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

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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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, tiger-nut and pineapple drinks), produced and sold in Calabar metropolis.
Methodology: Samples (tiger-nut, zobo, kunu and pineapple drink) were collected from five different markets within the study area and evaluated using standard bacteriological techniques.

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Results: The mean aerobic bacterial ranged from 21.6 ± 0.8 x 10^5 CFU/mL to 208.8 ± 2.02 x 10^5 CFU/mL. The coliform counts ranged from 5.8 ± 0.49 x 10^5 CFU/mL to 187.2 ± 2.10 x 10^5 CFU/mL. The staphylococcal counts ranged from 29.8 ± 0.92 x 10^5 CFU/mL to 194.4 ± 1.64 x 10^5 CFU/mL. Coagulase-negative Staphylococci was the most predominant isolates (27.0%), followed by *Escherichia coli* (12.2%) and *Enterobacter* sp (10.4%). Other bacteria genera detected include *Bacillus* sp, *Lactobacillus* sp, *Pseudomonas* sp, *Proteus* sp, *Micrococcus* sp, *Klebsiella* sp, *Serratia* sp, *Citrobacter* sp, and *Streptococcus* sp and *Listeria* sp.
Conclusion: This study revealed that the bacteriological quality of the analyzed samples was above the acceptable standards and as such a potential risk to public health. Therefore, education for vendors on food safety and hygienic practices is essential to reduce contamination rate. Furthermore, regular inspection on food vending practices and safety of ready-to-eat foods is required to improve the health standards of consumers.

Keywords: Bacteriological; assessment; beverages; kunu; zobo; tiger-nut; Calabar.

1. INTRODUCTION

Beverages are foods usually consumed in a liquid form and may include alcoholic and nonalcoholic drinks. In recent time, locally prepared beverages are gaining prominence over the consumption of conventional carbonated drinks as a result of the perceived health implications of excess sugars [1,2]. In Nigeria, zobo, tiger-nut, kunu and pineapple juice are commonly sold at public places and roadside shops. Aside their thirst-quenching properties, they are good sources of nutrients and income for the producers [3].

Zobo drink is prepared by either steeping or boiling the calyces of *Hibiscus sabdariffa* in portable water to extract the juice. The red pigment is usually sweetened with sugar and served chilled to consumers. *H. sabdariffa* (red sorrel) are naturally rich in carbohydrate, protein, minerals and important pharmacological constituents [4].

Tiger-nut drink is produce from the tiger-nut (*Cyperus esculentus*) tuber. Its local production involves washing the tiger-nuts in clean water 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. After mashing, water is added in the right proportion and sieved. Known quantity of sugar or honey can be added to obtained the desired taste [5].

Kunu is a popular cereal based nonalcoholic 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 [6]. Traditional production of kunu involves wet milling of cereal grains with spices, wet sieving and partial gelatinization of the curry, sugar addition, bottling and sales [7].

Pineapple (*Ananas comosus*) juice is a nonalcoholic drink widely consume by different folks across the world. The fresh juice is an excellence source of mineral such as calcium, potassium, magnesium and manganese. The traditional method of preparation exposes the juice to microbial contamination through various means. Pineapple juice is normally dominated by the microbial genera of *Bacillus*, *Staphylococcus*, *Micrococcus*, *Aspergillus*, *Fusarium*, *Rhizopus* and *Penicillium* [8].

The microbiological status of locally prepared beverages depends highly on the production protocol and this varies among individuals and community, resulting in high microbial density seldom exceeds tolerant limits for ready-to eat food [9]. These pathogens which include bacteria, fungi, parasites and viruses can be transmitted via contaminated foods, and as such poses threat to consumers. In developed countries, local production of beverages is under some laws and regulations but in many developing and under developed countries including Nigeria, the production protocol is not standardized and the producers seems to have little or no knowledge of Good Manufacturing Practice (GMP), Good Hygiene Practice or Hazard Analysis Critical Control Points (HACCP), which are aimed to guarantee microbiological safety of food products, especially ready-to-eat foods. Hence, sources of microbial contamination may include unhygienic environment, contaminated water, prolonged storage without

refrigeration, contaminated utensils and flies that landed sporadically on the foods [10]. Therefore, it is necessary to verify the safety of consuming this food drinks. Hence, this research was conducted to evaluate the "kunu, zobo, tiger-nut" and pineapple drinks sold in Calabar metropolis, for the presence of bacterial contaminants.

2. MATERIALS AND METHODS

2.1 Sampling Technique

A total of one hundred (100) locally produced beverages were purchased randomly from five different markets in Calabar metropolis, namely Watt market, Marian market, Akim market, Goldie market, and Abasi Obori market. The samples which include tiger-nut; zobo, kunu and pineapple juice packaged in plastic bottle were purchase from vendors. Twenty (20) samples from each market (i.e. 5 each of kunu, zobo, tiger-nut and pineapple juice). The samples were transported to the laboratory for microbiological analysis within 1 hour of collection.

2.2 Isolation and Phenotypic Characterization of Isolates

The bacterial load of the samples was determined following standard microbiological methods. Briefly, serial dilutions of the various samples were made up to 10^{-7} with sterile distilled water. About 0.5 mL of dilution 10^{-5} was evenly spread on Nutrient agar, and incubated at 37^{o}C for 24 hours. Plates were screened for discrete colonies after incubation period and the actual numbers of bacteria (total bacterial counts) were estimated as colony forming unit per milliners (Cfu/mL) of the sample [11]. Total coliform count (TCC) and total Staphylococcal count (TSC) were performed in similar manner using MacConkey agar and Mannitol Salt agar (MSA) mediums respectively. Discrete representatives of each unique colony type were selected for purification, characterization and identification using conventional microbiological and biochemical tests.

3. RESULTS

3.1 Bacterial Load Obtained from Samples

The bacterial loads of various locally made beverages obtained from five markets in Calabar is shown in Tables 1. The mean aerobic bacterial ranged from 21.6 ± 0.8 x 10^5 CFU/mL to 208.8 ± 2.02 x 10^5 CFU/mL. The coliform counts ranged from 5.8 ± 0.49 x 10^5 CFU/mL to 187.2 ± 2.10 x 10^5 CFU/mL. The staphylococcal counts ranged from 29.8 ± 0.92 x 10^5 CFU/mL to 194.4 ± 1.64 x 10^5 CFU/mL (Table 1).

3.2 Characterization and Identification of Isolates

A total of 115 bacterial isolates which made up thirteen genera were recovered. Bacteria was more frequently detected in tiger-nut drink (31.3%, 36/115) followed by zobo (28.7%, 33/115), kunu (21.7%, 25/115) and pineapple drink (18.3%, 21/115) (Table 2).

Coagulase-negative Staphylococci was the most predominant isolates (27.0%, 31/115) followed by *Escherichia coli* (12.2%,14/115) and *Enterobacter* sp (10.4%, 12/115). However, *Listeria* sp (2.6%, 3/115) was the least isolated bacteria (Table 2). Other bacteria genera detected include *Bacillus* sp, *Lactobacillus* sp, *Pseudomonas* sp, *Proteus* sp, *Micrococcus* sp, *Klebsiella* sp, *Serratia* sp, *Citrobacter* sp, and *Streptococcus* sp (Table 2). From the analyzed samples, tiger-nut had the highest bacterial contaminants, and the least bacterial contaminant was recorded in pineapple drink (Table 2).

4. 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, its microbiological quality and safety is of utmost importance to ensure the wellbeing of consumers [9].

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

The mean aerobic bacterial, coliform and staphylococcal counts obtained in this study ranged from 21.6 ± 0.8 x 10^5 CFU/mL to 208.8 ± 2.02 x 10^5 CFU/mL, 5.8 ± 0.49 x 10^5 CFU/mL to 187.2 ± 2.10 x 10^5 CFU/mL and 29.8 ± 0.92 x 10^5 CFU/mL to 194.4 ± 1.64 x 10^5 CFU/mL respectively. The counts recorded in this study

Table 1. Mean bacterial counts (x 10^5 $CFUml^{-1}$) obtained from locally produced beverages from different markets in Calabar

Location	Sample	Mean heterotrophic bacteria counts	Mean coliform counts	Mean staphylococcal counts
Watt market	Kunu	206.0 ± 2.07	118.4 ± 1.68	133.0 ± 1.82
	Zobo	235.8 ± 1.77	187.2 ± 2.10	149.2 ± 2.03
	Tiger-nut	247.8 ± 2.01	121.2 ± 1.40	115.8 ± 1.70
	Pineapple	181.8 ± 1.18	76.2 ± 1.78	128.3 ± 1.70
Marian market	Kunu	207.2 ± 2.00	119.2 ± 1.73	127.2 ± 0.91
	Zobo	280.8 ± 2.02	160.4 ± 1.97	154.8 ± 1.42
	Tiger-nut	117.2 ± 2.16	115.6 ± 1.89	194.4 ± 1.64
	Pineapple	177.2 ± 1.45	91.4 ± 1.82	74.6 ± 1.45
Akim market	Kunu	48.8 ± 1.24	74.8 ± 1.68	63.2 ± 0.91
	Zobo	45.0 ± 1.16	16.6 ± 1.13	44.2 ± 1.42
	Tiger-nut	61.8 ± 1.68	53.4 ± 1.33	56.8 ± 1.64
	Pineapple	54.6 ± 1.43	59.6 ± 0.46	53.4 ± 1.45
Goldie market	Kunu	115.4 ± 1.70	91.4 ± 1.39	67.8 ± 1.43
	Zobo	92.2 ± 1.62	17.2 ± 0.59	103.6 ± 2.02
	Tiger-nut	151.0 ± 1.97	5.8 ± 0.49	83.0 ± 1.71
	Pineapple	71.0 ± 1.59	44.2 ± 1.48	102.2 ± 2.05
Abasi-Obori market	Kunu	109.2 ± 1.97	38.0 ± 1.47	133.8 ± 2.22
	Zobo	72.2 ± 1.29	21.6 ± 0.81	55.4 ± 1.33
	Tiger-nut	74.6 ± 1.62	16.6 ± 1.13	29.8 ± 0.92
	Pineapple	21.6 ± 0.8	5.8 ± 0.51	74.6 ± 1.49

Table 2. Bacterial isolates with respect to types of locally produced beverages

	Types of beverages				
Identified organisms	**Kunu**	**Zobo**	**Tiger-nut**	**Pineapple**	**Total isolates**
Enterobacter sp	2 (16.7%)	3 (25.0%)	3 (25.0%)	4 (33.3%)	12 (10.4%)
Serratia sp	1 (25.0%)	1 (25.0%)	2 (50.0%)	0 (0.0)	4 (3.5%)
Klebsiella sp	0 (0.0)	2 (40.0%)	3 (60.0%)	0 (0.0)	5 (4.3%)
Pseudomonas sp	1 (14.3%)	5 (71.4%)	1 (14.3%)	0 (0.0)	7 (6.1%)
Lactobacillus sp	2 (22.2%)	3 (33.3%)	1 (11.1%)	3 (33.3%)	9 (7.8%)
Bacillus sp	1 (10.0%)	5 (50.0%)	2 (20.0%)	2 (20.0%)	10 (8.7%)
CoNS	8 (25.0%)	7 (22.6%)	10 (32.3%)	6 (19.4%)	31 (27.0%)
Micrococcus sp	3 (60.0%)	0 (0.0)	1 (20.0%)	1 (20.0%)	5 (4.3%)
Streptococcus sp	2 (50.0%)	0 (0.0)	1 (25.0%)	1 25.0%)	4 (3.5%)
Proteus sp	4 (57.1%)	2 (28.6%)	0 (0.0)	1 (14.3%)	7 (6.1%)
Escherichia coli	0 (0.0)	4 (28.6%)	7 (50.0%)	3 (21.4%)	14 (12.2%)
Listeria sp	0 (0.0)	1 (33.3%)	2 (66.7%)	0 (0.0)	3 (2.6%)
Citrobacter sp	1 (25.0%)	0 (0.0)	3 (75.0%)	0 (0.0)	4 (3.5%)
Total	**25 (21.7%)**	**33 (28.7%)**	**36 (31.3%)**	**21 (18.3%)**	**115 (100)**

KEY: CoNS: Coagulase-negative Staphylococci

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 [7,12]. A similar study by Badua *et al*. [13] 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*. [11], 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*. [1] 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 [14] 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 [15].

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 [2,13,15].

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*. [3] 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, Escherichia coli, Enterobacter, Bacillus, Lactobacillus, Pseudomonas, Proteus, Micrococcus, Klebsiella, Serratia, Citrobacter, Streptococcus* and *Listeria*. This is in accordance with previous findings in the same study area [7,15]. The bacterial diversity reported in this study is similar to other studies carried on street vended beverages outside Calabar metropolis. Thus, Ogodo *et al*. [11] 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*. [2] also reported *Staphylococcus aureus, Pseudomonas aeruginosa, Micrococcus luteus, Escherichia coli, Bacillus subtilis, Enterobacter aerogenes, Enterobacter cloacae, proteus vulgaris*, *Aspergillus flavus, Mucor* sp*, Cladosporium* sp*, Penicillium* sp*, Candida* sp *and Rhizopus* sp as microbial diversity found in zobo drinks prepared from different sources within Yenagoa city Bayelsa state, Nigeria. Badua *et al*. [13] reported microbial species of tiger nut sold in university of Maiduguri as *Staphylococcus aureus, Salmonella* sp*, Shigella* sp, *Pseudomonas* sp, *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 (*Escherichia coli*, *Enterobacter* and *Klebsiella* sp) is an indication of contaminants from fecal origins, packaging materials; water used for processing and washing etc. [1]. 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 [11]. Occurrence of *Staphylococcus* sp and *Bacillus* sp may result to food poisoning [2]. Also, some species of *Streptococcus* can cause sinusitis,

sore throat [16]. The occurrence of the organisms could put potential consumers of the drink at risk of disease condition especially in immune compromised individuals.

5. CONCLUSION

The findings of this research showed that the bacterial content of kunu, zobo, tiger nut and pineapple 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 bacterial 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 street vended foods is essential in order to reduce microbial contamination and improve the final product quality.

6. 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.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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