Nutritional, Sensory and Bacteriological Quality of Two Varieties of Locally Prepared Zobo (*Hibiscus sabdariffa*) Drink

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Abstract: The nutritional composition, sensory attributes and bacterial quality of two varieties of locally prepared zobo drinks was investigated. Dry calyces of *H. sabdariffa* and fresh wet form of ginger rhizomes and garlic bulbs were obtained. The dark red zobo (DRZ) had the highest percentage of vitamin C (8%) and calcium (4.5 ppm) when compared to vitamin C (5.5%) and calcium (2.5 ppm) in bright red zobo (BRZ). The BRZ recorded high value in magnesium (13.75 ppm) while DRZ, had high values of sodium (36.28 ppm) and potassium (220.5 ppm). Protein and iron were the same (0.5875 and 1.17 ppm) in both products. The pH values were all on the low side (3.03), confirming the high acidity usually noticed in zobo drink. The DRZ had 73.3% colour and 80% taste acceptability while the BRZ drink gave 26.7% colour with a 20% taste acceptance level. An average total heterotrophic bacterial counts of 1.87E+06 and 1.49E+05 CFU/g were obtained for dark red and bright red samples with total coliform counts of 1.63E+04 CFU/ml (DRZ) and 1.56E+03 CFU/ml (BRZ). The DRZ variety with high retention vitamin C content and its acceptance by evaluators should be consumed more. The DRZ is cheaper and produced more quality of zobo than the BRZ at the same concentration. Although, DRZ had significantly ($p<0.05$) higher bacterial counts, both met the ICMSF limit of $1x10^7$ CFU/ml set for total aerobic plate counts and so, should be consumed to boost local production and a healthy lifestyle.

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Keywords: Zobo, nutritional composition, local production, ICMSF limit, healthy lifestyle.

1. Introduction

There are different beverages that are consumed in the world depending on their nutritional composition. Most of these beverages are produced locally while others are widely produced in the world. Zobo is a locally produced beverage that has greater acceptance and is widely consumed in Rivers State, Nigeria.

It is locally called "Zobo rodo" (Hausa), "Isapa" (Yoruba) and Sorrel in English and is a delicacy in many parts of Nigeria (Adebayo-tayo and Samuel, 2008). Zobo drink is a non-alcoholic local beverage made from different varieties of dried, acid-succulent calyces of the flower *Hibiscus sabdariffa* by boiling and filtration (Ogiebor *et al*., 2008; Kolawole and Okeniyi, 2007). The calyces (Fig. 1) have been found to be rich in vitamins, natural carbohydrate, protein, vitamin C and other vital antioxidants (Wong, 2002).

Various medicinal uses of infusions of leaves or calyces have been reported such as being a diuretic, cholerectic, febrifugal, hypertensive, anti-helminthic, and antimicrobial, decreasing viscosity of the blood and stimulating intestinal peristalsis (Morton, 1987; Delgado-Vargas and Paredes-López, 2003).

Fig. 1: Dried calyces of *Hibiscus sabdariffa* (left) and the finished product- Zobo (right)
Source: Source: (Ezearigo *et al*., 2014)

The increasing level of soft drink consumption by children and teens is one of many barriers to their achieving an adequate diet and a healthy lifestyle and changes in beverage consumption patterns over the past several decades may be related to the high prevalence of obesity related diseases (Harnack *et al*., 1999; Ludwig *et al*., 2001; French *et al*., 2003; Forshee and Storey, 2003).

Demand for zobo is largely based on its nutritive value, flavour, aroma and colour (Adenipekun, 1988).

More importantly, its consumption will take an active role in bone and teeth formation as it is a rich source of vitamin C, calcium, magnesium and zinc (Babalola *et al.,* 2001).

1.1 Bacteriological Quality of Zobo Drink

In spite of its health and nutritional benefits, Zobo drink is often contaminated with enteropathogenic microorganisms with as much as $2.49x10^4$ CFU/ml, which could be harmful to persons who consume large quantities of the zobo drink (Foster, 2003; Bukar *et al.,* 2009).

Major points of contamination of the Zobo drink include: the packaging material, as most retailers package the drink in already used plastic bottles and polyethene bags, which are not properly, disinfected prior to packaging (Nwafor and Ikenebomeh, 2009).

The dried calyces are also a major point of contamination as they harbor spoilage organisms including as bacteria (Amusa *et al*., 2005) and the retailers, who seldom prepare the drink under aseptic conditions and often do not do enough boiling to reduce the microbial load in the preparation of the beverage.

Some of the microorganisms commonly found in the drink include *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* amongst a host of others (Nwachukwu *et al.*, 2007).

The shelf life of the Zobo drink depends on various factors such as the packaging material, contamination during preparation and refrigeration to mention a few, however, it has an average shelf life of 24 to 48 hrs after which spoilage organisms may begin to reduce the quality of the Zobo (Nwafor and Ikenebomeh, 2009).

The continuing increase in soft drink consumption among adolescents raised a national concern about the health effects of soft drinks as sugar-containing soft drinks can be cariogenic.

Consequently, it became imperative to determine the level of awareness of benefit of zobo among the youths and the frequency of consumption of the drink (Ezearigo *et al.*, 2014).

The study seeks to locally prepare two varieties of zobo drink, evaluate their organoleptic/sensory attributes, determine their nutritional properties, estimate the total heterotrophic bacterial and coliform bacteria counts of the drinks and to make recommendations based on findings.

2. Materials And Methods

2.1 Collection of Samples

Dry calyces of *H. sabdariffa* and fresh wet form of ginger were obtained from local fruit market in D-Line, Port Harcourt, Rivers State, Nigeria. The samples were manually cleaned by handpicking stones and other unwanted debris. The samples was then thoroughly washed separately using sterile de-ionized water and allowed to air dry under the sun at 40°C.

2.2 Preparation of Ginger and Extracts

One hundred grams (100g) of each of the spices was separately chopped into small pieces with a clean stainless steel knife. The chopped spices were then blended into 50 ml de-ionized water with a Kenwood blender having stainless steel blades until smooth pastes was obtained.

The pastes was diluted further with 100 ml de-ionized water and filtered using a clean Muslin cloth. The resulting extract was then stored in clean bottles inside a refrigerator (5°C) until needed for use in zobo preparation.

2.3 Preparation of Zobo Drinks

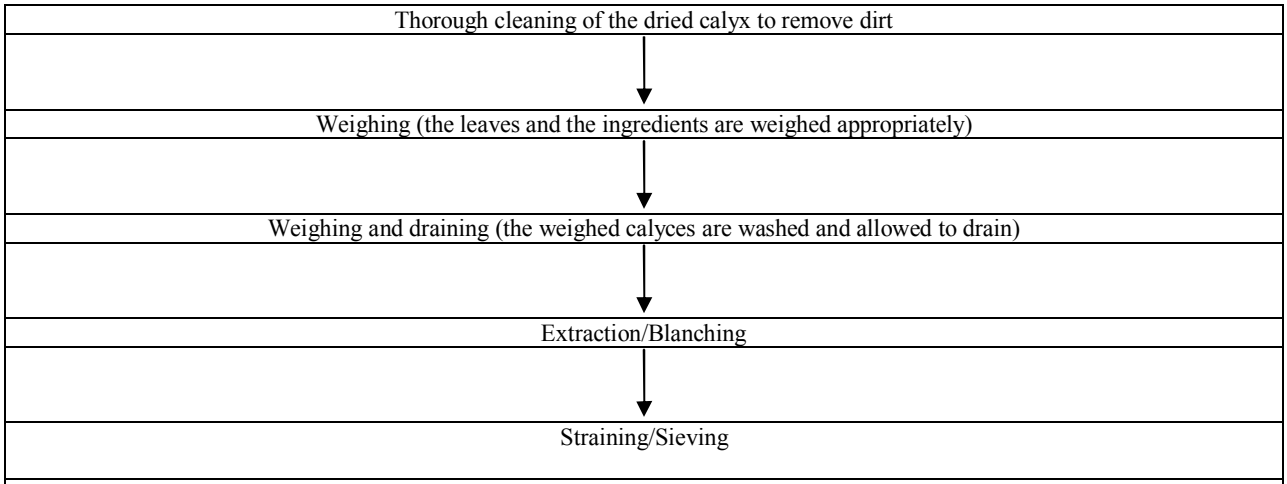

Fig. 2: A flow diagram for the preparation of Zobo drink.
Source: (Ezearigo *et al.*, 2014)

Six hundred grams of dried calyces of *Hibiscus sabdariffa* was boiled with 10 litres of clean water. Three whole pineapples and ginger were thoroughly cleaned, cut with a clean knife and the peels were boiled along with calyces to give the drink pineapple flavour.

The mixture was then boiled for 30 minutes and sieved. Immediately, 1075g of granulated sugar was added to taste and stirred to dissolve while the drink was still hot. It was kept to cool in a clean bowl. A litre each of this stock was partitioned into 8 separate clean bowls and labeled with each treatment that was applied.

For the spice treatments, the ginger was grated using a clean grater and 100g of each spice was added to each bowl according to their labels to give 1% w/v concentration per spice. Each Zobo drink was stored at ambient temperature.

The samples were taking to the laboratory for nutritional composition, sensory attributes and bacterial quality analyses following standard laboratory procedures. Fig. 2 shows the flow diagram indicating the steps for Zobo preparation.

2.4 Nutritional Composition

The nutritionally essential elements (Na, K, Ca and P) was determined using Atomic Absorption Spectrophotometer (AAS), Shimadzu model AA-7000. The pH of the zobo samples were measured using pH meter (Micron pH meter). Appropriate procedure of AOAC (1990) was used to determine the metallic ion contents, crude protein and total sugar content. Flame photometry was used to determined Na, K and Fe while Buck scientific atomic absorption spectrophotometer (model 200A) was used to determine Ca, Mg and Zn.

2.4.1 Organoleptic/Sensory Evaluation

For organoleptic evaluation, a 15 member panel consisting of students whom are regular Zobo drinkers was used to evaluate the drink. The panel tested the drink by sipping on the drink, then rinsing their mouth with water after testing each drink. It was ranked by the colour (appearance), taste (flavor), taste and overall acceptability on a modified 2-point Liker scale of 1-2 as represented as: 1= Like, 2= Like very much.

2.4.2 Determination of Bacterial Quality

The prepared Zobo drink samples were diluted using tenfold serial dilution. The spread plate technique was used and 0.1ml of the dilution required was plated in duplicate for greater accuracy of plate count. Dilutions of 10^{-1} to 10^{-4} were used for both samples and plated on Nutrient agar and MacConkey agar for total bacterial and total coliform counts respectively. Incubation was done for 24 hours at 37^{O} C and thereafter, the total number of colonies was counted. Red colonies on MacConkey agar represented coliforms. Pure colonies were preserved on nutrient agar slants for identification. Bacterial load of samples were calculated as colony forming units per milliliter (CFU/ml).

2.5 Statistical analysis

The data generated in the study were subjected to statistical analysis to determine level of significance using the students''t' test. A value of $P<0.05$ was accepted as significant and $P>0.05$ was considered as not significant.

3. Results

3.1 Nutritional Composition

Table 1 shows the nutritional composition of two varieties of locally prepared Zobo drink. It was observed that the dark red zobo drink had the highest percentage of vitamin C (8%) and calcium (4.5 ppm) contents as against vitamin C and calcium values of 5.5% and 2.5 ppm obtained in the bright red zobo drink respectively.

The bright red zobo drink recorded a high value in only magnesium (13.75 ppm) while the dark red zobo however, recorded high values of sodium (36.28 ppm) and potassium (220.5 ppm). The results indicated that the pH, protein, and iron contents were in the same range of 3.03, 0.5875 ppm and 1.17 ppm. The pH was high in both samples, indicating acidity.

Table 1: Nutritional Composition of two Varieties of Locally Prepared Zobo Drink

Sample code	pH (ppm)	Protein (ppm)	Calcium (ppm)	Magnesium (ppm)	Sodium (ppm)	Potassium (ppm)	Iron (ppm)	Vitamin (%)
DRZ	3.03	0.5875	4.5	6.25	36.28	220.5	1.17	8.00
BRZ	3.00	0.5875	2.5	13.75	25.61	219.5	1.17	5.5

Key: ppm = Parts per million (10^{-6}), **DRZ**=Dark red zobo, **BRZ**=Bright red zobo

2.4.3 Sensory Attributes

Table 2 shows the sensory characteristics of the two varieties of locally prepared Zobo drink. A 15 member panel assessed the zobo drink for taste and colour attributes. From the table, the dark red zobo had the highest percentage of colour acceptability (73.3%) as against the bright red zobo drink which had 26.7% acceptability. Also, the dark red zobo (DRZ) drink recorded 80% taste acceptability over the bright red zobo (BRZ) drink with 20% taste acceptance level (Fig.2). The Fig. 3 shows the percentage acceptance level of sensory attributes of the two varieties of locally prepared zobo drink by evaluators.

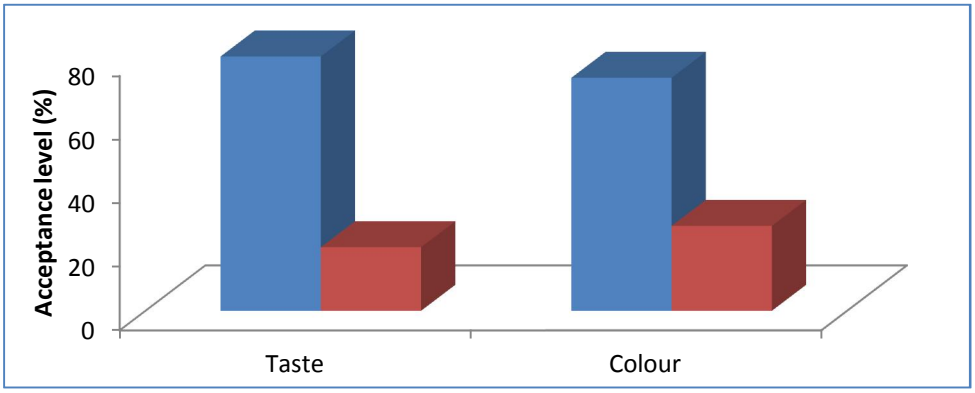

Fig. 3: Percentage of taste and colour acceptability of DRZ and BRZ by evaluators

The results obtained indicated that the dark red zobo drink had the highest percentage of vitamin C (8%) and calcium (4.5 ppm) contents as against vitamin C and calcium values of 5.5% and 2.5 ppm obtained in the bright red zobo drink respectively.

The bright red zobo drink recorded a high value in only magnesium (13.75 ppm) while the dark red zobo however, recorded high values of sodium (36.28 ppm) and potassium (220.5 ppm). The results indicated that the pH, protein, and iron contents were in the same range of 3.03, 0.5875 ppm and 1.17 ppm.

The pH was high in both samples, indicating acidity according to Jay (1996). The sensory evaluation result (Table 2) revealed that the dark red zobo drinks was rated higher than the other zobo drink in terms of taste/flavour, general acceptability and in colour.

A 15 member panel carried out a sensory evaluation to assess the taste and colour of the zobo and the dark red zobo had the highest percentage of colour acceptability (73.3%) as against the bright red zobo drink which had 26.7% acceptability. Also, the dark red zobo drink recorded 80% taste acceptability over the bright red zobo drink with 20% taste acceptance level. Wong (2002) has earlier noted that zobo contains vitamin C which raises the immune system of the body and this study confirmed it with the presence of moderate amount of vitamin C which is higher in dark red variety.

The pH of the zobo drinks were all on the low side indicating and confirming the high acidity usually noticed in zobo drinks, it is found to be a natural acidic fruit rich in organic acids: Oxalic, tartaric, malic and succinic (Wong, 2002).

Anyanwu *et al.* (2016) has earlier shown the phytochemical, proximate composition and antimicrobial potentials of *Pleurotus tuber-regium* sclerotium to contain these vital properties which are antimicrobial to food-borne pathogens.

The high acid level will also inhibit the growth f some microorganisms that are not tolerant to it. The excellent keeping quality of fruits and soft drinks is due to low pH. This is because low pH tends to inhibit bacterial growth.

There was only a little difference in the amount of protein in the samples although the samples are generally low in the protein as recorded in the two samples of zobo tested but the two zobo samples were found to be rich in the various minerals tested in this daily requirement in juices and natural fruits (Holden *et al*., 1999).

Potassium which is a principle intracellular cation of most body tissues and participates in a number of essential physiological processes (Kolawole and Okeniyi, 2007) was found to be highest in the dark red variety. However, consuming a high concentration of potassium from drugs, foods or drinks could result into cardiac arrest and small bowel ulcer.

Morton (1987) also observed the relatively low concentration of sodium ion in the two varieties (i.e. 35.76 and 25.11 in dark red and bright red, respectively) coupled with higher concentration of potassium has been shown to possibly be involved in reduction of blood pressure hence the antihypertensive effect of zobo drinks generally (Omemu *et al*., 2006). In term of cost of production, dark red zobo is cheaper and produced more quality of zobo drinks than other samples used at the same concentration.

3.3 Bacteriological Quality

Results of bacterial quality of the samples gave an average total heterotrophic bacterial count of 1.87E+06 and 1.49E+05 CFU/ml for dark red zobo (DRZ) drink and bright red zobo (BRZ) respectively.

The dark red zobo drink had an average total coliform count of 1.63E+04 CFU/ml while the bright red sample gave an average count of 1.56E+03 CFU/ml for coliform bacteria. Both the dark red and bright zobo drinks had bacterial contaminants.

The result obtained corroborated with that of Agwa *et al.* (2016), who observed the bacteriological contamination of foods are a reflection of the harvesting environment and post-process contamination through unhygienic handling, processing, packaging and storage as well as the filthy environment of the market place where the dry calyces of *H. sabdariffa* and fresh wet form of ginger rhizomes and garlic bulbs were purchase.

Solomon and Ibe (2012) has earlier reported that unhygienic practices are major factors contributing to high bacterial load of food samples sold in retail markets in Port Harcourt and our findings is in agreement to that findings.

The high level of contamination of zobo drink up to 1.8 x 10^6 CFU/ml for average total bacterial load supports the existence of unhygienic environment of Nigerian markets and the lack of monitoring of food standards by the National Agency for Foods and Drugs Administration and Control (Solomon and Ibe, 2012).

Although the dark red zobo drink had higher total heterotrophic and coliform counts than the bright red zobo drink, both samples met the International Commission on the Microbiological Specifications for Foods (ICMSF) recommended limit of 1x10^7 CFU/ml standards for total aerobic plate counts (ICMSF, 1986) and as such, can be consumed and exported in international trade to enhance revenue earnings for a country and boast her Gross Domestic Products (GDPs) in a recessive economy.

Conclusion

Demand for zobo drink is largely based on its nutritive value, flavour, aroma and colour. More importantly, its consumption will take an active role in bone and teeth formation as it is a rich source of vitamin C, calcium, magnesium, iron and zinc. Both varieties have their own peculiar nutrients embedded.

The consumption of variety of zobo drink should be based on the choice of individual producer and consumer. However, the sensory attributes of the prepared zobo drinks indicated that the dark red was more preferred over the bright red zobo drink. Based on this research work, it is suggested that the dark red zobo variety with high retention vitamin C content after 10 min of boiling and its acceptance by evaluators should be consumed more than the bright red variety.

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