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Research Article

Comparative Evaluation of Enterotoxigenic Bacteria in Kunu and Zobo Drinks

Smart Enoch Amala[1*], Daw Mercy Omeremu[1], Moore Ikechi Mike-Ogburia[1], Tombari Pius Monsi[1]

[1]*Microbiology Unit, Department of Medical Laboratory Science, Rivers State University, Nkpolu-Oroworukwo, Port Harcourt, Nigeria*

ABSTRACT

Introduction: Kunu and zobo are popular non–alcoholic drinks marketed all over Nigeria. They are made from grains and calyx of hibiscus and they are marketed in offices, schools, motor and parks, and used in festive celebrations.

Aim: This study aimed to isolate and identify bacteria associated with kunu and zobo contamination, identify potentially pathogenic strains and determine the enterotoxin production abilities of some bacteria strains.

Methodology: A total of 150 bottles each of kunu and zobo were purchased and examined. Out of the 150 bottles of kunu and zobo, 50 each was purchased from Yenegoa, Sagbama, and Ogbia respectively. Each bottle of the beverage was mixed by gentle inversion several times and 1 ml of it was pipette and added to an ml of peptone water. Subsequent serial dilutions were made to 10^5 for kunu and 10^3 for zobo and 0.1 ml of each was cultured on an agar plate in duplicate. The plates were incubated at 37 ^{0}C for 18–24 hrs and examined for growth.

Results: The percentage occurrences rod-shaped bacteria isolated from kunu were *E. coli* 150(27.8%), *Salmonella sp.* isolated from zobo were 90(18.6%), while *Bacillus sp.* isolated from kunu were 150(27.8%) and from zobo were 150(31.3%). The percentage occurrence of cocci bacteria isolates from kunu were *S. aureus* was 150(27.8%) and from zobo *S. aureus* 120(25.0%); coagulase-negative *Staphylococci sp* 120(25.0%) and *Streptococci sp,* isolated from kunu was 90(16.7%) respectively. The percentage of enterotoxins producing *E. coli* isolated from was 19(12.7%), while the percentages of S. aureus producing enterotoxins from kunu was 25(16.7%) and from zobo was 18(15.0%) respectively. The counts of bacteria in refrigerated kunu were 7.5 x 10^5 to 8.7 x 10^5 and at ambient temperature the counts were 7.5 x 10^5 to 1.3 x 10^6 CFU/ml while zobo refrigerated were 1.1 x 10^3 to 1.6 x 10^3 and at ambient temperature 1.1 x 10^3 to 2.5 x 10^3 CFU/ml

Conclusion: The bacteriological quality of kunu and zobo produced in Bayelsa are poor, the keeping quality of both beverages are very poor at ambient temperature such their consumption beyond 24 hrs is at the consumers risk especially due to the presence of enterotoxin producing *E. coli* and *S. aureus* isolated from the beverages.

Keywords: Purchased; Examined; Percentage; Enterotoxin; Consumption

INTRODUCTION

Kunu (kununzaki) a beverage drink made from grains of millet, sorghum and maize and combinations. It's popular in northern parts of Nigeria. Kunu produced from sorghum is milky light-brown coloured, while the product from maize is whitish coloured [1,2]. The grain seeds used for the production of kunu drink were allowed to germinate while steeped in water for few days and after which blended with sweet potatoes and ginger or pepper to form a smooth paste. The paste is divided into two, one part is placed in a vessel and boiled water is added to it to form a thick mixture. The unheated half is added to the previous and stirred to give a thick mixture. The mixture is left for a day or two for the grain husk to settle. The husk and other sediments are filtered out of the mixture and the filtrate is boiled for consumption.

Correspondence to: Smart Enoch Amala, Microbiology Unit, Department of Medical Laboratory Science, Riv ers State Univ ersity, Nkpolu-Oroworukwo, Port Harcourt, Nigeria, Tel: 2348055362681; E-mail: smrtamala@gmail.com

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Zobo, a non-alcoholic beverage is made from dried petals, acid-succulent calyxes of Hibiscus sabdariffa by boiling and filtrating [3,4]. Hibiscus sabdariffa is an annual erect, herbaceous shrub with smooth or almost smooth, cylindrical and typically red stem. The flowers are mostly cultivated in Northern, Nigeria, the beverage is popular in West Africa, in schools and social gatherings as an alternative, cheap, relaxing non-alcoholic [5]. Zobo a red coloured non-alcoholic local beverage made from different varieties of dried, succulent aqueous acid extract of Roselle carlyx [6]. The beverage has a 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 the Caribbean [7].

Zobo as a non-alcoholic beverage is more popular in Northern Nigeria [8]. It was reported that zobo is a good traditional medicine for the treatment of several diseases such as hypertension, UTI, etc. [9].

Kunu is a non-alcoholic beverage marketed in several public places such as offices, markets, schools, motor parks and a very commonly consumed beverage during festivities such as weddings, naming ceremonies, birthday celebrations, etc. [10].

Kunu is an appetizer, food complement and refresher to quench thirst [11,12,13]. The proximate analysis of Kunu was determined and the content includes; protein 2.31–3.63%, fats 3.35–3.65%, ash content 1.16–1.21% and carbohydrates 82.92–83.55% [2].

Religious and health campaign against alcoholic beverages in Nigeria and the subsequent decrease in the intake of alcoholic beverages in some areas have made zobo drink an alternative to alcoholic beverages. Zobo is known to be rich in carbohydrates, protein, calcium, vitamin, minerals, iron and antioxidants. Aside from this, it was used in folk medicine as a diuretic, mild laxative, treatment for cardiac and nerve diseases and management of cancer. It was reported that zobo is good traditional medicine for the treatment of diseases such as hypertension, UTI, etc. [9].

The high bacterial content of kunu is an indication of poor hygiene, poor quality cereals and water used in preparation and packaging processes [14]. The bacteria isolated from kunu were *E. coli* 33.3%, *S. aureus* 26.7%, *Streptococcus sp*. 23.3%, *Pseudomonas sp*. 10% and *Bacillus sp*. 6.7% [14]. Etang, [15] investigating locally produced kunu isolated *Bacillus sp*. 15%, *E. coli* 15%, *Salmonella sp.* 12.5%, *Streptococcus sp*.10%, *Pseudomonas sp*. 7.5%, *Proteus sp*. 7.5%, *Lactobacillus* 22.5% and *S. aureus* 10% respectively. Bacterial isolates from kunu might be linked with food spoilage, food infections and poisons [16].

Bacteria isolated from zobo include, *S. aureus*, *Bacillus sp*, *Lactobacillus sp*, *Escherichia coli*, *Pseudomonas sp Enterobacter sp*. [17]. Amusa et al., [18] isolated *S. aureus*, *Streptococci sp*. and *Proteus sp*. Other bacteria isolated from zobo were *S. aureus, Bacillus sp*. *Micrococcus, Proteus sp*. *Streptococci sp, E. coli* [19]. The study aims are to determine the bacteriological quality of kunu and zobo marketed in Bayelsa, the percentage occurrences of isolated bacteria, the pathogenic potential of *E. coli* and *S. aureus* isolates and the shelf life of the beverages.

MATERIALS AND METHODS

Study Area

The research was carried out in Bayelsa State, Nigeria. Samples of Zobo and Kunu drinks were procured from the three (3) senatorial district headquarters in Bayelsa, namely; Yenagoa, Sagbama and Ogbia town. Bayelsa State was created from River State in 1996. Bayelsa State is situated 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 the Atlantic Ocean on the West and South. Bayelsa has the largest wetland in the West African sub-region. Its population is about 1.7 million people [20].

Sample Collection

A total of 150 bottles of kunu and 150 bottles of Zobo were bought and out of these fifty (50) bottles of each were purchased from the senatorial headquarters namely, Yenegoa, Sagbama and Ogbia town respectively. The samples were transported to the laboratory in a cooler of icebergs for examination. Kunu and Zobo drinks were sold in recycled bottles of 35 cl soft drinks at the cost of fifty naira (#50.00) each.

Bacteriological Examination of Samples

Each sample of kunu and Zobo were mixed by gentle inversion several times and 1 ml of the sample (neat) was added to 9 ml of sterile peptone water in a test tube (sterilized by autoclaving at 121°C for 15 minutes). Subsequent serial dilutions were made to 10^5 and 0.1 ml of the last dilution (10^5) of each was dispensed on already prepared and dried agar plates in duplicates (nutrient, MacConkey and salmonella/shigella). These were uniformly spread on agar media with the aid of a sterile glass rod (sterilized by dipping in absolute alcohol and flaming in Bunsen flame). The inoculated plates were allowed to dry and incubated at 37°C for 18-24 hrs and examined for growth.

Bacterial Replication in Refrigerated Compared to Unrefrigerated Kunu and Zobo

A set of freshly prepared Kunu and Zobo were kept at room temperature and another set refrigerated at about 4°C after the initial determination of the bacteria counts in CFU/ml. The counts from the preserved Kunu and Zobo at room and refrigeration temperature were re-determined on the second and third day of storage at both conditions.

Identification of Isolated Bacteria

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

Enterotoxicity Testing for *S. aureus*

The Prolex™ Staph Latex Kit provides a rapid platform for the identification of Staphylococcal isolates particularly *S. aureus*

that produces enterotoxin. The Prolex™ Staph Latex Kit utilizes blue polystyrene latex particles that have been sensitized with fibrinogen and IgG

Detection of Enterotoxin Producing *E. coli* from Kunu

PROTM 0157 KIT can identify enterotoxin producing E. coli. Hardy Diagnostics PROTM 0157 KIT provides a rapid latex agglutination method to detect *E. coli* serogroup 0157 antigens from colonies isolated in the laboratory. These were *E. coli* producing verotoxin (VT-producing pathogen). Hardy diagnostic *E. coli* PROTM 0157 Kit contains due latex particles coated with an antiserum against *E. coli* 0157 antigens. When the coated latex particle is mixed with fresh colonies of *E. coli* serotype 0157, the bacteria will bind to the antiserum, causing the latex particles to visibly agglutinate, which was indicative of a positive result.

RESULTS

Percentage occurrences of Bacteria Isolated from Kunu and Zobo

A total of 150 samples of kunu were examined out of which 50 samples were examined from each location. The total number of *E. coli* isolated from kunu purchased from Yenagoa was 50(27.8%), Sagbama 50(27.0%) and Ogbia 50(27.8%) respectively. *E. coli* were not isolated from zobo 0(0.00%). The number of *Salmonella sp*. isolated from kunu was 0(0.00%) and *Salmonella* from zobo isolated from Yenagoa was 25(16.7%), Sagbama 35(20.0%) and 30(19.4%) respectively but no *Salmonella sp.* was isolated from kunu. *Bacillus sp.* recovered from kunu in Yenagoa was 50(27.8%), Sagbama 50 (27.0%) and Ogbia 50(28.6%) while from zobo Yenagoa 50(33.3%), Sagbama 50(28.6%) and Ogbia 50(32.3%) respectively.

Table 1: Percentage Occurrences of Rods Shaped Bacteria Isolated from Kunu and Zobo

Organism	***E. coli***			***Salmonella sp.***			***Bacillus sp.***		
Location	kunu	zobo	p-value	kunu	zobo	p-value	kunu	zobo	p-value
Yenegoa	50(27.8)	0(0.00)	<0.0001	0(0.00)	25(16.7)	<0.0001	50(27.8)	50(33.3)	<0.0001
Sagbama	50(27.0)	0(0.00)	<0.0001	0(0.00)	35(20.0)	<0.0001	50(27.0)	50(28.6)	<0.0001
Ogbia	50(28.6)	0(0.00)	<0.0001	0(0.00)	30(19.4)	<0.0001	50(28.6)	50(32.3)	<0.0001
Total	150(27.8)	0(0.00)		0(0.00)	90(18.6)		150(27.8)	150(31.3)	
Numbers in Parenthesis = Percentages									

Percentage Occurrences of Cocci Bacteria Isolated from Kunu and Zobo

The percentage occurrences of *S. aureus* from Kunu in Yenagoa was 50(27.8%), Sagbama 50(27.8%) and 50(27.8%) and zobo Yenagoa was 40(26.7%), Sagbama 40(25.7%) and Ogbia 35(22.6%) respectively. Coagulase negative *Staphylococci* from zobo were Yenagoa 33(23.3%), Sagbama 45(25.7%) and Ogbia 40(25.8%) respectively. Coagulase negative *Staphylococci* were not isolated from kunu.

Streptococci sp. isolated from kunu bought from Yenagoa were 30(16.7%), Sagbama 35(18.9%) and Ogbia 25(14.3%) respectively whereas there was *Streptococci* isolated from zobo.

Table 2: Percentage Occurrences of Cocci Bacteria Isolated from Kunu and Zobo

Organism	***S. aureus***			**Coagulase -ve *Staphylococci***			***Streptococci* sp.**		
Location	kunu	zobo	p-value	kunu	zobo	p-value	kunu	zobo	p-value
Yenegoa	50(27.8)	40(26.7)	>0.9999	0(0.00)	33(23.3)	<0.0001	30(16.7)	0(0,00)	<0.0001
Sagbama	50(27.8)	40(25.7)	>0.9999	0(0.00)	45(25.7)	<0.0001	35 (18.9)	0(0.00)	<0.0001
Ogbia	50(27.8)	35(22.6)	0.4639	0(0.00)	40(25.8)	<0.0001	25 (14.3)	0(0.00)	<0.0001
Total	150(27.8)	120(25.0)		0(0.00)	120(25.0)		90 (16.7)	0(0.00)	

Percentages of Enterotoxin Producing *S. aureus* and *E. coli* from Kunu and Zobo

A total of 50 *S. aureus* were isolated from kunu in Yenegoa out of which 7(28%) were enterotoxin producing strains and 50 *S. aureus* were isolated from Sagbama, 10(40%) produced enterotoxin, while in Ogbia Town 50 *S. aureus* were isolated, 8(32%) were positive for enterotoxin production respectively. The overall total of *S. aureus* that produced enterotoxin were 25(33.2%). The number of *E. coli* isolated were 150, 50 each from Yenegoa, Sagbama and Ogbia, 7(37%) produced enterotoxin from kunu bought from Yenegoa and Ogbia while 5(26%) were from kunu purchased from Sagbama respectively.

Out of the 40 isolates of *S. aureus* obtained from zobo drinks, 4(22%) produced enterotoxin from the Yenegoa zone, out of 45 *S. aureus* isolated from zobo purchase in Sagbama, 6(33%) produced enterotoxin, while in Ogbia, 35 *S. aureus* were isolated and 8(37%) were capable of producing enterotoxin respectively.

Table 3: Percentages of Enterotoxin Producing S. aureus and E. coli from Kunu and Zobo

Organisms	***S. aureus***			***E. coli***		
Location	Kunu	Zobo	p-value	Kunu	Zobo	p-value
Yenegoa	7 (28)	4 (22)	0.5818	7 (37)	0(0.00)	0.0165
Sagbama	10 (40)	6 (33)	0.5644	5 (26)	0(0.00)	0.0435
Ogbia	8 (32)	8 (45)	0.6447	7 (37)	0(0.00)	0.0165
Total	25(16.7)	18(15.0)		19(12.7)	0(0.00)	
Numbers in Parenthesis Percentages						

Bacterial counts in preserved Kunu and Zobo from zero to 48 hrs

The count of bacteria isolated from kunu after preparation (ready to drink) at 0 hr was $7.48x10^5$ CFU/ml, at 24 hrs it was $8.40x10^5$ CFU/ml and at 48 hrs $8.72x10^5$ CFU/ml respectively. The count bacteria from kunu preserved at ambient temperature was $7.48x10^5$ CFU/ml at 0 hr, $11.96x10^5$ CFU/ml at 24 hrs and 13x105 CFU/ml at 48 hrs respectively. The total counts of 13x 105 CFU/ml at 48 hrs respectively. The total counts of bacteria isolated from preserved zobo at refrigeration temperature were; CFU/ml at 0 hr, CFU/ml at 24 hrs and CFU/ml at 48 hrs respectively. The count obtained from the set preserved at ambient temperature were; CFU/ml at 0 hr, CFU/ml at 24 hrs and CFU/ml at 48 hrs respectively.

Table 4: Counts of bacteria in refrigerated and unrefrigerated kunu and zobo (0 hr- 48 hrs)

	0 hr		**24 hrs**		**48 hrs**	
	Counts in CFU/ml		**Counts in CFU/ml**		**Counts in CFU/ml**	
Temperature	Kunu	Zobo	Kunu	Zobo	Kunu	Zobo
4ºC	$7.5x10^5$	$1.1x10^3$	$8.4x10^5$	$1.5x10^3$	$8.7x10^5$	$1.6x10^3$
Ambient	$7.5x10^5$	$1.1x10^3$	$1.2x10^6$	$2.3x10^3$	$1.3x10^6$	$2.5x10^3$

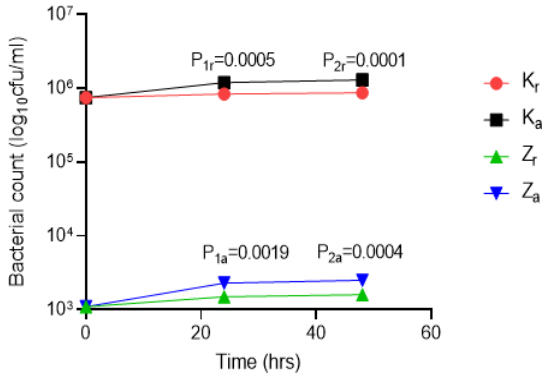

Figure 1: Time-dependent growth response of Kunu and Zobo at refrigerated and ambient temperature K= Kunu, Ka=Ambient, Kr=Refrigerated, Z= Zobo, Za=Ambient, Zr= Refrigerated.

Post-hoc Analyses of the Effect of Time on the Growth Response of Bacteria

Turkey's multiple tests at 95% Cl showed there was no significant difference between the growth of bacteria in kunu at 0 hr *vs* 24 hrs, 0 hrs and 48 hrs, 24 *vs* 48 hrs respectively and in Zobo at 0 hrs *vs* 24 hrs, 0 hrs *vs* 48 hrs, and 24 hrs *vs* 48 hrs respectively. The others were significant.

Table 5: Post-hoc analyses of the effect of time on growth response of bacteria in Kunu and Zobo

Tukeys multiple comparisons test	95.00% CI of diff.	P- value
0k *vs* 24k	-911247 to 1461247	0.6073
0k *vs* 48k	-931247 to 1441247	0.4181
0k *vs* 0z	-432397 to 1940097	0.0250
0k *vs* 24z	-433147 to 1939347	0.0251
0k *vs* 48z	-433297 to 1939197	0.0252
24k *vs* 48k	-1206247 to 1166247	0.9979
24k *vs* 0z	-707397 to 1665097	0.0057
24k *vs* 24z	-708147 to 1664347	0.0057
24k *vs* 48z	-708297 to 1664197	0.0058
48k *vs* 0z	-687397 to 1685097	0.0042
48k *vs* 24z	-688147 to 1684347	0.0042
48k *vs* 48z	-688297 to 1684197	0.0042
0z *vs* 24z	-1186997 to 1185497	>0.9999
0z *vs* 48z	-1187147 to 1185347	>0.9999
24z *vs* 48z	-1186397 to 1186097	>0.9999

Keys: z represents Zobo and k represents Kunu. ANOVA p=<0.0001.

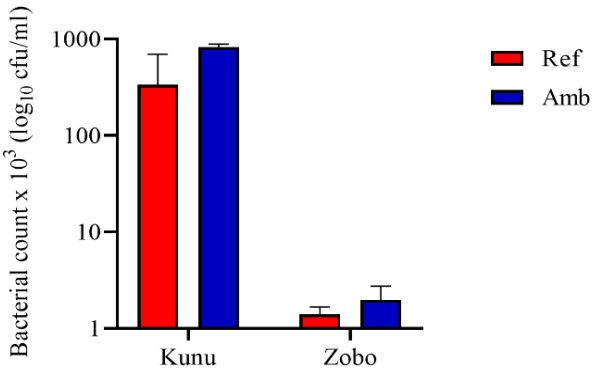

Figure 2: Effect of temperature on growth response of bacteria in Kunu and Zobo

Post-hoc Analyses of the Effect of Temperature on Growth Response of Bacteria

Turkey's multiple comparison showed at 95% CI, Rk *vs* Ak and Rz *vs* Az were not significant respectively. The other was significant.

Table 6: Post-hoc analyses of the effect of temperature on growth response of bacteria in Kunu and Zobo

Tukey's multiple comparisons	95.00% CI of diff.	P-Value
Rk *vs* Ak	7.857 to 965.5	0.2159
Rk *vs* Rz	339.8 to 1297	0.0007
Rk *vs* Az	339.2 to 1297	0.0007
Ak *vs* Rz	-146.9 to 810.7	<0.0001
Ak *vs* Az	-147.4 to 810.2	<0.0001
Rz *vs* Az	-479.4 to 478.2	>0.9999

Keys: z – Zobo and k – Kunu, R – refrigeration temperature, A – ambient temperature. ANOVA p= <0.0001

DISCUSSIONS

The rods shaped bacteria isolated from kunu and zobo were *E. coli, Salmonella sp*. and *Bacillus sp. Bacillus sp*. were isolated from both kunu and zobo non-alcoholic beverages. There was a significant difference among the isolates using chi-square at>0.05. E. coli was isolated from kunu only, while *Salmonella sp.* is isolated from zobo only. The presence of *E. coli* and *Salmonella sp*. indicates contamination with human or animal faeces during processing. Similar rod-shaped bacteria were isolated by [21,22,16]. The spices used to boost the nutritional and sensory qualities of kunu and zobo drinks (ginger, garlic etc.) at the concentrations used may exhibit inhibitory effects on *E. coli* and Salmonella sp. Zobo and kunu drinks are always spiced with ginger, garlic or a mixture of ginger and garlic and other spices [23]. These spices are also known to improve the pH level to slightly alkaline which may be hostile to some bacteria especially with the garlic and ginger combined as spices [23]. The spices aside from improving the nutritional properties of kunu or zobo, have been shown to possess antimicrobial properties [24,21,22]. Zobo drinks have high phenolic and flavonoids, mainly polyphenol which have been identified to possess antimicrobial activities [25]. Their proximate composition includes alkaloids, saponins and tannins at different concentrations [26,27] The extracts from Hibiscus sabdariffa used for zobo preparation may possess minimal antimicrobial properties but when combined with spices (ginger, garlic etc.) might exhibit better antimicrobial activity. The addition of spices contributes to the nutritional quality of kunu and zobo and on the other hand, might influence the developments of resistance to the spices and possibly trigger resistance against some therapeutic agents. The presence of *Bacillus sp*. in kunu and zobo concomitantly suggest contamination with the soil because *Bacillus sp.* are geophilic and the spores can survive boiling (sterilization) at 100^{o}C and germinate later to cause spoilage [28]. All the rods isolated from kunu and zobo were of public health importance and it calls for caution to consumers of the beverages. *Bacillus* is associated with food poisoning and infections.

The most predominant bacterium isolated from kunu and zobo were *Staphylococci* sp. *Staphylococci* are normal human flora carried on the palms, skin, nostrils etc. transmission to food can occur *via* human contacts, nasal droplets, desquamated skin dropping. The heavy growth observed in zobo and kunu may portray laxity in hygiene and deficiencies in food preservation techniques [29,30]. *Streptococci sp.* is of disquiet because it is associated with health conditions such as impetigo, erysipelas, cellulitis, puerperal sepsis, acute rheumatic fever etc.

There was a significant difference in the counts of bacteria isolated from kunu and zobo preserved at ambient temperature as compared to the refrigerated. This suggests the rapid proliferation of bacteria in both beverages at ambient temperature compared to refrigeration temperature. The isolation of *S. aureus*, Salmonella sp., *E. coli* and *Bacillus sp*. might predict the beverages are a good medium for the growth

of entero-pathogens capable of causing gastroenteritis. Therefore, should bear the risks associated in mind. Other workers also remarked that local produced kunu and zobo preserved at ambient temperature for 24 hrs and beyond may pose a public health risk to the consumers. Both Ndidi and Omeremu [31,28] noted that the shelf life of kunu depends on the temperature of storage. The microbiological quality of locally produced drinks is determined by total microbial counts and the pathogenic bacteria present in it.

The ability of a strain of bacterium to produce toxin enhances its pathogenic ability, for example, the production of enterotoxin associated with gastroenteritis. Foodborne infections are of public health concern worldwide. One of the commonest foodborne illnesses is staphylococcal food poisoning due to enterotoxin preformed in food by enterotoxigenic strains of coagulase-positive *S. aureus. Staphylococci* can survive desiccation and halophilic conditions but are destroyed by heat. The already produced enterotoxin in food can withstand approved irradiation and some recommended heat treatments including pasteurization [32, 33]. Kunu and zobo non- alcoholic beverages supported the growth of *S. aureus* and can be implicated in food poisoning. The isolation of 25(16.7%) and 18(15%) of enterotoxin producing *S. aureus* from kunu and zobo concomitantly was an indication of serious health risks that may be associated with consumers of foods, for example, food-borne intoxication. Staphylococcal enterotoxin (SEs) is secreted proteins that use superantigenic and emetic activities, they are resistant to proteolytic enzymes which enable them to maintain their activity in the digestive tract. The property enables staphylococcal SEs to cause severe gastroenteritis with nausea, emesis, and diarrhoea [33].

Enterotoxigenic *E. coli* (ETEC) are *E. coli* strains that produce a toxin that stimulates the intestine causing it to produce excess fluid (diarrhoea). The main source of ETEC is human and or animal wastes. Infection ensues when a person eats food, drink water or ice contaminated with ETEC. ETEC is a major cause of traveller's diarrhoea and diarrhoea in children mostly in developing countries like Nigeria. Other researchers have isolated *E. coli* from kunu [31,16]. The percentage of ETEC 19(27.7%) isolated from kunu calls for caution in the consumption of kunu and zobo non-alcoholic drinks.

Kunu is produced from the combinations of millet, sorghum, maize etc., they were steeped for few days and allowed to sprout and blend with sweet potatoes, after which spices such as ginger, pepper, garlic etc. were added. The multiple raw materials used to compound kunu make it a more nutritious and good medium for bacterial growth. The multiple steps involved in kunu preparation and indigenous microbiota of different raw materials exposes the final product to microbial contamination as compared to zobo which uses only the carlyx of Hibiscus sabdariffa and spices. The reasons might account for the higher counts observed in kunu at 0 hrs, 24 hrs and 48 hrs respectively, coupled with insufficient knowledge in food preservative techniques might have aided the high microbial load noticed in both beverages. The result of Post-hoc analysis of the effect of time on the growth response to bacterial at 95% C was significant for kunu and zobo. Post-hoc analyses of the effect of temperature on growth response of bacteria in Kunu and Zobo was also significant.

CONCLUSIONS

The microbiological quality of locally produced kunu and zobo in Bayelsa State is poor. The isolation of potentially pathogenic bacteria capable of producing toxins calls for caution to consumers because of the risks that may be associated. The shelf life of both kunu and zobo is just one day.

LIMITATION OF STUDY

This study did not assay for the presence of enterotoxins produced by the bacteria in the beverages nor determine the amounts of enterotoxins liberated into beverages by the organisms.

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