^5-3.42×10^5)	1.22×10^5 (5.4×10^5-2.04×10^5)	1.36×10^5 (5.9×10^5-2.42×10^5)
	Zobo	2.68×10^5 (1.12×10^5-4.56×10^5)	1.73×10^5 (7.4×10^5-3.11×10^5)	1.63×10^5 (4.7×10^5-4.22×10^5)
	Tiger-nut	1.99×10^5 (8.2×10^5-4.32×10^5)	1.28×10^5 (5.0×10^5-2.40×10^5)	9.4×10^5 (7.1×10^5-1.09×10^5)

The range values are given in (parentheses).

3.3 Percentage Occurrences of Bacteria Isolated from the analyzed samples

From the different samples evaluated, zobo drink had the highest number of isolates, 16(44.4%) while tiger-nut and kunu 10(27.8%) each (Table 2).

Figures 1 presents the results of frequency and percentage occurrences of bacteria isolates from the analyzed samples. It showed that *Staphylococcus* spp had the highest percentage occurrence (22.2%), while the least frequency was recorded for *Klebsiella* and *Streptococcus* spp 1(2.8%) respectively (Figure 1).

3.4 Occurrence of Isolates from different Locations and the Percentage Occurrences

The frequency and percentage of occurrence of bacterial isolates per locations are presented in Figure 2. Watt market had 20 isolates; *Pseudomonas* spp 4(11.1%), *Staphylococcus, Lactobacillus* and *Enterobacter* spp 3(8.3%) each, *Micrococcus* and *Bacillus* spp 2(5.6%) each, *Serratia*, *Klebsiella* and *Streptococcus* spp 1(2.8%) each.

Marian market had 2(5.6%) each of *Enterobacter Pseudomonas* and *Bacillus* spp*,* 5(13.9%) *Staphylococcus spp*, 1(2.8%) each of *Micrococcus* and *Serratia* spp, 3(8.3%) *Proteus* spp (Figure 2).

Table 2: Cultural, morphological and biochemical characteristics of bacterial isolates

												Triple Sugar Iron				
S/N	Isolate Code	Colonial Appearance	Gram	Shape	Mot	Cat	Cit	In	MR	VP	Ox	Slant	Butt	H_2S	Gas	Presumptive Organism
1.	T1	Round, shiny, convex, pink	-	Rods	+	+	+	-	-	+	-	Y	Y	-	+	*Enterobacter* spp
2.	T2	Round, raised, colourless colony	-	Rods	+	+	-	-	-	+	-	R	Y	-	+	*Serratia* spp
3.	T3	Circular, convex, pink colony	-	Rods	-	+	+	-	-	+	-	Y	Y	-	+	*Klebsiella* spp
4.	T4	Oval, mucoid, wavy, raised colony	-	Rods	+	+	+	-	-	-	+	R	R	+	-	*Pseudomonas* spp
5	Z1	Round, smooth, convex colony	+	Rods	+	+	-	-	-	+	-	R	Y	-	-	*Lactobacillus* spp
6	Z2	Circular, smooth, convex colony	+	Rods	+	+	-	-	-	+	-	R	Y	-	-	*Lactobacillus* spp
7	Z3	Irregular, wavy, flats, cream colony	+	Rods	+	+	+	-	+	+	-	R	Y	-	+	*Bacillus* spp
8	Z4	Irregular, wavy, raised, white colony	+	Rods	+	+	+	-	+	+	-	R	Y	-	+	*Bacillus* spp
9	Z5	Round, shiny, convex, milky colony	-	Rods	+	+	+	-	-	+	-	Y	Y	-	+	*Enterobacter* spp
10	Z6	Round, shiny, convex, colony	-	Rods	+	+	+	-	-	+	-	Y	Y	-	+	*Enterobacter* spp
11	Z7	Oval, mucoid, wavy, raised colony	-	Rods	+	+	+	-	-	-	+	R	R	+	-	*Pseudomonas* spp
12	Z8	Oval, mucoid, wavy, raised colony	-	Rods	+	+	+	-	-	-	+	R	R	+	-	*Pseudomonas* spp
13	K1	Circular, smooth, convex, yellow colony	+	Cocci	-	+	+	-	-	+	+	Y	Y	+	-	*Staphylococcus* spp
14	K2	Oval, mucoid, wavy, raised colony	-	Rods	+	+	+	-	-	-	+	R	R	+	-	*Pseudomonas* spp
15	K3	Circular, smooth, convex colony	+	Rods	+	+	-	-	-	+	-	R	Y	-	-	*Lactobacillus* spp
16	K4	Circular, smooth, bright yellow colony	+	Cocci	-	+	+	-	-	-	+	Y	Y	-	+	*Micrococcus* spp
17	K5	Circular, smooth, bright yellow colony	+	Cocci	-	+	+	-	-	-	+	Y	Y	-	+	*Micrococcus* spp
18	K6	Circular, smooth, convex, yellow colony	+	Cocci	-	+	+	-	-	+	+	Y	Y	+	-	*Staphylococcus* spp
19	K7	Circular, smooth, convex, yellow colony	+	Cocci	-	+	+	-	-	+	+	Y	Y	+	-	*Staphylococcus* spp
20	K8	Round, smooth, convex, raised colony	+	Cocci	-	-	-	+	-	+	-	Y	Y	-	+	*Streptococcus* spp
21	T5	Round, raised, colourless colony	-	Rods	+	+	-	-	-	+	-	R	Y	-	+	*Serratia* spp
22	T6	Circular, smooth, convex, yellow colony	+	Cocci	-	+	+	-	-	+	+	Y	Y	+	-	*Staphylococcus* spp
23	T7	Circular, smooth, bright yellow colony	+	Cocci	-	+	+	-	-	-	+	Y	Y	-	+	*Micrococcus* spp
24	K9	Round, entire, convex, mucoid colony	-	Rods	+	+	-	-	-	-	-	R	Y	+	+	*Proteus* spp
25	K10	Round, entire, convex, mucoid colony	-	Rods	+	+	-	-	-	-	-	R	Y	+	+	*Proteus* spp
26	T8	Round, shiny, convex, pink	-	Rods	+	+	+	-	-	+	-	Y	Y	-	+	*Enterobacter* spp
27	T9	Round, shiny, convex, pink	-	Rods	+	+	+	-	-	+	-	Y	Y	-	+	*Enterobacter* spp
28	T10	Round, entire, convex, mucoid colony	-	Rods	+	+	-	-	-	-	-	R	Y	+	+	*Proteus* spp
29	Z9	Circular, smooth, convex, yellow colony	+	Cocci	-	+	+	-	-	+	+	Y	Y	+	-	*Staphylococcus* spp
30	Z10	Oval, mucoid, wavy, raised colony	-	Rods	+	+	+	-	-	-	+	R	R	+	-	*Pseudomonas* spp
31	Z11	Oval, mucoid, wavy, raised colony	-	Rods	+	+	+	-	-	-	+	R	R	+	-	*Pseudomonas* spp
32	Z12	Circular, smooth, convex, yellow colony	+	Cocci	-	+	+	-	-	+	+	Y	Y	+	-	*Staphylococcus* spp
33	Z13	Circular, smooth, convex, yellow colony	+	Cocci	-	+	+	-	-	+	+	Y	Y	+	-	*Staphylococcus* spp
34	Z14	Irregular, wavy, flats, cream colony	+	Rods	+	+	+	-	+	+	-	R	Y	-	+	*Bacillus* spp
35	Z15	Circular, smooth, convex, yellow colony	+	Cocci	-	+	+	-	-	+	+	Y	Y	+	-	*Staphylococcus* spp
36	Z16	Irregular, wavy, flats, cream colony	+	Rods	+	+	+	-	+	+	-	R	Y	-	+	*Bacillus* spp

KEY: Mot *= Motility test;* ***Cat*** *= Catalase test;* ***Cit*** *= Citrate test;* ***In*** *= Indole test;* ***MR*** *= Methyl red test;* ***VP*** *= Voges-Proskaure test*
OX *= Oxidase test;* ***H_2S*** *= Hydrogen sulphide;* ***R*** *= Red;* ***Y*** *= Yellow;* ***-*** *= Negative;* ***+*** *= Positive.*

Table 3: Prevalence of bacterial isolates in relation to the samples

S/N	**Bacterial isolates**	**Sample**		
		Kunu	**Zobo**	**Tiger-nut**
1	*Enterobacter* spp	0	2	3
2	*Serratia* spp	0	0	2
3	*Klebsiella* spp	0	0	1
4	*Pseudomonas* spp	1	4	1
5	*Lactobacillus* spp	1	2	0
6	*Bacillus* spp	0	4	0
7	*Staphylococcus* spp	3	4	1
8	*Micrococcus* spp	2	0	1
9	*Streptococcus* spp	1	0	0
10	*Proteus* spp	2	0	1
	Total	**10**	**16**	**10**

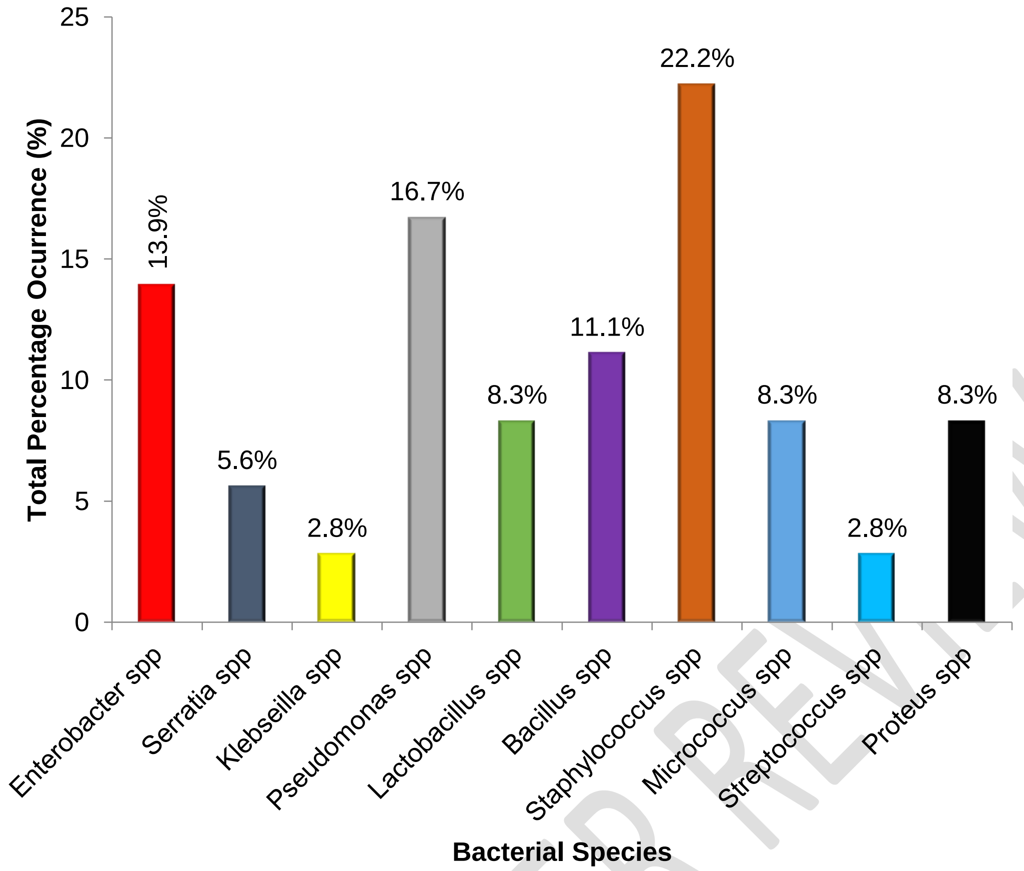

FIG 1: Percentage occurrence of bacteria isolates from the samples

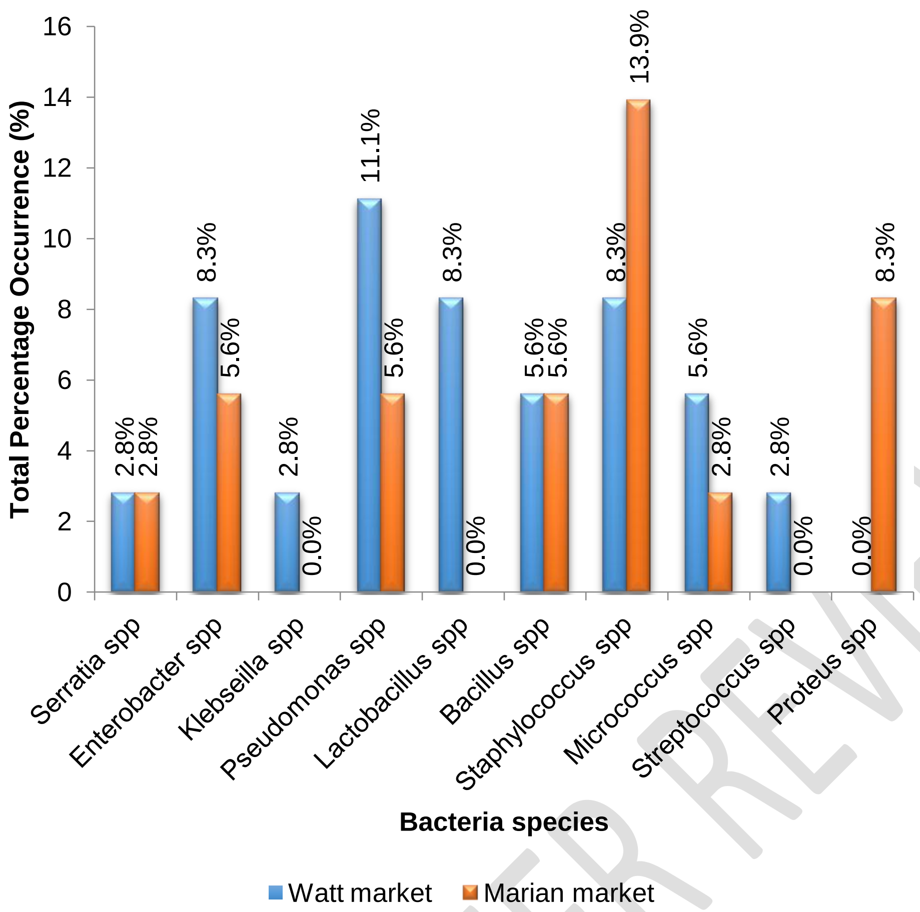

FIG 2: Frequency and percentage of bacterial isolates per location

4.0 DISCUSSION

This study evaluated the bacteriological quality of some locally made beverages sold in Calabar metropolis. Locally produced beverages are consumed by many people, especially around Cross River State and its environs, due to its readily availability and affordability. However, the microbiological quality and safety is of utmost importance to ensure the wellbeing of consumers (Izah *et al*., 2016).

The presence of microorganisms in locally made drinks is an indication that the raw materials might have been of poor quality, or that inadequate sanitary conditions were not observed during the handling, and production process.

The total bacterial and coliform counts obtained in this study ranged from 2.4 x 10^5- 4.56 x 10^5CFU/mL and 5.0 x 10^5 - 4.01 x 10^5CFU respectively. The counts recorded in this study were not surprising because, there is no authorized quality assurance agency to monitor the microbial quality and safety of these locally made drinks and beverages (in most part of Nigeria). So the production process is sometimes under unhygienic conditions (Asuquo *et al*., 2017; Ayandele *et al*., 2015). A similar study by Badua *et al*. (2018) in Maidugri, Nigeria, also showed high bacterial counts in kunu-aya in the range of 3.13 x 10^4 - 9.93 x 10^4CFU/mL, total coliform counts ranged from 1.56 x 10^4 - 6.0 x 10^4CFU/mL and total Staphylococcal counts ranged from 1.43 x 10^4 - 2.23 x 10^4CFU/mL. However, the above counts were below the finding of Ogodo *et al*. (2018), who reported bacterial counts of 1.3 × 10^7CFU/mL - 2.2 x 10^7CFU/mL, total coliform and Staphylococcal counts ranging from 1.0 x 10^3CFU/mL - 7.0 x 10^3CFU/mL and 4.0 x 10^4CFU/mL - 8.0 x 10^4CFU/mL respectively from kunu-aya drink sold in Wukari, Taraba state, Nigeria.

Kigigha *et al*. (2018) investigated the microbial quality of zobo drink sold in Yenagoa, Bayelsa state, Nigeria and recorded 4.16 x 10^2CFU/mL - 5.71 x 10^2CFU/mL total heterotrophic bacterial counts and 1.66 x 10^2CFU/mL - 2.63 x 10^2CFU/mL total coliform counts.

According to Food and Drug Administration (FDA, 2013) the standard limit of <10^5CFU/mL is permissible for aerobic mesophilic bacteria counts in food. However, according to the revised guidelines for the assessment of microbiological quality of processed foods (cereals/cereals products) by the Food and Drug Administration (FDA), the set down maximum level of permissible microbial load for finished cereal products have been stated to include 10^2 cells/g for aerobic mesophilic bacteria, 5.0 x 10^2 cells/g for molds, 0.5 x 10^2 cells/g for coliforms and 0 mg/ml for *Escherichia coli* above which, the food is unfit for human consumption (Etang *et al*., 2017).

The high bacterial counts observed in this study, might have originated from the source of water used in the preparation of the beverages, the poor personal hygiene practices by the producers, exposure of the drinks to dust and flies, poor storage conditions, lack of preservative measures, the machines used for grinding, as well as spoilage of the raw materials before use (Etang *et al*., 2017; Badua *et al*., 2018; Oku *et al*., 2018).

Moreover, these locally made drinks are usually packaged in recycled plastic bottles of water and soft drinks, which are most often picked from ceremonial grounds where they are found in large numbers. Thus, no proper sterilization process is often followed. The sterility of the water used for washing or rinsing is also questionable. A study conducted by Omeremu *et al*. (2019) revealed that locally made beverages marketed in recycled bottles harbor diverse microbial groups.

The bacterial species obtained from the analyzed samples included species of *Staphylococcus, Klebsiella, Pseudomonas, Micrococcus, Serratia, Bacillus, Enterobacter, Lactobacillus, Streptococcus* and *Proteus*. This is in accordance with the earlier findings by Etang *et al*. (2017) in the same study area but differ slightly from that reported by Asuquo *et al*. (2017) who isolated *Pseudomonas, Staphylococcus, Bacillus* and *Escherichia coli* from selected market in Calabar metropolis.

The microbial diversity reported in this study is similar to other studies carried on kunu, zobo and tiger nut drink outside Calabar metropolis. Thus, Ogodo *et al*. (2018) reported the presence of *Klebsiella, Bacillus, Staphylococcus, Citrobacter, Salmonella, Shigella, Micrococcus, Proteus, Pseudomonas, Enterococcus* species and *Escherichia coli* as bacterial isolates found in Kunun-aya sold in Wukari, Taraba state, Nigeria. Oku *et al*. (2018) also reported *Staphylococcus aureus, Pseudomonas aeruginosa, Micrococcus luteus, Escherichia coli, Bacillus subtilis, Enterobacter aerogenes, Enterobacter cloacae, proteus vulgaris*, *Aspergillus flavus, Mucor* spp, *Cladosporium* spp, *Penicillium* spp, *Candida* spp *and Rhizopus* spp as microbial diversity found in zobo drinks prepared from different sources within Yenagoa city Bayelsa state, Nigeria. Badua *et al*. (2018) reported microbial species of tiger nut sold in university of Maiduguri as *Staphylococcus aureus, Salmonella* spp, *Shigella* spp, *Pseudomonas* spp, *E. coli*, *Candida albicans, Saccharomyces cerevisiae,* and *Rhizopus oryzae*.

The organisms isolated from the analyzed samples are known to be important foodborne pathogens. The occurrence of coliforms (*Enterobacter* and *Klebsiella* spp) is an indication of contaminants from fecal origins, packaging materials; water used for processing and washing etc. (Kigigha *et al*., 2018). Others such as species of *Pseudomonas*, *Bacillus*, *Staphylococcus* and *Proteus* are important environmental isolates. Hence their presence may be due to environment factors such as exposure to air and water used in the processing. It could also be attributed to organisms inherent in the substrates used in production of the beverages as well as processing machine (Ogodo *et al*., 2018). Occurrence of *Staphylococcus* spp and *Bacillus* spp may result to food poisoning (Oku *et al*., 2018). Also, some species of *Streptococcus* can cause sinusitis, sore throat (Orutugu *et al*., 2015). The occurrence of the organisms could put potential consumers of the drink at risk of disease condition especially in immune compromised individuals.

4.1 CONCLUSION

The findings of this research showed that the microbial content of kunu, zobo and tiger nut drinks locally prepared and sold in Calabar is high and do not fall within the standard limit of $<10^5$CFU/mL for aerobic mesophilic bacteria in food as stipulated by FDA for permissible microbial load in cereals/finished cereal products. This implies that, consumption of these products may constitute a serious public health challenge. Hence, there is need for regular monitoring of the local production protocol by the food safety agencies. And also, sensitization on the importance of good hygiene and sanitation during preparation and processing of kunu, zobo and tiger nut drinks in order to reduce microbial contamination to acceptable level and improve the final product quality.

4.2 RECOMMENDATIONS

Based on the findings of the study, the following recommendations can be made:

1. Water used in processing the drink should be properly and thoroughly treated.
2. Personnel involved in processing the drink should be educated on good personal hygiene.
3. More research should be carried out on the extent at which microbes act on locally produced drinks.

REFERENCE LIST

Agbo V, Tahir F. Species of bacteria associated with laboratory and locally produced indigenous beverage, kununaya. *GSC Biological and Pharmaceutical Sciences*, 2018; 03(03): 047–053. DOI: https://doi.org/10.30574/gscbps.2018.3.3.0055.

Asuquo NE, Antai SP, Tiku R. Microbiological and nutritional quality characteristics of kunu drinks sold in Calabar metropolis. *International Journal of Science and Research Methodology*, 2017; 8(1): 221-235.

Ayandele A. Microbiological analysis of hawked kunu and zobo drinks within LAUTECH campus, Ogbomoso, Oyo state, Nigeria. *Journal of Environmental Science, Toxicology and Food Technology*, 2015; 9(10): 52-56.

Badua MH, Bilyaminu D, Ogori AF, Charles B, Joeguluba O. Microbial quality evaluation of tiger nut beverage (*kununaya*) processed sold in university of Maiduguri. *EC Nutrition*, 2018; 138-142.

Batool SA, Tahir SS, Rauf N, Kalsoom R. Microbiological analysis of pasteurized and fresh fruit juice sold in Rawalpindi of Pakistan. *Bangladesh Journal of Scientific and Industrial Research*, 2013; 48(3): 185-192.

Etang UE, Ikon GM, Udofia SM, Umo AN, Udo EE, Uyanga FZ, Ohagim PI. Microbiological analyses of kunu drinks locally produced and sold in Calabar, southern Nigeria. *Journal of Advances in Microbiology,* 2017; 5(2): 1-8.DOI: 10.9734/JAMB/2017/36169.

Foline O, Rachael A, Ruquayyah M, Eunice B. The Nutritional quality of 3 varieties of zobo (*Hibiscus sabdariffa*) subjected to same preparation method. *American Journal of food Technology*, 2011; 6(8): 705 – 708.

Food and Drug Administration. Revised guidelines for the assessment of microbiological quality of processed foods. *Food and Drug AdministartionPhillipines*, 2013: 1-11.

Izah SC, Orutugu LO, Kigigha LT. A review of the quality assessment of zobo drink consumed in Nigeria. *ASIO Journal of Microbiology, Food Science & Biotechnological Innovations (ASIO-JMFSBI)*, 2016; 1(1): 34-43.

Kigigha LT, Samson GA, Izah SC, Aseibai ER. Microbial assessment of zobo drink sold in some locations in Yenagoa metropolis, Nigeria". *EC Nutrition,* 2018; 13(7): 470-476.

Mohammed FS, Ismail BB. Comparison on two methods of preparation of zobo drink on the survival of *Bacillus* spp. *American Journal of Food Technology*, 2014; 9: 200-8.

Mohammed SF, Gimba IK, Bahago EJ. Production and quality evaluation of instant sorrel (zobo) drink produced by infusion, dehydration and size reduction methods. *Journal of Nutrition and Health Sciences*, 2017; 4(2): 205. doi: 10.15744/2393-9060.4.205.

Musa AA, Hamza A. Comparative analysis of locally prepared 'kununaya' (tiger-nut milk) consumed by students of Kaduna state university, Kaduna-Nigeria. *Science World Journal*, 2013; 8(2): 13-18.

Ogodo AC, Agwaranze DI, Nwaneri CB, Yakubu MN, Hussain ZJ. Comparative study on the bacteriological quality of kunun-aya sold in Wukari, Nigeria.*International Journal of Research Studies in Microbiology and Biotechnology*, 2018; 4(1): 23-29. DOI: http://dx.doi.org/10.20431/2454-9428.0401004.

Oku IY, Alagoa KJ, Daworiye PS, Izon-ebi BM. Microbial content of zobo drink from five different producers within Yenagoa city Bayelsa state, Nigeria. *International Journal of Advances in Scientific Research and Engineering (ijasre)*, 2018; 4(9): 73-89. DOI: 10.31695/IJASRE.2018.32878.

Omeremu DM, Enoch AS, Azuonwu O. Bacteriological quality assessment of zobo drink sold in Bayelsa state Nigeria. *Journal of Applied Life Sciences International*, 2019; 20(1): 1-8. DOI: 10.9734/JALSI/2019/v20i130073.

Orutugu LA, Izah SC, Aseibai ER. Microbiological quality of kunu drink sold in some major markets of Yenagoa metropolis, Nigeria. *Continental Journal of Biomedical Sciences*, 2015; 9(1): 9-16. doi:10.5707/cjbmsci.2015.9.1.9.16.

Sule IO, Saliu BK, Zakariyah RF, Adegbenro IO. (2016). Microbiological quality assessment of ‘’kunu” sold within Ilorin metropolis, Nigeria. *Biological and Environmental Sciences Journal for the Tropics*, 2016; 13(3): 135 – 140.