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Hawked Zobo Drinks Bacteriological quality in Oko, Anambra, Nigeria.

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Abstract

The bacteriology quality of hawked zobo beverages was conducted in oko, Anambra state, Nigeria. Zobo drinks were randomly bought from the popular market (Eke oko market), and their bacteriological quality was examined using standard microbiological procedures.The analysis showed that the samples' pH ranged from 2.9 to 3.2. While the total coliform count ranged from 0.3 x 10^4 cfu/ml to 5.6 x 10^4 cfu/ml, the total aerobic bacterial count varied from 0.5 x 10^5 cfu/ml to 6.0 x 10^5 cfu/ml. For each test sample, a count corresponds to an average value. With a total aerobic bacterial count of 1.3 x 10^5 cfu/ml and a total coliform count of 2.1 x 10^4 cfu/ml, the control sample also showed some levels of contamination. The total aerobic bacterial count in every sample that was examined, including the control sample, was higher than the acceptable limit of 10^4 cfu/ml. Since the required sanitary requirements were followed throughout preparation, it is probable that the ground spices and additives, which are typically applied uncooked, are what contaminated the control samples. Five different bacterial isolates, including *Escherichia coli*, *Bacillus* spp, *Staphylococcus aureus*, *Lactobacillus* spp, and *Pseudomonas* spp., were identified as a result of the morphological and biochemical characteristics of the bacterial isolates. The research revealed that zobo beverages sold in markets may serve as carriers of food-borne disease, necessitating the development of good manufacturing practices (GMP) and post-production preservation and packaging techniques.

Keywords: Bacteriological analysis, Hygiene, and *Hibiscus sabdariffa*

Introduction

In Nigeria and other areas of the world, drinking zobo drink, a non-alcoholic beverage, has become a widespread tradition among numerous tribes. To make zobo, pigments are extracted by boiling the dried calyces (sepals) of *Hibiscus sabdariffa* in water for 10 to 15 minutes.The Malvaceae family includes H. sabdariffa, sometimes known as the "red sorrel" or "roselle" (Izah *et al*., 2015). According to Ezearigo *et al.* (2014), roselle is a widely used plant in the creation of beverages, paint, and traditional remedies. Natural carbohydrate, protein, anthocyanin, antioxidants, vitamin C, calcium, magnesium, zinc, and other minerals are abundant in the plant. In addition to its impressive nutritional and therapeutic qualities, Zobo beverage has a low glycemic index (Risiquat, 2013).The term Zobo is taken from the Hausa word for Hibiscus sabdariffa in Northern Nigeria,

"zoborodo." Particularly in Northern Nigeria, the non-alcoholic beverage known as zobo is highly popular and is often offered chilled at different social occasions (Osuntogu and Aboaba, 2004). The inexpensive cost, nutritional value, and medical benefits of zobo have increased consumer demand. The state of Nigeria's economy has contributed to the zobo drink's increased popularity on a number of occasions. In the West African sub-region, it is drank by many millions of individuals from various socioeconomic groups and backgrounds, particularly among young people who also see zobo as an alternative source of affordable and soothing non-alcoholic drink in social settings (Izah *et al* 2015). It is also offered in market places to a variety of customers for usage as refreshments, entertainment during gatherings, or appetizers before the main course is served.In order to extract the flower's pigments, the dried calyces (sepals) of *Hibiscus sabdariffa* are cooked in water to make zobo drink. The extract may be consumed either hot as tea or cold as a cool beverage. Typically, sugar cane, granulated sugar, pineapple, oranges, or other fruits are used to sweeten the raw extract's sour flavor. However, zobo preparation methods and variety might differ from one region to another, which can affect the product's nutritional and microbiological properties as well as its look (Ezearigo *et al.*, 2014). Both food borne microorganisms that cause sickness and rotting may be present in zobo drinks. Therefore, the drink's quick degeneration is the biggest obstacle to the large-scale manufacture of zobo. If not refrigerated, it has a shelf life of around twenty-four hours after manufacturing. The beverage includes certain microorganisms that might ruin food. Additionally, the method of packaging juice in nylon or plastic containers before selling it, poor hygiene standards, and a lack of portable restrooms, proper storage, and waste disposal facilities at preparation and service points have all contributed to unsanitary conditions that have served as potential contaminants and increased risks to the public's health, increasing the likelihood of microbial contamination(Singh *et al* 2012).The outbreaks of food poisoning caused by ready-to-eat meals have been associated to bacterial species from the genera *Streptococcus, Proteus, Klebsiella, Micrococcus, Enterobacter, Bacillus, Campylobacter, Salmonella* serotypes, as well as *Listeria monocytogene*s, *Escherichia coli*, and *Staphylococcus aureus*. According to Chukwu *et al*. (2020), the most often reported fungal genera are *Aspergillus, Rhizopus, Mucor, Candida, Penicillium,* and *Fusarium*. Hawked zobo drink is a ready-to-eat meal, and ready-to-eat meals have been associated to incidents of poisoning.

Therefore, the aim of the study was to evaluate the bacteriological safety of zobo beverages sold in Oko, Anambra state, Nigeria.The food safety organizations will use it as a crucial tool in their job to make sure that proper production procedures are followed.

METHOD AND MATERIALS

Sample Collection

Five distinct zobo drink samples were bought at random from various vendors and hawkers,in eke Oko,Anambra state.Nigeria.It was placed in sterile polyethylene bags, and transported to the microbiological Laboratory for microbial analysis.To prepare the control sample for a comparative study, dried roselle calyces from one of the neighborhood markets were bought and brought to the lab in a sterile cellophane bag. The required sanitary requirements were followed in the preparation of the control sample.

Preparation of Zobo Drink

To make the zobo drink, Hibiscus sabdariffa's dried calyces was boiled in water for ten to fifteen minutes to extract the pigments. The filtrate was spiced after extraction and may be consumed hot as tea or allowed to cool and packed in plastic sachet containers and consumed as a cold beverage. After which pineapple was used to sweeten the raw extract's harsh sour flavor.

Microbiological Media

The study employed two distinct media, including MacConkey agar and Nutrient agar. The media were set up in accordance with the manufacturer's instructions.It was then sterilized with an autoclave at 121 ^{0}C at 15psi for 15 minutes.

Biochemical Analysis

In sterile test tubes, 1ml of each sampling zobo beverage was added to 9ml of sterile distilled water, mixed, and then serially diluted, 0.1 ml of the diluted sample was inoculated separately on the solidified Nutrient agar and MacConkey agar plates and then was aseptically spread with a sterile bent glass rod,. To get a mean standard value of the colony forming units (cfu/ml) on the plates, duplicates of each experiment were performed. Plates of inoculated Nutrient agar and MacConkey agar were incubated for 24 hours at 30°C and 35°C, respectively. Colonies on the plates were enumerated and reported as colony forming unit per milliliter (cfu/ml) and coliform, respectively, after the incubation time. Each bacterial colony on the agar plates was sub-cultured in order to achieve the pure culture. Gram staining, spore staining, and biochemical assays such catalase, coagulase, oxidase, citrate utilization, indole, methyl red, urease, VogesProskauer, and sugar fermentation were used to identify the isolates.

RESULTS

Five samples in all were analyzed, and the findings indicate that their pH ranged from 2.9 to 3.2 (Table 1). The total number of aerobic bacteria and coliforms were shown in Table 2. The results showed that whereas the total coliform count varied from 0.3 x 10^4 cfu/ml to 5.6 x 10^4 cfu/ml, the total aerobic bacterial count ranged from 0.5 x 10^5 cfu/ml to 6.0 x 10^5 cfu/ml. For each test sample, a count corresponds to an average value. With a total aerobic bacterial count of 1.3 x 10^5 cfu/ml and a total coliform count of 2.1 x 10^4 cfu/ml, the control sample also showed some levels of contamination. Table 3 displays the cultural and biochemical characteristics of the bacterial isolates. Five different strains of bacteria, including *E. coli*, *Bacillus* spp, *Staphylococcus aureus*, *Lactobacillus* spp, and *Pseudomonas* spp., were found. The percentage of each isolate in each sample was shown in Table 4. *Staphylococcus aureus* (87%) was the most common, followed by *Lactobacillus* spp (93%) and *E. coli* (92.0%), *Bacillus* spp (54.0%), and *Pseudomonas* spp (23%).

Table 1: The pH values of the Zobo drinks

SAMLPLES	pH VALUES
A	2.9
B	3.0
C	3.2

D	3.1
E	2.9
Control	3.0

KEY ; A to E are the five samples of zobo drinks randomly selected from different locations at Oko.

Table 2: Total Aerobic and Coliform Counts (cfu/ml)

Sample	**Aerobic bacterial count(cfu/ml**	**Total coliform count (cfu/ml)**
A	0.5×10^5	0.3×10^4
B	2.2×10^5	3.2×10^4
C	2.3×10^5	2.5×10^4
D	7.1×10^5	4.5×10^4
E	6.0×10^5	5.6×10^4
CONTROL	1.3×10^5	2.1×10^4
AVERAGE	3.2×10^5	3.0×10^4

KEY ; A to E are the five samples of zobo drinks randomly selected from different locations at Oko.

Table 3: Morphological and Biochemical characteristics of the bacterial isolates

Isolates	morphology	Gram reaction	catalase	oxidase	Sporestain	coagulase	indole	citrate	Methyl red	V-P	Urease	Glucose	Lactose	mannitol	Sucrose	Probable microbes
A	Long rods chains	+	_	_	_	_	_	_	+	-	_	+	+	+	+	*Lactobacillus spp*

B	Long rods single	+	_	_	_	_	_		+	_	_	+	+	_	_	*Bacillus spp*
C	Rod shaped(bacillus)	_	+	_	_	_	+	_	+	_	_	+	+	+	_	*Escherichia coli*
D	Cocci - cluster shape	+	+	_	_	+	_	+	+	+	+	+	+	+	+	*Staphylococcus aureus*
E	Rod shaped (bacillus)	_	_	_	_	_	_	+	_	_	_	+	_	+	_	*Pseudomonas spp*

Key: V.P - Voges Proskauer, A to E - isolates gotten from the different samples

Table 4: The percentage occurrence of isolates

Sample	*Lactobacillus spp*	*Bacillus spp*	*Staphylococcus aureus*	*Escherichia coli*	*Pseudomonas spp*
A	20	7	20	14	5
B	30	10	24	20	2
C	10	12	14	10	6
D	20	15	10	18	6
E	8	10	11	30	4
Control	5	_	8	_	_
Total(%)	93	54	87	92	23

DISCUSSION

The study's findings revealed that the samples' pH ranged from 2.9 to 3.2.

Additionally, microorganisms such as *E. coli, Staphylococcus spp., Lactobacillus spp., Bacillus spp., and Pseudomonas spp*. were isolated from the analyzed zobo beverages. *Staphylococcus spp., Lactobacillus spp., and E. coli* were present in every sample. The results of some authors, such as Adebayo and Samuel (2009), Rami (2013), and Nwachukwu *et al* (2007), are in agreement with this conclusion. Surprisingly, the control sample that was generated under normal hygienic settings was also contaminated. All of the tested samples, including the control sample, had total aerobic bacterial counts that were higher than the permitted limit of 10^4 cfu/ml (Public Health Laboratory Services (PHLS).(2010). The raw materials (dry calyces and additive) that were obtained from the open market are likely what caused the contamination of the control sample. Hibiscus sabdariffa calyces are vulnerable to microbial contamination when they are sold in the open market and hawked for potential customers in bottles and plastic bags. The calyces are often boiled or steeped in hot water to extract the red pigments; however, adding raw, powdered spices to the drink after boiling might introduce contaminants. The presence of several bacterial isolates in zobo beverages is significant for public health. These bacteria are a sign of pollution, hence their existence is important. The presence of *E. coli*, *Bacillus spp*., and *Staphylococcus spp.* found in this samples is a sign that the beverage was not handled with good hygiene. These bacteria come from untreated water or infected containers that are often used to prepare zobo, which as food and beverages are excellent indicators of the quality of the environment in which they are made or served, the high prevalence of bacterial contamination found in this research is mostly attributable to the unclean and generally unhygienic state of the drink preparations and surroundings.Additionally, the average total counts for aerobic bacterial and coliform counts were found to be 3.0 x 10^4 and 3.2 x 10^5 cfu/ml, respectively. According to Public Health Laboratory Services (PHLS.(2010), the range of bacterial counts is higher than the permitted upper limit of $>10^4$. All of the zobo beverages that were analyzed were identified with varied degrees of unacceptable bacteria counts. The average coliform count is far higher than the suggested safe level of zero. Therefore, the following recommendations are made as follows

• It is important to maintain personal and environmental cleanliness while preparing zobo beverages.

• Additives and packaging components need to be properly sterilized.

. The processing of zobo should be done using potable water, and zobo producers should be made aware of the value of adhering to quality control procedures.

CONCLUSION

The study's findings revealed a higher than expected level of contamination in the zobo drink. Public health is at risk because these germs may cause food poisoning and other illnesses of varied severity. The hygienically produced control sample likewise revealed contamination. The sources of water utilized, unsterilized raw materials, and a lack of personal and environmental cleanliness are all potential

causes of contamination in all the examined samples. As a result, the National Agency for Food and Drug Administration and Control (NAFDAC) and other regulatory bodies should regulate beverages like zobo, which are widely consumed by Nigerians of all ages and are regarded as an alternative source of inexpensive, non-alcoholic beverages by the youth. Producers of the beverages and dried calyces should have proper training in personal and environmental cleanliness in order to enforce regulation. Before using, the additives and additions should be sterilized. It is crucial to properly supervise manufacturers and if possible some punitive measures should be employed to discipline the offenders.

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