

# Bacteriological Quality of Zobo Drink Sold in Bayelsa Isolated and Enterotoxin Production by some strains

## ABSTRACT

**Introduction:** Zobo is a non-alcoholic locally produced beverage from dried petals, acid-succulent calyxes of *Hibiscus sabdariffa* by boiling and filtration. Zobo is rich in carbohydrates, proteins, calcium, vitamins, minerals, iron and antioxidant. The consumption of zobo may be associated with food infection and/ or food borne illness arising from unhygienic processes.

**Aim:** The aim of this study is to determine the bacterial quality of zobo sold in Bayelsa, identify the bacteria isolated and determine the enterotoxin producing ability of some strains.

**Materials and Methods:** A total of 150 bottles of zobo were examined, 50 were purchased from each zone (Yenagoa, Sagbama and Ogbia). Each bottle of zobo was well mixed by gentle inversion and 1mL of the zobo was added to 9mL of sterile peptone water in a test tube. Serial dilution was made to  $10^5$  and 0.1mL of the last dilution ( $10^5$ ) was inoculated on already prepared and dried media (nutrient, MacConkey and salmonella/shigella agar) in duplicate and spread evenly with sterile glass rod. The plates were incubated at 37°C for 18-24 hours and examined for growth. Commercial purchased kits were used to test for enterotoxin production of some isolated strains.

**Results:** Out of the 150 zobo samples examined, the bacteria isolated were *S. aureus* 120 (25%), *Coagulase negative Staphylococci sp.* 120 (25%), *Bacillus sp.* 150 (31.3%) and *Salmonella sp.* 90 (18%) respectively. Out of 120 *S. aureus* isolated, 18 (15%) produced enterotoxin.

**Conclusion:** Regulatory Agencies should as a matter of urgency consider the regulation of zobo production for public consumption and producers should be instructed on the principles of food preservation, sanitation and hygiene. The consumption of locally produced zobo is a public health concern in Nigeria.

**Keywords:**

## 1. INTRODUCTION

Zobo is a non-alcoholic local beverage made from dried petals, acid-succulent calyxes of *Hibiscus sabdariffa* by boiling and filtration [1, 2]. *Hibiscus sabdariffa* is an annual erect, herbaceous shrub with smooth or almost smooth, cylindrical and typically red stem. The flower is mostly cultivated in northern Nigeria and zobo is now popular in West African Sub Region, especially among the youths who see zobo as an alternative, cheap, relaxing non-alcoholic drink in social gatherings [3].

Zobo is a red coloured non-alcoholic local beverage made from different varieties of dried, succulent aqueous acid extract of Roselle calyx.[4]. The beverage has soured taste but

often sweetened. The name zobo is derived from zoborodo in Hausa, goneura in Hindi, krajeab in Thailand, bissap in Senegal and sorrel in Caribbean [5]. Zobo as non-alcoholic beverage is quite popular in northern part of Nigeria.[6].

Religious and health campaigns against alcoholic beverages in Nigeria and the subsequent decrease in intake of alcoholic beverages in some areas have made zobo drink an alternative to alcoholic beverages. Zobo is known to be rich in carbohydrate, protein, calcium, vitamin, minerals, iron and antioxidants. Aside this, it was used in folk medicine as diuretic, mild laxative, treatment for cardiac and nerve diseases and management of cancer. It was reported that zobo is a good traditional medicine for the treatment of diseases such as hypertension, UTI etc.[7] The danger of food infections and food borne illness that may be associated with zobo outweighs the benefits derived. Bacteria isolated from zobo drink includes, *S. aureus*, *Bacillus sp*, *Lactobacillus sp*, *Escherichia coli*, *Pseudomonas sp*, *Enterobacter sp* [8]. Other researchers isolated *S. aureus*, *Streptococci sp* and *Proteus sp*. [9] Bacteria isolates from Zobo include *S. aureus*, *Bacillus sp*, *Micrococcus*, *Proteus sp*, *E. coli* [10]. It was noted that the consumption of zobo might serve as vehicle for food borne disease agents such as *S. aureus*, *Salmonella sp*, *Brucella sp*, *Mycobacterium* and *E. coli* [9,11]. In a study conducted on the microbiological quality of zobo in Aba South East Nigeria, the bacteria isolated were *Staphylococcus sp*, *E. coli*, *Lactobacillus*, *Bacillus sp* and *Pseudomonas sp*. The level of contamination was attributed to lack of personal and environmental hygiene in the processing, packaging and preservation [12]. Looking at the nutritional, sensory and microbiological assessment of zobo, the following bacteria were isolated, *E. coli*, *Klebsiella sp* and *Bacillus sp*. which was linked to contamination from vendors and materials used for production [13]. In assessing the microbial quality of zobo sold in Yenagoa metropolis the bacteria isolated were *E. coli*, *S. aureus*, *Enterobacter sp*, *Micrococcus* and *Proteus sp*. [14]. The poor bacteriological quality was attributed to poor handling, and poor quality materials used for production, unhygienic processing and vendors. Other researchers also isolated similar organisms [15,11]. It was noted that lack of proper hygiene and sanitary measures in processing and packaging of kunu were responsible for contamination.

The aim of this research is to determine the bacteriological quality of zobo drinks marketed in Bayelsa State, Nigeria, identify bacteria isolated and the enterotoxin producing ability of some strains.

## 2. MATERIAL AND METHODS

### 2.1 Study Area

The study was conducted in Bayelsa State, Nigeria. The samples of Zobo drink were purchased from the three (3) senatorial district headquarters of Bayelsa State, namely; Yenagoa (capital), Sagbama and Ogbia town. Bayelsa state was carved out of River State in 1996. Bayelsa is located in latitude 4°15' North, latitude 5°23' South and longitude 5°22' West and longitude 6°45' East. It is bound by Delta State on the North, River State on the East and Atlantic Ocean on the West and South. Bayelsa has the largest wetland in West African Sub-Region. It has a population of about 1.7 million.

### 2.2 Collection of Samples

A total of 150 bottles of zobo were purchased, out of which fifty (50) were bought from each of the senatorial headquarters. The samples of zobo were transported to the laboratory in a cooler of ice-packs for examination. Zobo drinks were packaged and sold in recycled coke, fanta, sprite or medium water bottles of 35cl at 50 naira each.

### 2.3 Bacteriological Examination of Samples

Each sample of Zobo was mixed properly by gentle inversion several times and 1ml of the sample (neat) was pipetted and transferred to 9mL of sterile normal saline (sterilized by autoclaving at 121° C for 15 minutes). Subsequent serial dilutions were made up to 10<sup>5</sup> and 0.1ml of the last dilution (10<sup>5</sup>) was placed on already prepared and dried nutrient, MacConkey and *Salmonella Shigella* agar plates in duplicates. These were spread evenly with the aid of sterile glass rod (sterilized by dipping in absolute alcohol and flaming in Bunsen flame). The inoculated plates were incubated at 37° C for 18 - 24 hours and examined for growth.

## 2.5 Identification of Isolated Bacteria

The bacteria isolated were identified using morphology, cultural, Gram's stain reaction, chemical and biochemical reactions such as citrate, VP, Methyl red, indole, catalase, coagulase and carbohydrates fermentation etc.

## 2.5 Test for Bacterial Load in stored Zobo

A set of purchased zobo were kept at room temperature and another in the refrigerator at 4°C after the initial determination of the bacterial counts in CFU/mL. The counts from the preserved zobo at ambient and refrigerator temperature were determined on the second and third day respectively.

## 2.6 Test for Toxigenicity of *S. aureus*

The Prolex™ Staph Latex Kit is used to detect *S. aureus* that produce enterotoxin. The Kit uses blue polystyrene latex particles that had been sensitized with fibrinogen and IgG.

### 2.6.1 Procedure

The latex kit were removed from refrigerator and allowed to attain room temperature before use. The reagent was re-suspended by gentle inversion several times. A drop of the Staph test Latex reagent was placed in a circle on test card and colony of *Staphylococcus aureus* was mixed with the reagent on the entire circle. This was rocked gently by allowing the mixture flow the entire area. Strong agglutination within 20 seconds was regarded as positive. Negative control Latex reagent was included as quality control.

## 3 RESULTS

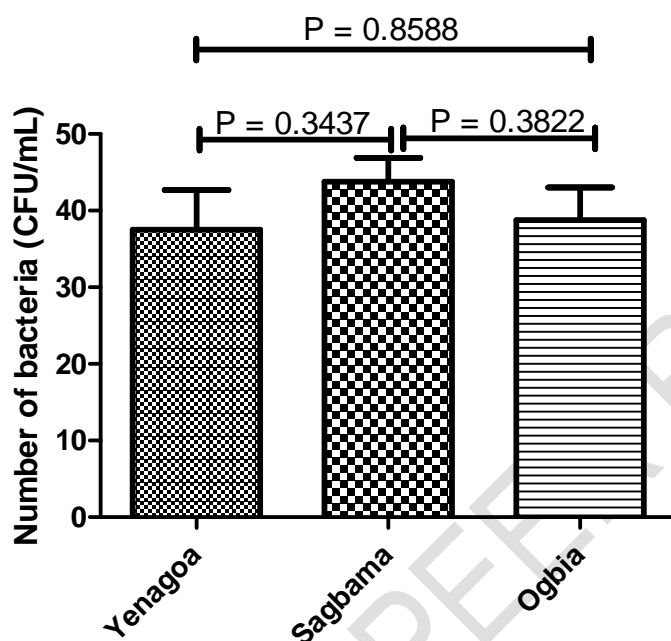
### 3.1 Percentage occurrences of isolated bacteria from zobo

Out of the 150 samples of zobo purchased, 50 were purchased from Yenegoa, Sagbama and Ogbia town respectively. In zobo from Yenegoa *S. aureus* were 40 (26.7%), coagulase negative *Staphylococci* were 35 (23.3%), *Bacillus sp.* 50 (33.3%) and *Salmonella sp.* 25 (16.7%). In Sagbama *S. aureus* 45 (25%), *Bacillus sp.* 50 (28.6%), Coagulase negative *Staphylococci* 45 (25.7%) and *Salmonella sp.* 35 (20.0%) respectively. In Ogbia town *S. aureus* 35 (22.6%), coagulase negative *Staphylococci* 40 (32.3%), *Bacillus sp.* 50 (32.3%) and *Salmonella sp.* 30 (19.4%) respectively. The overall percentage occurrences of *S. aureus* was 120 (25.0%), coagulase negative *Staphylococci* 120 (25%), *Bacillus sp.* 150 (31.3%) and *Salmonella sp.* 90 (18.6%) respectively.

131 **Table 3.1.** Percentage occurrence of Bacteria Isolated from Zobo in Yenegoa, Sagbama and  
 132 Ogbia Town

Location	<i>S. aureus</i>	Coagulase negative <i>Staph.</i>	<i>Bacillus sp.</i>	<i>Salmonella</i> <i>sp.</i>	Total
<b>Yenegoa</b>	40 (26.7)	35 (23.3)	50 (33.3)	25 (16.7)	150 (31.3)
<b>Sagbama</b>	45 (25.7)	45 (25.7)	50 (28.6)	35 (20.0)	175 (36.6)
<b>Ogbia</b>	35 (22.6)	40 (25.8)	50 (32.3)	30 (19.4)	155 (32.3)
<b>Total</b>	120 (25.0)	120 (25.0)	150 (31.3)	90 (18.6)	480

133 Numbers in parenthesis = percentages



134  
 135  
 136  
 137 **Figure3.1** The difference in the counts of bacteria isolated from different locations.

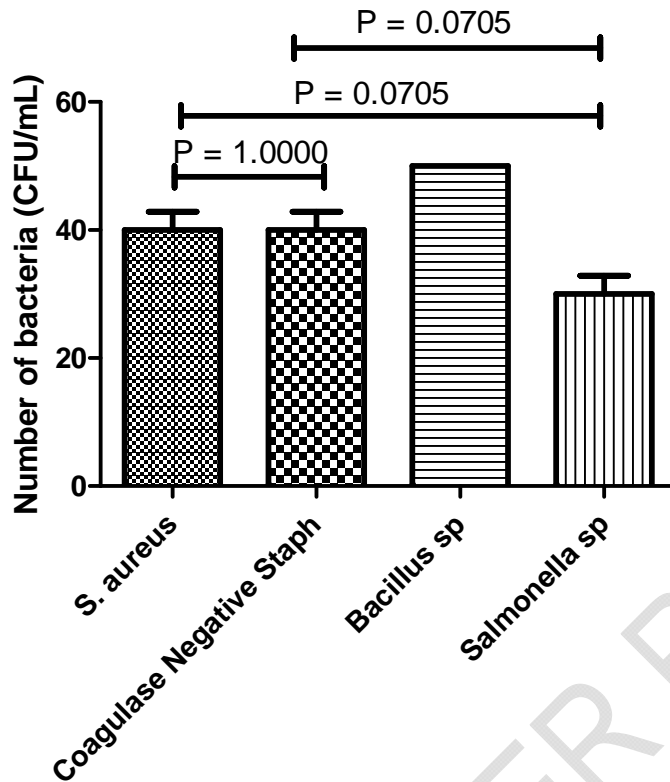


Figure 3.2 The difference in the counts of bacteria species isolated from zobo.

### 3.1 The percentage of enterotoxin producing *S. aureus*

Out of the 40 isolates of *S. aureus* obtained from zobo drinks, 4 (10%) produced enterotoxin from Yenegoa zone, out of 45 *S. aureus* isolated from zobo purchase in Sagbama, 6 (13.3%) produced enterotoxin, while in Ogbia, 35 *S. aureus* were isolated and 8 (22.8%) were capable of producing enterotoxin respectively.

**Table 3.2.** Percentage occurrence of enterotoxin producing *S. aureus* isolated from zobo drink from each zone.

Location	No. Of <i>S. aureus</i> Isolated	No. Positive
Yenegoa	40	4 (10)
Sagbama	45	6 (13.8)
Ogbia	35	8 (22.8)
Total	120	18 (15)

Numbers in parenthesis = percentages

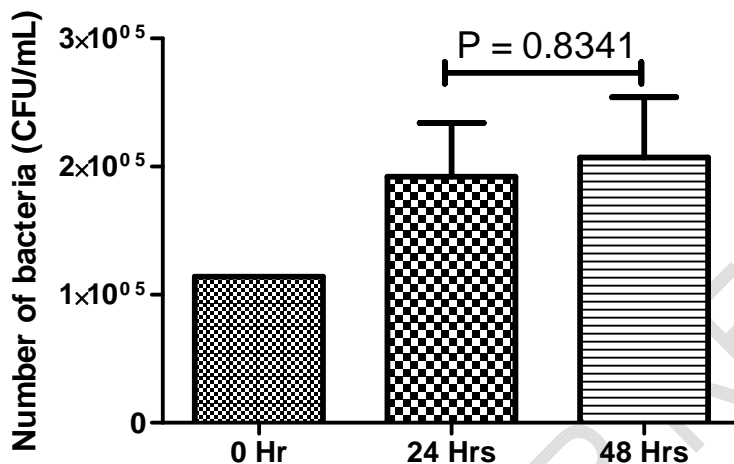
### 3.3 Bacterial counts in preserved zobo from zero to 48Hrs

The total count of bacteria isolated from zobo sample immediately after preparation were;  $1.14 \times 10^3$  cfu/ml on Day 1,  $1.56 \times 10^3$  CFU/MI on Day 2 and  $1.60 \times 10^3$  CFU/MI on Day 3 respectively. The counts obtained from room temperature storage were;  $1.14 \times 10^3$  CFU/MI on Day 1  $2.34 \times 10^3$  CFU/MI on Day 2 and  $2.54 \times 10^3$  CFU/MI on Day 3 respectively.

160  
161  
162

**Table3.3** Bacteria counts in zobo preserved at room and refrigeration temperature for 3 days.

Temperature	Day 1 Counts in CFU/MI	Day 2 Counts in CFU/MI	Day 3 Counts in CFU/MI
Refrigeration	$1.14 \times 10$	$1.5 \times 10^3$	$1.6 \times 10^3$
Room	$1.14 \times 10^3$	$2.34 \times 10^3$	$2.54 \times 10^3$



163  
164  
165  
166  
167  
168

Figure 3.3: Mean values of bacterial counts in zobo.

Figure3.4: Identification of isolated bacteria from zobo

S/No	Colour	Surface	Edge	Translucency	Texture	Gram Rxn	Size	Shape	Motility	Methyl Red	Voges Proskauer	Oxidase	H <sub>2</sub> S Production	Indole	Coagulase	Catalase	Citrate	Urease	Starch Hydrolysis	Glucose	Lactose	Sucrose	Maltose	Galactose	Mannitol	Arabinose	Oxidative	Fementative	Bacteria
1	M	R	E	C	D	+	Md	Rd	+	-	+	-	-	-	-	+	-	-	+	A	A	A	A	A	±	-	+	+	<i>Bacillus sp.</i>
2	M	S	E	C	Mt		Md	Rd	+	+	-	-	-	+	-	+	-	-	+	A	A	A	A	A	A	A	+	+	<i>E. coli</i>
3	M	S	E	C	Mt	-	Md	Rd	+	+	-	-	+	-	-	+	+	-	-	+	-	-	+	-	-	-	+	+	<i>Salmonella sp.</i>
4	Cr	S	E	C	Mt		Md	Cc	-	+	+	-	-	-	-	+	N	N		A	A	A	-	A	-	-	+	+	<i>Strptococcus sp.</i>
5	Cr	S	E	C	Mt	+	Md	Cg	-	+	+	-	-	-	+	+	N	N	-	A	A	A	A	A	A	-	+	+	<i>S. aureus</i>

169  
170  
171  
172  
173  
174  
175  
176

Key: M=Milky, Cr=Creamy, R=Rough, S=Smooth, E=Entire, C=Clear, D=Dry, Mt=Moist, Md=Moderate, Cc=Cocci in chain,, Cg=Grape like cocci Rd=Rod, N=Not determined

#### 4. DISCUSSION

The most prevalent bacteria isolated from zobo were *Staphylococci* followed by *Bacillus sp.* and *Salmonella sp.* *Staphylococcus* is a common contaminant of foods and other similar preparations if good hygienic practices are not employed. *Staphylococci* as normal flora of

human inhabit the nostrils, hands, skin, mouth and dresses etc. They might easily gain access to zobo without good sanitary practices. Those who prepare zobo locally do not consider the use of good water as a means of reducing contamination therefore water may also serve as source of contamination introducing *Staphylococcus* and other bacteria into zobo, especially water used for washing and rinsing of recycled containers for packaging. *Bacillus* sp. were the second most prevalent bacteria, the calyxes may have come in contact with soil during harvesting and the dusts blown by the wind during drying in the sun might deposit both bacteria and/ or their spores on the calyxes. *Bacillus* is geophilic and the above process may favour their deposition on the calyxes. Some of the spores may survive the only Critical Control Point (CCP) in the preparation of zobo (boiling at about 100° C for about 30 minutes). The spores might germinate and re-contaminate zobo. *Salmonella* sp. might be present as a result of the handling processes from contaminated hands and water. These bacteria were also isolated by other researchers from zobo drink as contaminants with similar prevalence [12]. Zobo drinks were marketed on recycled bottles of water, fanta, coke, sprite etc. These bottles were only washed or rinsed without any sterilization process. The sterility of the water used for washing or rinsing is questionable. The ready to drink zobo were just transferred directly into the bottle for sale. This process might aid contamination of zobo drinks. The percentage occurrence of bacteria isolated from zobo is an indication of poor hygienic handling [16].

Other workers also isolated *Escherichia coli* from zobo drinks, but from our study, *E. coli* was not isolated. It might be possible that the concentration of extracted *Hibiscus* calyxes used for zobo preparation might possess antimicrobial activity against *E. coli* or the phytochemical substances in the extracted calyxes and/or other spices used with the calyxes may have inhibitory action on *E. coli*.

Zobo had been noted to harbour bacteria associated with food borne illness and the health implication of consuming contaminated zobo outweighs the benefits.[17,18] The spices added to zobo were mainly agricultural produce which had been noted to contain heavy microbial load.[11,19] The consumption of local drinks such as zobo is of public health concern. Zobo might serve as a vehicle of transferring bacteria associated with food borne illness.[9,20].

One of the challenges of zobo is the preservation of finished product. The total bacterial counts from freshly prepared zobo were  $1.41 \times 10^5$  CFU/ mL and after 18 – 24 hours the count rose to  $2.34 \times 10^5$  CFU/ mL and on the third day  $2.54 \times 10^5$  CFU/mL. Freshly prepared zobo should be consumed the same day, except when preserved in refrigerator at a temperature of 4°C to control the proliferation of contaminating bacteria. In a similar estimation of total heterotrophic counts in zobo, a count of  $4.02 \times 10^5$  CFU/mL was obtained [10]. Post preparation contamination from personnel and packaging might play major role in contamination of zobo.

The test for the production of enterotoxin by *S. aureus* isolated from zobo showed that 15% of *S. aureus* were capable of producing enterotoxin. This clearly showed that the consumption of locally produced zobo drink is a public health concern because the bacteria isolated were associated with food infections and food poisoning (foodborne illnesses).

#### 4. CONCLUSION

With the increasing interest in consumption zobo drink, there is need for regulatory agencies to regulate the production zobo. The isolation of enterotoxin producing *S. aureus* is of major public health concern associated with the consumption of zobo drinks. Left over zobo from previous day sale should be confiscated, unless preserved at 4° C.

#### REFERENCES

1. Ogiehor IS, Nwafor DE, and Owhe-Ureghe UB Changes in the quality of zobo beverage produced from *Hibiscus sabdariffa* (Unn roselle) and the effect of ginger alone or in combination with refrigeration. *African Journal of Biotechnology*. 2008; 7(8):1176 -180.
2. Kolawole JA and Maduenyi A Effect of zobo drink (*Hibiscus sedbrariffa* water extract) on the pharmacokinetics of acetaminophen in human volunteers. *European Journal of Drug Metabolism and Pharmacokinetics* .2004; 29(1): 25-29.
3. Ogiehor IS, Nwafor OE. Associated microbiological, biochemical and chemical quality changes in zobo beverages produced from *Hibiscus sabdariffa* Linn, Niger. *Ann. Nat. Sci.* 2004;5:1-10.
4. Zumbes TO, Kerfeld CA, Dakul DA, Afolabi SA and Dapiya MS. Enteropathogenic bacteria contamination of some ready to eat foods sold in Jos Metropolis, Nigeria. *Indian Journal of Applied Research*. 2014;4(7): 456 – 458.
5. Okereke CN, Iroka FC and Chukuruma MO . Phytochemical analyses and medicinal uses of *Hibiscus sabdariffa*. *International Journal of Herbal Medicine*. 2015; 2(6):16-19.
6. Egbere OJ, Anuonye JC, Challom PF, and Kpara PV. Effect of some technique on the quality and storage stability of zobo drink, a Nigerian non- alcoholic beverage from *Hibiscus sabdariffa*. *Journal of Food Technology*.2007; 5(3): 225-228.
7. Bamishaiye EL, Olayemi FF, Bamishaiye OM. Effect of boiling time on minerals and vitamin content of three varieties of drink from *Hibiscus sabdariffa*. *Journal of Food Technology*.2011; 3(5): 225-228.
8. Nwachukwu E, Onovo OM, Ezeama CF. Effect of lime juice on the bacterial quality of zobo drinks locally produced in Nigeria. *Research Journal of Microbiology*.2007;2:787-791.
9. Amusa, N. A. Ashaye, O. A. Aiyegbajo, A. A., Oladapo, M.O and Oni, M.O. Microbiological and Nutritional Quality of Hawked Sorrel Drink (Zoborodo) Widely Consumed and Notable in Nigeria. *Journal of Food Agriculture and Environment* 2005; 3(3-4): 47-50.
10. Ibitoye FO, Oyetayo AM, Aribisa OK, and Giwa OE.. Investigation of bacteria associated with the spoilage of zobo drink fortified with scent leaf and ginger. *Archives of Current Research International*.2017; 9(1): 1-7.
11. Ayandele AA.. Microbial Analyses of Hawked Zobo and Kunu within LAUTECH Campus Ogbomoso , Oyo State, Nigeria. *IOSR Journal of Environmental Science, Toxicology and Food Technology*.2015; 9 (11): 52 – 56.
12. Ezeigbo O.R, Uhiara S. Nwodu JA and Ekiako MU . Bacteriological Assessment of Hawked Sorrel Drinks (zobo drink) in Aba South East Nigeria. *British Microbiology Research Journal*. 2015; 5 (2): 146 -151.
13. Adeniji PO . Nutritional, Sensory and Microbiological of Fortified Zobo Drink, a Home Prepared Traditional Nigerian Beverage. *Journal of Nutrition and Food Science*. 2017; 7: 627 – 632.



- 270 14. Kigigha LT, Izah SC, Samson GA and Asiebai ER. Microbiological Assessment of  
271 Zobo Drinks Sold in Some Locations in Yenagoa Metropolis, Nigeria. EC – Nutrition.  
272 2018; 13(7) :470 – 476.
- 273 15. Seiyaboh EI, Oku IY and Odogbo MO. Bacteriological Spoilage of Zobo (a Nigerian  
274 Drink Prepared from the Calyces of *Hibiscus sabdariffa* L. Malvaceae). International  
275 Journal of Engineering and Science. 2013; 2(11): 46 -51.
- 276 16. Oku IY, Alagoa KJ, Daworiye PS and Izah-ebi BM (2018).Microbial content of zobo  
277 from five different producers withi Yenagoa city Bayelsa State, Nigeria. International  
278 Journal of Advances in Scientific Research and Engeneering. 2018; 4(9): 74-89.
- 279 17. Balakarami AU, Umarazulum and Mustafa AI (2016). Microbiological analyses of  
280 drinking water in Maiduguri Metropolis, Nigeria. International Journal of Research,  
281 2016; 3(8):13: 93-97.
- 282 18. Amusa, N. A. Ashaye, O. A. Aiyegbajo, A. A., Oladapo, M.O and Oni, M.O.  
283 (2005). Microbiological and Nutritional Quality of Hawked Sorrel Drink  
284 (Zoborodo) Widely Consumed and Notable in Nigeria. Journal of Food  
285 Agriculture and Environment, 3(3-4), 47-50.
- 286 19.
- 287 20. drink consumed predominantly in Northern Nigeria. Internet Journal of Food  
288 Safety.2016; 3(8):13: 93-97.
- 289 21. Musa, A.A. and Hamza, A. Comparative Analysis of Locally Prepared  
290 ‘Ogbonna, I. O., Opobiya, M. Y., Katuka, B. and Waba, J. T. .Microbial  
291 evaluation and proximate composition of kunu-zaki, an indigenous fermented  
292 food Kunun Aya’ (Tiger-Nut Milk)Consumed By Students of Kaduna State  
293 University, Kaduna-Nigeria. Science World Journal. 2013; 8 (2): 13 – 18.
- 294 22.

295  
296  
297  
298  
299  
300  
301

## **DEFINITIONS, ACRONYMS, ABBREVIATIONS**

Here is the Definitions section. This is an optional section.

**Term:** Definition for the term

## **APPENDIX**