

Preservation of Zobo Drink (Calyces of Hibiscus Sabdariffa) Using Kolanut

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Doi:10.5901/ajis.2013.v2n10p17

Abstract

This study investigate the efficacy of two species of kolanut being Kola acuminata (ABATA) and Kola nitride (GBANJA) as antimicrobial agent in the preservation of Zobo drink from the calyces of Hibiscus sabdariffa which is commonly called ZOBO. 5mls extracts of the caffeine was added to 50mls of prepared Zobo drink using Escherichia coli (ATCC 23716) indicator organisms for 96hours. Physiochemical analysis for pH, Brix content, growth measurements of the indicator organisms were carried out at 24hourly interval starting at zero hour. The ash and caffeine contents were also noted for the Kolanut samples only. The results obtained from the various analyses were of immense benefit at enhancing the shelve stability of the drink and thereby making it available all the year round for its numerous consumers.

Keywords: Preservation, Zobo-Drink, Kolanut, Extract, Consumer.

1. Introduction

Additives are defined under the Food Labeling Regulations (1995) as any substances that are not commonly regarded or used as food which are added to, or used in or on food at any stage to affect its keeping qualities. Preservatives are chemical additives that are used to make foods have a desired shelf-life by preventing them from bacterial spoilage, retarding growth of molds and prevention from other chemicals that will change the food quality in terms of color, taste, aroma and texture.

In recent years, Nigeria obtains her food colors from the developing countries and regulations of these countries have been modified to a level that the conventional sources of food colors are limited. Reducing the supply of certain food colors with a polyphenol content of 67%. Kolanut could serve as a potential source of food color. When the red nut is extracted with 50% methanol, it yields a reddish extract, which becomes deeper when extracted with 1% hydrochloric acid in methanol. The pod husk and also the testa contain high amount of pectin, which can be extracted and used in jam and jelly production.

Kolanut belongs to the family of *Sterculiaceae*. Its tree is an evergreen tree native to tropical Africa and it grows well in soils suitable for cocoa and coffee. The seeds are extensively used as a condiment by the natives of Western and Central tropical Africa, Kolanut constitute an important

article to trade in West Africa because they are rich in caffeine.

There are different types of cola seeds derived from different species but there are just three edible ones in Nigeria. These include *Kola acuminata* popularly called Abata in Yoruba land and usually cherished for ceremonial uses. *Kola nitida*, which is known as the one used commercially because its consumption is very high and it is cherished amongst the Northerners. It is referred to as Gbanja amongst the Southerners. It consists of the separated cotyledons of the kernel of the seed; when fresh, it nearly white and on drying it undergoes a fermentation turning reddish brown and losing much of its astringency. *Kola verticillata*, this type is not eaten by many people especially Nigerians because of its sting taste but it can also be mentioned amongst the others.

Zobo, a non-alcoholic beverage popularly consumed in northern Nigeria is produced from the dried calyces of the roselle plant *Hibiscus sabdariffa* by boiling and filtration (Ameh *et al*, 2009). It is gaining wide acceptance, being consumed by severe millions of people from different socio-economic classes and background. Zobo drink has been shown to be good source of natural carbohydrate, protein and vitamin C which constitutes the major reason for consuming soft drink and fruit juice (Braide, Oranusi and Peter-Ikechukwu, 2012). Several researchers have study the preparation and preservation of zobo drinks with different food items, such as lime. Nwachukwu, Onovo and Ezeama (2007) revealed that total coliforms and total viable counts generally decreased in values following treatment of zobo drink samples with lime juice.

Efforts have been made by researchers to find various uses of Kolanut but it has not been really satisfied in the area of food preservation. Kolanut contains caffeine serve as a stimulant and decreases fatigue. Although some studies have been employed in preservation of some fruit juices but its use as preservatives has not been fully exhausted. The use of chemical preservatives at small allowable concentrations to control the growth of microorganisms in beverages is desirable and gaining research interest worldwide (Nwafor and Ikenebomeh, 2009). It is on the basis of the preservative role of the caffeine from Kolanut that this study is conducted. Braide, *et al* (2012) stated that in spite of the increasing popularity of Zobo beverage, one of its limitations for large scale production is that it deteriorates rapidly. The drink contains microorganisms (*Bacillus*, *Streptococcus*, *Staphylococcus*, *Leuconostoc*, *Lactobacillus*, *Aspergillus*, *Penicillium*, *Geotrichum*, *Fusarium* and *Alternaria*). These lead to its spoilage. Therefore, the study will specifically addresses the following objectives:

1. To determine the physiochemical analysis on two popular species of Kolanut available in Nigeria.
2. To determine the preparation of caffeine extract from the two species of Kolanut.
3. To determine the preservative effect of the extract on zobo drink by studying the antimicrobial effects of the caffeine on selected strains of micro-organisms.

2. Methods and Procedure

Samples of the calyces of *Hibiscus sabdariffa* and sugar were obtained at Mile-12 market, Lagos state. Kolanut samples, *Abata* and *Gbanja* were also obtained from this same market. The samples were kept in clean transparent polythene bags and stored at room temperature of 28°C. The calyces sample was prepared by sorting which involves the removal of unwanted particles such as dirt, stones, etc. The calyces of *Hibiscus sabdariffa* was extracted for its anthocyanin components using the hot water extraction method. 1000mls of hot water was added to 200g of the calyces of *Hibiscus sabdariffa*, then boil for 5 minutes. This was left for 30 minutes to cool for removal of calyces using fine seive. Thereafter, 100g of sugar was added to produce the Zobo drink.

The two species of Kolanut of interest *Abata* and *Gbanja* were extracted separately using the wet milling procedure. The Kolanut were washed and Cleaned, then weighed 80g. These 80g was wet milled by adding 100ml distilled water and then strained with fine seive to remove unwanted solids. Centrifugation at 10,00r.p.m for 20 minutes was done to extract Kolanut and stored in refrigerator. Indicator organism for anti-microbial experimentation was *Escherichia coli* (ATCC

23716) and was obtained from the culture collection unit, in the Department of Biotechnology, Federal Institute of Industrial Research, Oshodi. The organisms were maintained on Nutrient Agar slants at 4°C in the refrigerator.

2.1 Experiments on the Antimicrobial Effects of Kolanut Extracts on Zobo Drink

50mls of Zobo drink was accurately measured into boiling tubes and 5mls of the Kolanut extracts was added to each tube and later autoclaved at 5kg for 5 minutes using the autoclave. The tubes were allowed to cool to room temperature. On cooling, they were aseptically inoculated with colonies (2.0×10^8 cfu/ml) of the test organisms. The inoculated tubes were allowed to remain at room temperature ($28 \pm 2^\circ\text{C}$) for 96 hours. At 24 hourly intervals, parameters which include brix reading, pH measurements, growth of the indicator organism were monitored and recorded.

2.1.1 Determination of pH from samples

The pH of the experimental samples were determined using the pH meter (unicam 9450 model) after its initial standardization to pH 4.0 and 7.0

2.1.2 Brix reading determination

This was determined using the hand refractor-meter.

2.2 Growth of indicator organisms: spectrophotometry method

The growth of indicator organisms for antimicrobial experimentation was carried out using the spectrophotometer (spectronic 20D model) at 540nm throughout the period of experimentation. The experimental sample was determined against the control sample that is, without the Kolanut extracts.

2.2.1 Direct plate count method

This was carried out by serial dilution of both the control sample and the experimental sample (Kolanut extracts) and placing out from 10 dilutions onto Nutrient agar plates using the pour plate method. The surface organisms were counted and recorded.

2.3 Analysis of Kolanut Samples

2.3.1 Determination of the ash content

5g of each Kolanut sample was separately weighed into porcelain crucible previously ignited and weighed organic matter was charred by igniting the material on a hot plate in a fume cupboard. The crucibles were placed in the muffle furnace and maintained at 600°C for 6 hours. They were then cooled in a desiccators and weighed immediately (AOAC, 1990).

$$\% \text{ Ash} = \frac{(\text{weight of crucible} + \text{ash}) - (\text{weight of empty crucible})}{\text{sample weight}} \times 100$$

2.4 Determination of the Caffeine Contents from Kolanut Samples

2.4.1 Gravimetric method

This was carried out as described by Akoh, (1981) where 5.0g of each Kolanut sample was separately weighed into 300ml flask. 10g of magnesium oxide, 200ml was added and later boiled gently under reflux for 2 hours. This was later cooled and transferred to a 500ml volumetric flask, made up to mark with distilled water, mixed and later filtered. 300mls of the filtered sample was boiled with 15ml of 10% sulphuric acid until the volume drops to 100ml. To this was added 10ml chloroform. The chloroform extract was later washed with 5ml of 1% potassium hydroxide solution. The chloroform was later evaporated to dryness in an oven at 100°C and weighed. The residue was later transferred to Kjeldahl flask with small portions of concentrated sulphuric acid. This was later digested using Kjeldahl catalyst (5 selenium tablets). This was later followed by distillation for 15minutes (AOAC, 1990). The filtrate was then titrated against 0.05N HCL.

% Total Nitrogen = Sample titer (V2) – Blank titer (V1) x 0.1 x 0.014 x 100

Sample weight of Caffeine = % N2 x 3.464.

3. Results

3.1 Physiochemical Analysis of Kolanut Samples

The Ash contents for the two (2) varieties of kola were calculated thus;

$$\% \text{ Ash} = \frac{\text{weight of crucible ash} - \text{weight of empty crucible}}{\text{sample weight}} \times 100$$

$$\begin{aligned} \% \text{ Ash for Abata} &= \frac{(65.06 - 65.0)}{5} \times 100 \\ &= \frac{0.06}{5} \times 100 \\ &= 1.2\% \end{aligned}$$

$$\begin{aligned} \% \text{ Ash for Gbanja} &= \frac{65.08 - 65.0}{5} \times 100 \\ &= \frac{0.08}{5} \times 100 \\ &= 1.6\% \end{aligned}$$

3.2 Caffeine Content Determination

Caffeine = % N2 x 3.464

% N2 = Sample titer (V2) – Blank titer (V1) x 0.1 x 0.014 x 100

Sample weight for *Abata*:

$$\begin{aligned} \% \text{ N2} &= (18.5 - 12.40\text{mls}) \times 0.1 \times 0.014 \times 100 \\ 1.00 &= 0.854\% \end{aligned}$$

$$\% \text{ Caffeine} = 0.854 \times 3.464 = \underline{2.958\%}$$

Sample weight for *Gbanja*

$$\begin{aligned} \% \text{ N2} &= 20.2 - 12.40 \times 0.1 \times 0.014 \times 100 \\ 1.00 &= 1.092\% \end{aligned}$$

$$\% \text{ Caffeine} = 1.092 \times 3.464 = \underline{3.782\%}$$

Table 1: pH determination of samples

Samples	pH Readings
<i>Gbanja</i>	5.66
<i>Abata</i>	5.29
Zobo drink	2.94
Zobo drink + Abata Extracts	3.42
Zobo drink + Gbanja extracts	3.50

Table 2: Changes in the pH reading during antimicrobial experiment with zobo drink. pH reading/Time (Hour)

Samples	0	24	48	72	96
<i>Gbanja</i> extracts with zobo drink	3.50	3.20	2.70	2.50	2.50
<i>Abata</i> extracts with zobo drink	3.42	3.30	3.24	3.20	3.18
Zobo drink without extracts	2.94	3.38	4.50	6.20	6.54

Table 3: Changes in the brix content during antimicrobial experiment with zobo drink

Samples	Brix Reading/Time (Hours)				
	0	24	48	72	96
<i>Gbanja</i> extracts with zobo drink	15.00	14.50	14.50	14.50	14.00
<i>Abata</i> extracts with zobo drink	15.00	14.80	14.50	14.80	14.50
Zobo drink without Extract (control)	15.00	8.00	6.00	4.00	2.00

Table 4: Changes in the growth of *Escherichia coli* in zobo drink

Samples	Growth (540nm)/Time (Hours)				
	0	24	48	72	96
<i>Gbanja</i> extracts with zobo drink	1.340	1.300	1.200	1.090	0.940
<i>Abata</i> extracts with zobo drink	1.340	1.280	1.200	1.070	0.870
Zobo drink without extracts	1.340	1.540	1.610	1.840	1.985

Table 5: Changes in the Colony Forming Units (CFU) of *Escherichia coli* in zobo drink. Colony forming unit cfu/time (hours)

Samples	0	24	48	72	96
<i>Gbanja</i> extracts with zobo drink	2.0×10^4	1.8×10^4	1.5×10^3	1.8×10^2	1.5×10^2
<i>Abata</i> extracts with zobo drink	2.0×10^4	1.9×10^4	1.4×10^4	1.2×10^4	1.0×10^3
Zobo drink without extracts	2.0×10^4	3.2×10^4	2.5×10^5	3.2×10^5	4.2×10^5

3.3 Physiochemical Analysis of Kolanut Samples

The physiochemical analysis of the kolanut samples as earlier calculated revealed that the ash content for *Gbanja* and *Abata* were 1.2% and 1.6% respectively. The caffeine content from *Gbanja* and *Abata* samples were 2.958% and 3.782% respectively. The result showed that *Gbanja* sample was high in both ash and caffeine content when compared to *Abata*. However, the caffeine content obtained disagreed with the result obtained by (Kordylase, 1990) with caffeine content of 2%.

3.4 pH Determination of Samples

The pH reading of all the samples investigated was as shown in Table 1. The result showed that both the Kolanut extracts and the zobo drink samples were within the acidic range for food samples. pH measurement is important physiochemical parameter that tends to ensure good shelf stability of food samples. High acid range food confers more antimicrobial effects on contaminating organisms.

3.5 Changes in the pH Reading during Antimicrobial Experiment with Zobo Drink

Table 2 revealed that there was a gradual reduction in the pH of the medium to an acidic range. The gradual acidic release might prevent the multiplication of the *Escherichia coli* which is the indicator organisms. The control samples however, showed an increase in pH with time.

3.6 Changes in the Brix Content during Antimicrobial Experiment with Zobo Drink

Changes in the Brix content revealed that the Kolanut extracts sample were able to maintain the sugar level throughout the period of experiment. The caffeine content from the Kolanut samples might have hindered the fermentation by the indicator organism (*Escherichia coli*). The control utilization of the sugar in the medium and hence there was a sharp reduction in the sugar content from 15 Brix to 2.0. Brix has shown in Table 3.

3.7 Changes in the Growth of *Escherichia coli* in Zobo Drink

The result revealed that Zobo drink containing the Kolanut extracts showed a downward trend in the growth of the *Escherichia coli* over time. The control sample however, showed an increased growth of the *Escherichia coli* over time. The fall in the growth of *Escherichia coli* in the Zobo drink with Kolanut extracts might have been due to the caffeine; the active ingredients for its antimicrobial effects. The caffeine like other forms of chemical preservatives must have prevented the growth of the indicator organism from growing.

3.8 Changes in the Colony Forming Unit of *Escherichia coli* in Zobo Drink

Table 5 showed that Zobo drink containing the Kolanut extract had a gradual fall in the growth number of inoculated *Escherichia coli* when compared to the control (without the growth) that had an increased number in the growth of the inoculated *Escherichia coli*.

4. Conclusion

Zobo drink is a product made from the calyces of *Hibiscus sabdariffa* using hot water extraction. The product was formulated and prevention of the product with kolanuts of *Kola acuminata* and *Kola nitida* was very effective using *Escherichia coli* as the indicator organism. The use of preservatives in the preservation of Zobo drink is highly very necessary since the drink is prone to fermentation during storage. The use of the Kolanut extracts has helped to arrest the growth of the micro-organisms and hence, helped in the maintenance of the product during storage.

5. Recommendation

Zobo drink is a highly refreshing drink that is consumed by majority of Nigerians. The use of Kolanut extracts will therefore help to increase the shelf life of the products for its consumption at anytime. The use of Kolanut extracts will also help to retain the quality of the products. It is on the

basis of this that the use of Kolanut extracts is therefore recommended in the preservation of Zobo drink.

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