

Bacteriological and Fungal Assessment of Sorrel (Zobo) Drinks sold in Oba and Ojoto Markets in Idemmili North Local government Area, Anambra State, Nigeria.

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ABSTRACT

This study was on bacteriological and fungal assessment of sorrel (zobo) drinks sold in some markets in Idemmili North Local Government Area, Anambra State, using standard microbiological and biochemical procedures. Total of Ten (10) different samples were collected from different vendors in two different markets (Afor Oba Market, and Ojoto Market), in the local government area. Enumeration of coliform, and aerobic bacterial counts, were carried out using most probable number (MPN) and pour-plate techniques respectively. Enumeration of fungi was also carried out using pour-plate technique. Specific pathogens were also isolated and characterized using standard procedures. The results showed that the MPN Per Milliliter (MPN/ml) of all the samples was less than three (<3). The aerobic bacterial counts ranged between 1.1×10^5 cfu/ml and too numerous to count (TNTC), while the fungal counts ranged between no growth (NG) and TNTC. The isolated organisms include: *Escherichia coli*; *Samonella species*, *Aspergillus species*, *Penicillium species*, *Mucor species*, *Rhizopus species*. The findings revealed that the samples were grossly contaminated with bacterial and fungal agents. Hygiene practices in every stage of Sorrel drinks preparations by the handlers is recommended to reduce the level of contamination to ensure their safety for human consumption.

Keywords: Sorrel (zobo) drinks, microbial counts, assessments, markets, Anambra.

INTRODUCTION

The economic and religious situations in Nigeria has made the Sorrel (zobo) drink gain wide acceptance indifferent occasions. It is used as refreshment, entertainment in parties or as appetizers before the main dish is served and it is also sold in the market to various consumers. The zobo drink is a red liquid drink and tastes like fruit punch, served as a fair source of vitamin A, riboflavin, niacin, calcium and iron, and is low in sugar content [1]. The drink also contains anthocyanins and vitamin C, among others and it is used in curing minor stomach ailment, sore throat and strengthening the heart among other uses [2]. Zobo drink is extracted from the dried reddish purple calyces of the plant *Hibiscus sabdariffa*. Zobo is being consumed by several millions of people from different socio-economic classes and background in the West Africa sub-region, especially amongst the youths, who see zobo drink as an alternative source of cheap and relaxing non-alcoholic drink in social gathering [3]. With recent government policy emphasizing the need to be less depended on foreign food items and drink and the outright ban on the importation of formulated soft drinks and juices, zobo beverage appears to be promising economically and socially. Zobo drink has been shown to be a good source of natural

carbohydrate, protein and vitamin C (which constitute the major reasons) for consuming soft drink and fruit juice [4,5,6]. The name "zobo" is derived from local Hausa (Northern Nigeria) name for the Roselle plant that is "zoborodo". The Calyx contains about 8.3% moisture, 4% citric acid, 1.5% pigment (mainly anthocyanin), 6.9% protein and about 9% soluble solid with a PH of about 2.7. *Hibiscus sabdariffa* (sorrel) is an annual shrub that belongs to the Malvaceae plant family. It is cultivated in both tropical and subtropical regions for its popular edible calyces, stem fiber leaves and seed [7,8]. According to [9], sorrel is considered to be one of the most important and popular medicinal plant and it has several properties such as antiseptic, aphrodisiac. The drink is made by boiling the dried calyces in water for about 15 minutes from which the pigment, aroma and unique characteristic flavor embedded is extracted, after extraction, the rich filtrate may be taken hot as a refreshing drink [10]. Some locally beverage drinks are often accompanied with poor sanitary practices resulting in the spoilage micro-organism. The attributes tends to limit its acceptability, after the nutritional quantity and reduces the shelf life of the drink [10].

Materials and Methods

The sampling sites were Afor Oba Market and Ojoto Market both in Idemmili North Local Government Area, Anambra State. The sites were chosen because, they serve as outlets of

various aqueous preparations including Sorrel (zobo) drinks. Samples were collected on market day basis.

Sample Collection

The temperatures of the samples were recorded at the time of collection. All the samples

containers were labeled immediately before the samples were taken following the [5] protocols.

Physicochemical Parameters

Temperature: A liquid in glass thermometer was used which was put in contact with the

zobo (Sorrel) samples and the temperature recorded [6].

PH

A digital PH meter, model Jenway 3305 was used to determine the PH of the samples.

Colour

The colours of the samples were assessed and recorded. Enumeration of aerobic bacteria: Enumeration of viable aerobic mesophilic bacteria was carried out following the FAO 1979 protocol. Enumeration of coliform bacteria using most probable numbers (MPN) techniques: The Enumeration of coliform bacteria was carried out following the FAO 1979 protocol.

Biochemical characterization of medically important isolates; detection of *Escherichia coli*: These were carried out according to the procedure specified by the FAO 1979 protocol. Enumeration of Fungi: This was carried out according to the procedure specified by the FAO 1979 protocol

RESULTS

The physicochemical features of the samples were shown in table 1. The temperature of the samples ranged between 31.1°C and 33.1°C, while the PH ranged between 3.1 and 5.0 all the samples have neither NAFDAC number nor Batch number. In terms of packaging 100% of the samples were packaged in used transparent, plastic containers. The colours were all deep red. The most probable number (MPN) counts for coliform, aerobic bacteria counts, and their safe levels were shown in table 2. The MPN/ml of all the samples was <3. The aerobic bacterial counts ranged between 1.1×10^5 CFU/ml and too numerous to count (TNTC), while the fungal counts ranged between no growth (NG) and TNTC. Based on European pharmacopeia (2011) criteria, 70% of the total samples were found unsafe for aerobic bacterial count, while 30% appeared safe for human consumption. For fungal counts, 60% of the samples were found unsafe, while 40% appeared safe (table 2). The organisms isolated include: *Escherichia Coli*, *Staphylococcus species*, *Mucor Species*, *Rhizopus Species* (Table 3). The most probable number (MPN) counts or coliform, mean aerobic bacterial counts, mean

fungal counts, and their safe levels are shown in table 2. The MPN/ml of all samples were less than 3 (<3). The mean aerobic bacterial count ranged between $<1 \times 10^1$ CFU/ml and too numerous to count (TNTC) CFU/ml while the fungal counts ranged between $<1 \times 10^1$ CFU/ml and TNTC. following the European pharmacopeia (2011) criteria for non-sterile preparations, seventy percent (70%) of the total samples were found unsafe, while 30% appeared safe for human consumption. In terms of fungal counts, 60% of the total samples were found unsafe, while 40% appeared safe. Table 3 shows the pathogenic bacterial and fungal contaminants detected in various samples. *Escherichia Coli* was detected in 60% of the total samples, *Shigella Species* was not detected in any of the samples. *Salmonella Species* was detected in 1% of the samples, *Aspergillus Species* (70%), *Penicillium Species* (50%), *Mucor Species* (40%) and *Rhizopus Species* (40%). Table 1: The physicochemical features of sorrel (zobo) drinks sold in Afor Oba and Ojoto markets in Anambra state.

S/N	sample	PH	Temp (°C)	Colour	NAFDAC NO	Batch No	Package
1	OB1	4.0	32.6	Deep red	Nil	Nil	Recycled plastic container
2	OB2	4.8	32.5	Deep red	Nil	Nil	Recycled plastic container
3	OB3	3.7	32.6	Deep red	Nil	Nil	Recycled plastic container
4	OB4	4.0	33.1	Deep red	Nil	Nil	Recycled plastic container
5	OB5	4.0	33.0	Deep red	Nil	Nil	Recycled plastic container
6	OJ1	5.0	32.0	Deep red	Nil	Nil	Recycled plastic container
7	OJ2	4.7	31.1	Deep red	Nil	Nil	Recycled plastic container
8	OJ3	3.1	31.2	Deep red	Nil	Nil	Recycled plastic container
9	OJ4	3.2	32.2	Deep red	Nil	Nil	Recycled plastic container
10	OJ5	3.4	32.0	Deep red	Nil	Nil	Recycled plastic container

Key: S/N = serial number, OB1 = Oba sample 1, OB2 = Oba sample 2, OB3 = Oba sample 3, OB4 = Oba sample 4, OB5 = Oba sample 5, OJ1=Ojoto sample 1, OJ2 = Ojoto sample 2, OJ3 = Ojoto sample 3, OJ4 Ojoto sample 4, OJ5 Ojoto sample 5 . NAFDAC = National agency for food and drugs Administration and control.

Table 2: Bacterial and fungal counts in different Sorrel (zobo) samples sold in AFor Oba and Ojoto markets in Anambra State.

S/N	Sample	MPN/ml (for coliform)	Remarks for MPN	Mean aerobic count (cfu/ml)	EP criterion (10^2)	Fungal count (in cfu/ml)	EP criterion (10^1)
1	OB1	<3	Safe	1.5×10^3	Unsafe	1.0×10^4	Unsafe
2	OB2	<3	Safe	2.2×10^3	Unsafe	1.8×10^4	Unsafe
3	OB3	<3	Safe	1.1×10^4	Unsafe	1.1×10^3	Unsafe
4	OB4	<3	Safe	2.1×10^4	Unsafe	1.2×10^3	Unsafe
5	OB5	<3	Safe	1.5×10^4	Unsafe	2.0×10^3	Unsafe
6	OJ1	<3	Safe	TNTC	Unsafe	$<1 \times 10^1$	safe
7	OJ2	<3	Safe	3.7×10^2	Unsafe	TNTC	Unsafe
8	OJ3	<3	Safe	$<1 \times 10^1$	Safe	3×10^1	safe
9	OJ4	<3	Safe	$<1 \times 10^1$	Safe	$<1 \times 10^1$	safe
10	OJ5	<3	Safe	$<1 \times 10^1$	Safe	$<1 \times 10^1$	safe

Key: S/N = serial number, OB1 = Oba sample 1, OB2 = Oba sample 2, OB3 = Oba sample 3, OB4 = Oba sample 4, OB5 = Oba sample 5, OJ1 = Ojoto sample 1, OJ2 = Ojoto sample 2, OJ3 = Ojoto sample 3, OJ4 = Ojoto sample 4, OJ5 = Ojoto sample 5. MPN = Most Probable Number, EP = European Pharmacopoeia

Table 3: The pathogenic Bacterial and fungal contaminants in the Sorrel Samples Sold in Oba and Ojoto markets in Idemmili North Local Government Area, Anambra state

S/N	Sample	<i>Escherichia</i>	<i>Shigella</i> species	<i>Salmonella</i> species	<i>Aspergillus</i> species	<i>Penicillium</i> species	<i>Mucor</i> species	<i>Rhizopus</i> species
1	OB1	+	-	+	+	+	-	-
2	OB2	-	-	-	-	-	+	+
3	OB3	+	-	-	+	-	+	-
4	OB4	-	-	-	+	+	-	-
5	OB5	+	-	-	+	-	-	+
6	OJ1	+	-	-	+	+	-	-
7	OJ2	-	-	-	-	+	+	-
8	OJ3	-	-	-	+	-	+	-
9	OJ4	+	-	-	+	-	+	-
10	OJ5	+	-	-	-	+	-	+

Key: S/N = serial number, OB1 = Oba sample 1, OB2 = Oba sample 2, OB3 = Oba sample 3, OB4 = Oba sample 4, OB5 = Oba sample 5, OJ1 = Ojoto sample 1, OJ2 = Ojoto sample 2, OJ3 = Ojoto sample 3, OJ4 = Ojoto sample 4, OJ5 = Ojoto sample 5. + = Presence, - = Absence.

DISCUSSION

The study confirmed a preponderance of Sorrel (zobo) drinks in Oba and Ojoto markets in Idemmili North Local Government Areas. The questions on the use of safety codes and NAFDAC regulations as well, were not answered. This was given by lack of any trace of pharmacopoeia details on the samples which also accounted for the high recovery rates of pathogenic and fungal contaminants which rendered the products virtually fearful. The samples were found not safe because, present investigation identifies high microbial load. The bacterial isolates were coliforms including *E. coli* while the fungal isolates include *Aspergillus* species, *Penicillium* species, *Mucor* species and *Rhizopus* species. The presence of coliforms indicated faecal pollution, which

indeed, may indicate possible presence of harmful disease-causing organisms [6]. The presence of bacterial contaminants in the Sorrel (zobo) drinks is related to source of poor quality of water used for the processing. Another source of contamination could be from the packaging materials which are not usually or poorly sterilized. The coliform associated with the sold Sorrel (zobo) drinks created suspicion not only on the water used during preparation, but the handlers, as well as the raw Sorrel materials during collection, drying, processing and dispensing. This may mean exposing consumers to dangers of gastroenteritis [8]. The presence of *Aspergillus* species, *Penicillium* species, *Mucor* species and *Rhizopus* species were not surprising as they

are Mycoflora and common spoilage organism of carbohydrate containing foods [8]. The efforts of NAFDAC in ensuring safety of consumables have been tremendous. However, all the samples worked on, had no NAFDAC identity (Table 1) This, by implication, suggests that NAFDAC has no control over the Sorrel (zobo) drinks vended in Idemmili North local

As at the time of this investigation, sorrel drinks sold in Oba and Ojoto markets in Idemili North Local Government Area were microbiologically contaminated. Adequate

government area probably because of inadequate manpower provision or that policy and laws are not yet promulgated as to handle such products. Presence of contaminating and spoilage organisms and their high counts call for further researches in this area so as to provide means of encouraging a safer and better quality production of Sorrel drinks.

CONCLUSION

hygienic practices in every stage of sorrel preparation are recommended. This will reduce the microbial density of sorrel, as well as their safety for human consumption.

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