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Determination of the Microbiological Safety and Quality of Zobo Drink and Calyces from Two Communities, Kogi State, Nigeria

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ABSTRACT

About 600 million people worldwide develop an illness after ingesting a contaminated food or drink and 420 000 die every year. Random samples of zobo drink and calyces were collected from Idah and Ugwolawo communities and analysed for their microbiological safety and quality criteria, such as Total Viable Count (TVC) of bact, oreria, Total Mould and Yeast Count (TMYC), Total Coliform Bacterial Count (TCBC) and *Escherichia coli* detection. Safety and quality indicators were isolated on/in nutrient agar (TVC), sabouraud dextrose agar (TMYC), MacConkey broth (TCBC) and eosin methylene blue agar (*E. coli*). The results from the study showed that the samples had TVCs of 5.01 to 5.32 log CFU/mL, which exceed the acceptable limit of <104 CFU/mL for ready to eat foodstuff. The highest TMYC (log 3.24 CFU/mL) was obtained from dry zobo calyces while the high TCBCs recorded for the zobo drink (1.63 log MPN index/mL) and the dry zobo calyces (1.63 log MPN index/g) from the two study locations are indicative of contamination from intestinal or environmental sources. There was no significant difference (p=0.05) between counts from the two study locations. The isolates included bacteria: *Bacillus* sp., *Staphylococcus aureus*, *Proteus* sp. and *Lactobacillus* sp and fungi: *Aspergillus niger* and members of the genera, *Candida, Rhizopus, Penicillium* and *Saccharomyces*. These isolates are established foodborne pathogenic and spoilage microorganisms. In conclusion, the zobo drink samples do not meet the microbiological safety and quality criteria for ready to eat beverages, constituting a major challenge to public health.

Keywords: Illness; Microbiological; Quality; Ready to eat; Zobo

INTRODUCTION

The consumption of zobo drink, which is a non-alcoholic beverage, has become a common practice among many tribes in Nigeria and other parts of the world. Zobo is produced by boiling the dry calyces (sepals) of *Hibiscus sabdariffa* in water, for about 10 to 15 minutes to extract the pigments. *H. sabdariffa* is commonly referred to as the "red sorrel" or "roselle" and is a member of Malvaceae family [1]. Roselle is a popular plant in folk medicines, paint manufacture and beverage production [2]. The plant is rich in natural carbohydrates, protein, anthocyanin, antioxidants, vitamin C,

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calcium, magnesium, zinc and several other nutrients. Zobo beverage has a low glycemic index as well as striking nutritional and medicinal properties [3,4].

The pharmacological properties of the plant are partly traceable to the presence of phytochemicals, such as flavonoid and anthocyanin, organic acids and other constituents, many of which possess antioxidants effects [5].

H. sabdariffa thrives well in a loamy and well drained soil which receives an annual rainfall of about 1500-2000 mm^3 and the plant can tolerate high temperature, floods, heavy winds and is resistant to some pests [6]. Zobo is prepared with the addition of natural preservatives and flavour enhancers, such as orange or lime, pineapple, cinnamon, alligator pepper, garlic, scent leaf, ginger and moringa, in order to improve the taste, healing properties and keeping quality [7,8]. In Nigeria, two varieties of *H. sabdariffa* exist, including the red-brown and green varieties. The red-brown type is most common in Northern Nigeria, while the green variety is cultivated in Southern Nigeria. The calyces of the two varieties of *H. sabdariffa* are used in the preparation of soup, stew, sauces or zobo drink [9].

Zobo drink and calyces may habour both foodborne microorganisms which are responsible for poisoning and spoilage. The processes of zobo production and packaging in polythene bags or plastic bottles before retailing are unhygienic and the safety and quality of raw materials and other ingredients are not guaranteed, which may increase the chances of microbial contamination.

Bacterial species in the genera, *Streptococcus, Proteus, Klebsiella, Micrococcus, Enterobacter*, *Bacillus, Campylobacter, Salmonella* serotypes as well as *Listeria monocytogenes*, *Escherichia coli* and *Staphylococcus aureus* have been implicated in the outbreaks of poisoning due to ready to eat meals. The most frequently reported fungi genera are *Aspergillus, Rhizopus, Mucor, Candida, Penicillium* and *Fusarium* [10].

Food poisoning occurs from the ingestion of foodstuff or drinks contaminated by microbial agents, chemicals, toxins or other hazards in food at the point of consumption. Food safety, nutrition and food security are inextricably linked and unsafe food creates a vicious cycle of disease and malnutrition, particularly affecting the infants, young children, elderly, pregnant women and immune compromised group. Foodborne diseases impede socio economic development by straining healthcare systems, harming national economies, tourism and trade. An estimated 600 million (1 in every 10) people worldwide, develop an illness following the ingestion of a contaminated food or water and 420 000 die each year, resulting in the loss of 33 million disability adjusted life years.

Food safety concerns represent a major obstacle to socio-economic development and the attainment of sustainable development goals, 1 to 3: “No poverty,” “zero hunger” and “good health and well-being.” About US $110 billion is lost each year in productivity and medical expenses resulting from foodborne illnesses and outbreaks in low, middle and some high income countries.

Zobo is a preferred drink in social gatherings because it is economically affordable, nutritious and safe and more appealing compared to most traditional carbonated beverages. Religious beliefs, health factors and campaigns against alcoholic drinks in Nigeria, while limiting alcoholic beverage consumption has turned zobo drink to a cherished local alternative beverage. Most Nigerians can afford fifty to one hundred naira (#50 to #100) for a cold serving of zobo drink, especially during hot or warm weathers.

The production steps for zobo drink are not standardized and the producers seem not to understand the principles of Good Manufacturing Practice (GMP), good hygiene practice or Hazard Analysis Critical Control Points (HACCP), which are meant to guarantee microbiological product quality and safety. Zobo drink belongs to ready to eat foodstuffs which are not subjected to any form of post-production processing or heating before ingestion. The status predisposes zobo to contamination and proliferation by foodborne pathogens and spoilage microbial agents which might have survived the production processes.

Sensitization of the public and food handlers together with standardized hygienic processing procedures would guarantee the safety, quality, increased acceptability and marketability of zobo drinks in Nigeria and other developing countries.

The research, therefore, was designed to determine the microbiological quality and safety of zobo drink and calyces obtained from two communities in Kogi State, Nigeria.

MATERIALS AND METHODS

Sample Collection

Ten discrete zobo drink samples and six dry calyces were purchased from different sellers and hawkers and put into clean polyethylene bags and taken to the laboratory for analysis. The zobo drink samples were transported under an ice cold condition, while the zobo calyces were kept in good conditions and transported to the laboratory immediately for microbiological analysis.

Microbiological Analysis

The microbiological analysis was carried out according to standard methods. All media were prepared according to the manufacturer’s instructions. Preparation of sample homogenate and serial dilutions were carried out and microbiological quality and safety parameters, such as total viable count of aerobic mesophilic bacteria, total mould and yeast count, Total Coliform Bacterial Count (TCBC) and detection of *Escherichia coli* were investigated, using media, such as nutrient agar, Sabouraud dextrose agar, MacConkey broth and eosin methylene blue agar, respectively. Sterile Buffered Peptone Water (BPW, Oxoid) was used as diluent.

Identification of Microbial Isolates

Gram staining and biochemical tests (indole, catalase, coagulase, motility) of bacterial isolates were carried out

according to standards methods. Wet mount identification of fungal isolates was carried out using lactophenol cotton blue stain, followed by microscopic examination.

Statistical Analysis

All results were expressed as means ± SD of duplicate counts. The data were subjected to one-way Analysis of Variance (ANOVA) using SPSS version 16.0. The Duncan's multiple range tests was used to separate the means at 5% degree of probability.

RESULTS AND DISCUSSION

The microbial counts ranged from 5.01 to 5.38 log CFU/mL or g (Table 1), 2.00 to 3.30 log CFU/mL or g (Table 2) and 0.87 to 1.63 log MPN index/mL or g (Table 3). Bacterial isolates included *Bacillus, Proteus, Lactobacillus spp*. and *Staphylococcus aureus* (Table 4) while the fugal isolates were identified as *Candida, Saccharomyces, Penicillium, Rhizopus spp*. and *Aspergillus niger* (Table 5). The samples were negative for *E. coli* on EMBA.

Table 1: Total viable bacterial count of zobo drink and calyces.

Sample code	Total viable count (log CFU/mL or g)	
	Zobo drink	Calyces
A	5.03	5.38
B	5.1	5.33
C	5.15	5.36
D	5.11	5.38
E	5.07	5.32
F	5.01	5.38
G	5.31	-
H	5.31	-

Note: A to D: Samples from Idah LGA; E to H: Samples from Ofu LGA.

Table 2: Total Mould and Yeast Count (TMYC) of zobo drink and calyces.

Sample code	Zobo (log CFU/mL)	Calyces (log CFU/g)
A	1.99	3.24
B	2.16	2.24
C	3.12	3.3
D	2.05	3.2
E	2.05	3.09
F	2.05	2.08
G	3.16	-
H	2.13	-

Note: A to D: Samples from Idah LGA; E to H: Samples from Ofu LGA.

Table 3: Total Coliform Bacterial Count (TCBC) of zobo drink and calyces.

Sample code	Log_1 MPN drink (per mL)	Index calyces (per g)
A	1.63	1.63

B	1.63	1.63
C	1.2	1.2
D	1.63	1.63
E	1.04	1.04
F	0.87	0.87
G	1.58	-
H	1.3	-

Note: A to D: Samples from Idah LGA; E to H: Samples from Ofu LGA.

Table 4: Cultural, morphological and biochemical characteristics of bacterial isolates.

Isolates	Cultural characteristics	Cellular	Gram's reaction	Catalase	Indole	Coagulase	Motility	Probable bacterium
A	Irregular, wavy, lobate, flat colony	Large, single rods or chains	+	+	+	+	+	*Bacillus* sp.
B	Circular, convex, smooth, golden-yellow colonies	Large cocci in clusters	+	+	-	+	-	*Staphylococcus aureus*
C	Round, entire, yellowish colony	Convex rods	_	+	+	-	+	*Proteus* sp.
D	Gray, white, mucoid colonies	Rods in clusters	-	+	+	_	+	*Lactobacillus* sp.

Note: +, Positive; -, Negative; sp., species

Table 5: Identification of fungal isolates from zobo drink and calyces.

Isolates	Cultural charactertics	Wet mount morphology	Probable fungus
A	Large, creamy, white, smooth, flat colony	Oval budding cells, pseudo-hyphae	*Candida sp*
B	Smooth, cream, white and hairy colony	Budding cells	*Saccharomyces sp.*
C	Greenish colony with yellow reverse	Double branching septate hyphae short conidiophores	*Penicillium sp*
D	Dense grayish, cottony colony	Oval, non septate hyphae with sporangiophores	*Rhizopus sp*
E	Brown, black colony with white edges, yellow on the reverse of plate	Double branching septate hyphae, short conidiophores	*Aspergillus niger*

The high total bacterial loads (log 5.32 CFU/mL and log 5.39 CFU/g) obtained from the study on zobo drink and calyces, respectively are unacceptable, as they indicate bad manufacturing practices for a ready to eat beverage. The Total Viable Counts (TVCs) of aerobic mesophilic bacteria for the zobo drinks and calyces were in the range of 5.01 to 5.31 logs CFU/mL and 5.32 to 5.38 log CFU/g, respectively. The indirect TVC of food samples indicates the hygiene level in the food processing environment inclusive of processing procedures, personnel hygiene, the cleanliness of food contact surfaces, utensils, packaging materials and time-temperature control and storage conditions. TVC counts are important parameters in predicting the shelf life or keeping quality of food. The poor knowledge of food hygiene and lack/abuse of standard operating procedures may be responsible for the unsatisfactory data from zobo drink samples. The samples might have been contaminated from utensils, use of water and other additives and packaging materials. The results agreed with the reports of Ayandele and Nwachukwu and Aniedo, who obtained TVCs in the range of 0.4-15.0 × 10^5 CFU/mL and 2.79 to 2.62 log CFU/mL, respectively for zobo samples.

The zobo calyces which normally carry bacteria from the field due to improper cleaning might have contributed their quota to high level of bacterial contaminants. Some growers use untreated sewage and other organic fertilizer to condition the soil and researchers have reported that roselle calyx is the major source of contamination in the drink.

The bacterial flora of zobo drink and calyces were dominated by species of *Bacillus, Proteus, Lactobacillus* and *Staphylococcus aureus*. These pathogens have been implicated in several food poisoning incidences. In a related study, Nwachukwu and Aniedo, isolated bacteria such as *S. aureus*, *Bacillus cereus, Lactobacillus* sp., *E. coli, Pseudomonas aeruginosa*, *Enterobacter aerogenes* and *Klebsiella* sp. from zobo drinks. *Staphylococcus aureus* is the most resistant to adverse conditions of all non-spore formers. The presence of *S. aureus* in food above a certain critical number is objectionable because the bacterium has been implicated in several foodborne intoxications. The presence of *S. aureus* is probably due to improper handling and subsequent storage at elevated temperatures. The bacterium is an agent of food intoxication producing several enterotoxins with or without demonstrable emetic activity. *B. cereus* is not a competitive bacterium, but it grows after heating and cooling. Deaths due to the diarrhoeal and emetic toxin of *B. cereus* have been reported.

Coliform bacteria also form part of the microbial flora of locally processed RTE foods. The MPN count of coliform bacteria indicated a range of 0.87 to 1.63 log MPN index/mL and 0.87 to 1.63 log MPN/g, for the drink and calyces, respectively.

The microbiological content of raw materials can reflect in the end product quality and the point of raw material reception is often a Critical Control Point (CCP), under the concept of HACCP. Most biological, chemical and physical hazards in food cannot be prevented, reduced or eliminated if they escape the CCP measures.

The fungal counts in the current report ranged from 1.99 to 2.20 Log CFU/mL and 2.08 to 3.24 log CFU /g, for zobo drink and calyces, respectively. The identified fungal isolates belong to the genera, *Candida, Saccharomyces, Penicillium, Rhizopus* and *Aspergillus niger*. Isolation of fungi in the genera, *Penicillium, Saccharomyces* and *Aspergillus* has been reported by Ekanem in retailed zobo drinks. Field moulds follow raw materials from the farm to fork or straw and they are important fungi with respect to food illnesses and spoilage.

CONCLUSION

The determination of the microbiological safety and quality of zobo drink and calyces has been successfully carried out and the results indicate that the final product quality and safety are a function of the quality of raw materials and ingredients as well as the hygiene condition under which the zobo beverage is prepared. The microbiological criteria assessed showed that the raw materials and zobo beverage are unacceptable for any ready to eat beverage or product. The use of contaminated water as ingredient, additives, packaging materials and poor hygiene of food personnel and abuse of time temperature control during production, storage and retailing tend to increase the microbial load of zobo drink to unsafe levels. This, therefore, calls for sensitization and the need to standardize the procedures involved in zobo beverage production. Further research is required on zobo drink and preparation to generate enough data that would enhance the use of GMP, GHP and the HACCP concept which are meant to guarantee final product quality and safety.

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