# EFFECT OF *XYLOPIA AETHIOPICA* FRUIT EXTRACT AND CHEMICAL PRESERVATIVES ON ZOBO DRINK

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**Abstract**
*Zobo* drinks are non-alcoholic local beverages prepared from varieties of dried petals, acid succulent aqueous extract *Hibiscus sabdariffa* calyx. The shelf life of laboratory prepared zobo was evaluated for eight weeks using three different concentrations (0.25%, 0.5% and 1.00%) each *of Xylopia aethiopica* fruit extract, benzoic acid and sodium metabisulphite. Each of the treated *zobo* sample were divided into two portions and stored at 5$^{o}$C (refrigeration temperature) and 26±2$^{o}$C (ambient temperature) respectively while a portion of the zobo sample was left untreated and stored at ambient temperature (control). Total viable count was carried out using the spread and pour plate techniques for bacterial and fungal respectively at weekly intervals for the eight weeks period of storage. The result revealed that the preservatives were able to extend the shelf life of zobo for 3-4weeks with the benzoic acid treated sample having the least counts for both bacteria and fungi among all the treated sample, This was followed by the *Xylopia aethiopica* fruit extract treated samples and lastly the samples treated with sodium metabisulphite. All of the preservatives were most effective at 1.00%. *Enterobacter cloacae* strain VCRC B519, *Enterobacter* sp.AAP4, *Enterobacter cloacae* strain HR-1, *Bacillus subtilis* strain YX3, *Klebsiella pneumoniae* strain NF16 and *Salmonella enterica* subsp*. enterica* serovar *Typhimurium* strain SA40, *Aspergillus niger*, *Aspergillus fumigatus*, *Alternaria alternata* and *Acremonium strictum* were the isolated microorganisms from the zobo samples. All samples were found to be rich in vitamin C but low in protein. All preservatives used in this study were more effective at refrigeration temperature compared to ambient temperature. Phyto-chemical screening of *Xylopia aethiopica* fruit extract revealed the presence of alkaloids, glycosides, saponnins, flavonoids and tannins.

**Keywords**: Beverages, organoleptic, preservative, shelf life, storage, temperature

## Introduction

As a result of Nigeria's quest toward zero import dependency and food security, the food drink industry has come under scrutiny, as many imported drinks (especially "energy drinks") have almost no food value, contain harmful or even carcinogenic chemicals and have been shown to aggravate certain diseases e.g. diabetes and high blood pressure (Omemu *et al*., 2006). This has precipitated research into local drinks, for example, *burukutu*, *kunu* and *zobo*. Despite these obvious advantages that are attached to many of this local beverages, the leap from locally marketed products to standard industrial products is still relatively improbable due to its poor shelf life, which would require very little inventory and storage time (Paine, 1992). This is due to high microbial proliferation from unsanitary preparation, harsh storage conditions and improper packaging materials. The simplicity in production, availability of raw plant material as well as abject poverty in many rural communities' coupled with new economic revamping policies of the government has

resulted in increased consumption and merchandise of many traditional foods at cottage levels in Nigeria (Bola & Aboaba, 2004).

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

*Zobo* is a nutritional drink consumed by different classes of people irrespective of socio economic status (Izah *et al*., 2015), sex and age in Nigeria especially in the Northern region and other neighboring African countries (Izah *et al*., 2015). The most active ingredient used in the production of zobo drink is *Hibiscus sabdariffa,* which belongs to the Malvaceae family. *Hibiscus sabdariffa* are mainly cultivated as vegetables for soup preparations (Fasoyiro *et al*., 2005), folk medicine and tea preparations (Adesokan *et al*., 2013). *H. sabdariffa* is a tropical annual herb. *H. sabdariffa* is native to Asia (India and Malaysia) (Ezearigo *et al*., 2014).

This popular drink is called *Zobo* or *Yakwua* which could sometimes be spelled as *Sobo* (Egbere *et al*., 2007). However, based on the name of this useful drink in different languages, the popular name it is known in Nigeria is derived from its Hausa name (Ezeigbo *et al*., 2015). *Zobo* has gained prominence in several parts of the country and are sold in public places. *Zobo* is one of the nutritional drinks that are served during festivals and in a number of other ceremonies (Umaru *et al*., 2014) in different parts of Nigeria. The increased consumption of *zobo* is due to the nutritional, medicinal properties and low cost (Oboh & Elusiyan, 2004). At present, *zobo* drink is consumed by several millions of people cutting across different socio-economic classes in West African (Ogiehor & Nwafor, 2004).

The use of antimicrobial agents (preservatives) for the control of bacterial and mould associated with *zobo* drink in storage has not been given the deserved attention. Their use at low allowable concentrations either individually or in combinations will remove the risk associated with them (Leistner, 2000). Furthermore, the role of *A. niger in* deleterious changes in physio-chemical quality of *zobo* drink in stored condition has been reported (Nwafor & Ikenebomeh, 2009).

Considering the significant position being assumed by zobo drink among Nigerians of all ages and other African countries, the need to control spoilage due to the spoilage organisms becomes very imperative, hence, the need for this study. The important role of *zobo* among Nigerians of all ages and other African countries of the world has prompted many researchers to conduct a number of investigations to overcome the challenges associated with *zobo* as a conventional soft drink.

The aims and objectives of this study were: To determine the effectiveness of *Xylopia aethiopica* fruit extract and some chemical preservatives both at room and refrigeration temperatures on the total microbial load of *zobo*. To Isolate, characterize and identify the food borne microorganisms that are associated with the spoilage of *zobo*. To examine the effect of *Xylopia aethiopica*,chemical preservatives and refrigeration on the organoleptic quality (taste, color & flavour) of *zobo*. To examine the effect of *Xylopia aethiopica*, chemical

preservatives and refrigeration on some physiochemical parameters (pH and titratable acidity) on *zobo*.

## Materials and Methods

### Sample Collection and Identification

Dried *zobo* plant calyces, the red variety of *Hibiscus sabdariffa* as well as dried plant of *Xylopia aethiopica* were obtained from Oja-Oba Market in Ilorin, Kwara State and transported to the herbarium of the Department of Plant Biology, University of Ilorin where they were identified and authenticated with the voucher numbers UILH/101/646 and UILH/001/1089 respectively.

### Extraction of *Xylopia aethiopica* fruit

The purchased *Xylopia aethiopica* fruits were sorted to remove debris, The fruits were then thoroughly rinsed with sterile water and dried at indoor ambient temperature for one week. The dried fruits were then sliced into small pieces after which 80 g of the sliced fruit sample was transferred into a 1000 mL Erlenmeyer flask to which was added 800mL of sterile water and then sealed with an aluminium foil. The flask was kept at ambient temperature with intermittent shaking for three days. The solution was filtered through No.5C Whatman filter paper. The filtrate was concentrated to about one-third of the original volume in a water bath at 40 $^0$C. The filtrate was transferred into glass petri dishes and dried completely using a drier at 55 $^0$C.

### Preparation of *Zobo* drink

*Zobo* drink was produced in the laboratory using sterile water according to the methods of Bankole *et al*. (2013). The calyces sample was prepared by sorting which involved the removal of unwanted particles such as dirt, stones, etc. The calyces of *Hibiscus sabdariffa* were extracted using the hot water extraction method. One thousand millilitres of hot sterile water was added to 200g of the calyces of *Hibiscus sabdariffa*, then boil for 5 minutes. This content was then left for 30 minutes to cool for removal of calyces using fine sieve after which 100 g of sugar was added to produce the *zobo* drink.

### Treatment of *Zobo* drink

The *zobo* drink was then divided into three places with each having three divisions and treated with different concentrations, 0.25%, 0.5% and 1% (w/v) of benzoic acid, sodium metabisulphite and fruit extract of *Xylopia aethiopica* . Each of the divisions was then futher divided in two in which one will be kept at room temperature of 26±2$^o$C while the second part will be kept in the refrigerator at a refrigeration temperature of 5$^0$C. A portion of the laboratory prepared zobo sample was left untreated with neither any chemical or plant extract to serve as the first control. All treated *zobo* samples as well as untreated samples (control) were then packaged in sterile glass bottles for further use throughout the eight weeks investigation period.

The following were the various treatment and designations of a total of twenty samples which were examined throughout the study period:

1. *Zobo* sample treated with 1% *Xylopia aethiopica* fruit extract kept on the bench at room temperature of 26±2$^o$C: Sample A1
2. Zobo sample treated with 0.5% *Xylopia aethiopica* fruit extract kept on the bench at room temperature of 26±2$^o$C: Sample A2
3. Zobo sample treated with 0.25% *Xylopia aethiopica* fruit extract kept on the bench at room temperature of 26±2$^o$C:Sample A3

4. Zobo sample treated with 1% *Xylopia aethiopica* fruit extract kept in the refrigerator at refrigeration temperature of 5°C: Sample A4
5. Zobo sample treated with 0.5% *Xylopia aethiopica* fruit extract kept in the refrigerator at refrigeration temperature of 5°C: Sample A5
6. Zobo sample treated with 0.25% *Xylopia aethiopica* fruit extract kept in the refrigerator at refrigeration temperature of 5°C: Sample A6
7. Zobo sample treated with 1% benzoic acid kept on the bench at room temperature of 26±2°C:Sample B1
8. Zobo sample treated with 0.5% benzoic acid kept on the bench at room temperature of 26±2°C: Sample B2
9. Zobo sample treated with 0.25% benzoic acid kept on the bench at room temperature of 26±2°C: Sample B3
10. Zobo sample treated with 1% benzoic acid kept in the refrigerator at refrigeration temperature of 5°C: Sample B4
11. Zobo sample treated with 0.5% benzoic acid kept in the refrigerator at refrigeration temperature of 5°C: Sample B5
12. Zobo sample treated with 0.25% benzoic acid kept in the refrigerator at refrigeration temperature of 5°C:Sample B6
13. Zobo sample treated with 1% sodium metabisulphite kept on the bench at ambient temperature of 26±2°C:Sample C1
14. Zobo sample treated with 0.5% sodium metabisulphite kept on the bench at ambient temperature of 26±2°C: Sample C2
15. Zobo sample treated with 0.25% sodium metabisulphite kept on the bench at ambient temperature of C:Sample C3
16. Zobo sample treated with 1% sodium metabisulphite kept in the refrigerator at a refrigeration temperature of 5°C:Sampple C4
17. Zobo sample treated with 0.5% sodium metabisulphite kept in the refrigerator at a refrigeration temperature of 5°C:Sample C5
18. Zobo sample treated with 0.25% sodium metabisulphite kept in the refrigerator at a refrigeration temperature of 5°C:Sample C6
19. Zobo sample that was not treated with no chemical or plant extract left at ambient temperature of 26±2°C.Control

**Microbial Enumeration**

This was carried out by serial dilution of both the control sample and the experimental *zobo* samples according to the methods of Avishai and Charles (2014). One millilitre of the *zobo* drink sample was placed in 9 mL of sterile distilled water in sterilized test tubes and immediately covered with caps. The test tubes were shaken gently, then serially diluted. From it, 0.1 mL of $10^3$ dilutions was plated onto Nutrient Agar (NA) and Potato Dextrose Agar (PDA) using the spread and the pour plate methods respectively. Streptomycin was added to Potato Dextrose agar was to inhibit bacteria growth. The nutrient agar plates and the Potato Dextrose Agar plates were incubated at $37^0$C for 24hours and 28±2 $^0$C for 3days respectively. Bacterial and fungal count were carried using a colony counter.

**Characterization of Isolates**

Fungal isolates were stained with cotton-blue lacto-phenol and microscopically observed for cell shape, size, edge, elevation and sporulation. The cellular morphology of the bacteria isolates were determined by gram staining and spore staining. Biochemical analysis such as test for catalase and oxidase activities, nitrate reduction ,citrate utilization, oxygen utilization patterns of sugar utilization as well as urea and starch hydrolysis were all carried out according to the methods of Fawole and Oso (2004).

**Molecular Characterization Using 16S rRNA PCR Technique**

Molecular characterization of isolates were done molecularly for six bacterial isolates designated; B1, B2, B3, B4, B5 and B6 isolated from stored *zobo* samples over the eight weeks storage period (Pitcher *et al,* 1989).

**Organoleptic Evaluation of *Zobo* Samples**

Organoleptic qualities of both the treated and untreated (control) *zobo* samples were checked after few hours of preparation and subsequently at weekly interval for the eight weeks storage period. The *zobo* samples were analyzed by a panel of five members which comprises of students of the Department of Microbiology, University of Ilorin that consumes *zobo* regularly and are familiar with the sensory characteristics (taste, colour and flavour) of *zobo*. The samples were scored based on a five point hedonic scale where 5 = very good, 4 = good, 3 = fair, 2 = poor, 1 = very poor.

**Physicochemical Analysis of *Zobo* Samples**

The physicochemical analysis (pH and titratable acidity) of the *zobo* samples were conducted after two to three hours from the the onset of storage and then repeated weekly throughout the eight week storage period.

*Determination of pH of Zobo Samples*

The pH of the *zobo* samples were determined by measuring 1mL of each sample into a beaker containing 10 mL of distilled water, the pH was determined using phillip's PW 9418 pH meter, The instrument was initially standardized with a buffer solution.

*Determination of Titratable Acidity of Zobo Samples*

The titratable acidity of zobo samples were determined be measuring 4mls of *zobo* into 250mL conical flask and 200mL of distilled water was added. The flask was allowed to stand in a water bath at $40^0$C for 1 hour, it was then swirled occasionally to ensure complete mixing before filtration. Phenolphthalein was added after filtration and then titrated against 0.1NaOH solution. The titratable acidity was expressed as percentage ascorbic acid equivalent present in the beverage and calculated using equation 1

$$\text{Acidity (\% ascorbic acid)} = \frac{\text{volume of 0.1M NaOH used} \times 0.09 \times 100}{\text{Volume of sample}} \quad (1)$$

*Determination of Vitamin C content of Zobo Samples*

Vitamin C was determined by method of Roe and Kuether, (1943) using colourimetric method.1mL of *zobo* sample was diluted with 9ml of distilled water and homogenized with 10% trichloroacetic acid and 0.5mL chloroform. The mixture was centrifuged and allowed to settle. The clear supernatant liquid was taken out and mixed with 0.4mL freshly prepared colour reageant (5mL,2,4 dintrophenyl hydrazine,0.1mL of 5% cupric sulphate and 0.1mL of 10% (thiorea) and incubated for 56°C in a water bath for 1 hour. This was cooled in ice bath for 3 minutes. Ice cold sulphuric acid was added slowly with thorough mixing, It was then left at ambient temperature for 30 minutes. Absorbance was taken at 490nm while vitamin C was calculated equation 2:

$$\frac{\text{Absorbance of sample} \times \text{Concentration of standard solution} \times \text{Dilution factor}}{\text{Absorbance of standard solution} \times \text{Sample volume}} \quad (2)$$

*Determination of Percentage Moisture*

Moisture content will be determined by oven drying method in accordance with Shumaila and Mahpara (2009). Exactly 2mL of the sample was weighed in a dry crucible as initial weight. The crucible was placed in the oven for 8 hours at $100^o$C until a constant weight was

obtained. After eight (8) hours, the crucible was removed from the oven and cooled in a dessicator for 30 minutes. After cooling, the crucible was weighed again as final weight. The percentage moisture was calculated using equation 3:

$$\% \text{ moisture} = \frac{W_2 - W_3 \times 100}{W_2 - W_1} \quad (3)$$

W1 = weight of empty crucible
W2 = weight of sample and crucible before heating
W3 = weight of sample and crucible after heating

*Determination of Percentage Protein Content of Zobo samples*

Determination of crude protein was carried out using the Association of Official Analytical Chemists AOAC (2003) methods. Five millilitre of *zobo* sample was mixed with 15g of potassium sulphate ($K_2SO_4$ ), 0.7g of mercuric oxide, copper sulphate ($CuSO_4$) as a catalyst and digested in a long necked Kjeldhal bottles with 40millilitre of concentrated $H_2SO_4$ for 2 hours. Distilled water (200mL) and 25mL of sodium thiosulphate solution (80g/L) was added. The content of the digestive flask was mixed and boiled until 150mL was distilled into the receiver. Five (5) drops of methyl red indicator solution (0.5/100ml ethanol) were added to the mixture before titration with 0.1M sodium hydroxide. The percentage nitrogen obtained was multiplied by a conversion factor (6.25) to get the protein content using equation 4:

$$\%\text{Nitrogen} = \frac{\text{Volume of acid required to titrate sample} - \text{Volume of acid required to titrate blank} \times \text{Acid normality} \times 14.007 \times 1000}{\text{Weight of sample}} \quad (4)$$

$$\%\text{Protein} = \% \text{ Nitrogen} \times \text{conversion factor (6.25)}.$$

**Phytochemical Screening of *Xylopia aethiopica***

Phytochemical screening was carried out on the fruit extract of *Xylopia aethiopica* using standard procedures to identify the constituents present as described by Sofowora (1993).

**Results**

Cellular morphology, biochemical tests and polymerase chain reaction were used to identify bacteria and fungi isolated from zobo and these are presented in Tables 1 and 2 respectively. Altogether, six bacteria were isolated from the zobo drink samples which include *Enterobacter cloacae* strain VCRC B519, *Enterobacter sp.*AAP4, *Enterobacter cloacae* Strain HR-

1, *Bacillus subtilis* Strain YX3, *Klebsiella pneumonia* Strain NF16 and *Salmonella enterica* subsp. *enterica* serovar *Typhimurium* strain SA40 While four fungi were isolated and are comprised of *Aspergillus niger, Aspergillus fumigatus, Alternaria alternata and Acremonium strictum* as shown in Figures 1-4. DNA sequencing and amplification of all isolated bacterial are presented in Table 2.

The effects of the various treatment on the total viable counts on (bacteria and fungi) of the zobo samples over a period of eight weeks are depicted in Figures 1-5. Figure 6, 7, 8 shows the changes in the vitamin C content for the various treatment applied (*Xylopia aethiopica* fruit extract, benzoic acid and sodium metabisulphite). In Figures 9 and 10, the effects of the treatments on the $p^H$ and titratable acidity of the zobo are shown. Table 3 present the changes in organoleptic quality/ acceptability of the zobo samples over the period of eight weeks. The proximate analysis of zobo before and after the eight weeks storage period is presented in Table 4, while differerent phytochemicals present in *Xylopia aethiopica* is presented in Table 5.

**Table 1: The cultural, morpological and biochemical characteristcis of bacteria isolates from zobo samples**

| Isolate | B1 | B2 | B3 | B4 | B5 | B6 |
|---|---|---|---|---|---|---|
| Colonial morpohology | Medium mucoid rods with entire margin | Rod shaped yellow pigmented mucoid colonies | Medium mucoid rods with entire margin | Large flat rods with rough edges | Medium mucoid colonies with entire margin | Rod shaped |
| Gram reaction | – | – | – | + | – | – |
| Spore staining | – | – | – | + | – | – |
| Motility | + | + | + | + | - | + |
| Catalase | + | + | + | + | + | + |
| Urease | – | – | – | – | + | – |
| Citrate | + | + | + | + | + | – |
| Oxidase | – | – | – | + | – | + |
| Hydrogen sulphide from TSI. | – | – | – | – | – | + |
| Tentative Identity | *Enterobacter sp* | *Enterobacter sp* | *Enterobacter sp* | *Bacillus sp* | *Klebsiella sp* | *Salmonella Sp* |

**Key:**
(+): Present (-): Negative

**Table 2: Molecular characterisation of bacteria isolates from zobo samples**

| ISOLATE | ORGANISM | NUMBER OF BASES | IDENTITY (%) | ACCESSION NUMBER |
|---|---|---|---|---|
| B1 | *Enterobacter cloacae* strain VCRC B519 | 851 | 99 | KC119193.1 |
| B2 | *Enterobacter sp.* AAP4 | 852 | 99 | JF276427.1 |
| B3 | *Enterobacter cloacae* strain HR-1 | 796 | 96 | KX403447.1 |
| B4 | *Bacillus subtilis* strain YX3 | 856 | 89 | KM403447.1 |
| B5 | *Klebsiella pneumonia* Strain NF16 | 850 | 95 | KP772069.1 |
| B6 | *Salmonella enterica* subsp. *enterica* Serovar *Typhimurium* strain SA40 | 874 | 93 | KU843856.1 |

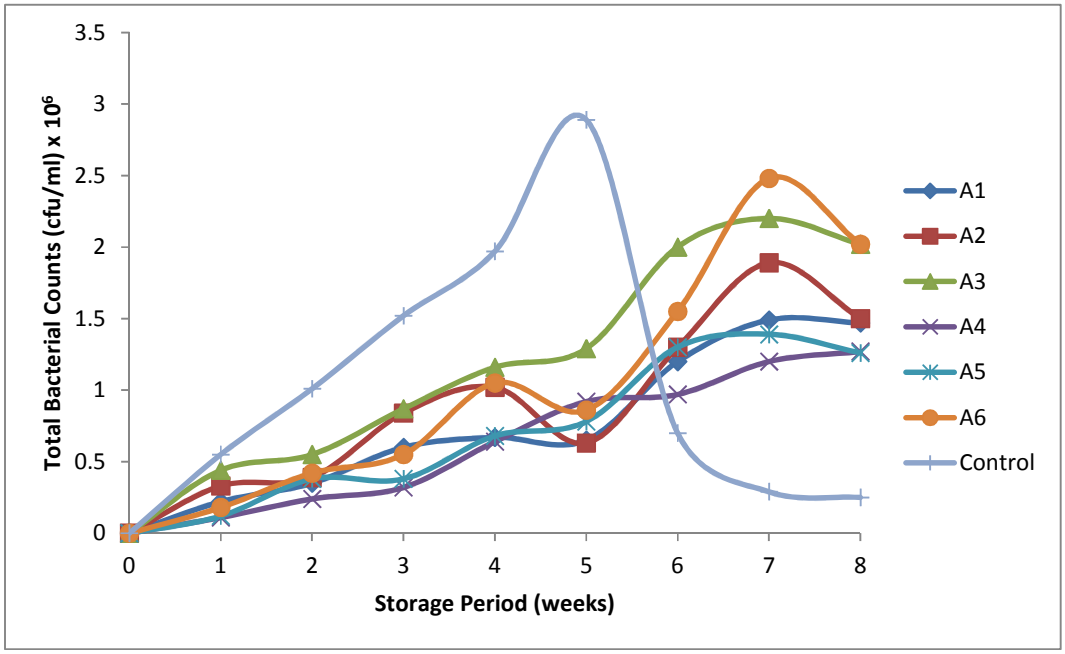


**Figure 1: Changes in viable bacteria count of zobo samples treated with *Xylopia aethiopica* fruit extract stored at room and refrigeration temperatures for eight weeks**

**Key:**
**A1** Zobo + 1% *X. aethiopica* (26±2°C), **A2** Zobo + 0.5 % *X. aethiopica* (26±2°C), **A3**- Zobo + 0.25% X. aethiopica (26±2°C), **A4** Zobo + 1% *X. aethiopica* (5°C), **A5** Zobo + 0.5% *X.aethiopica* (5°C), **A6** Zobo + 0.25% *X. aethiopica* (5°C)

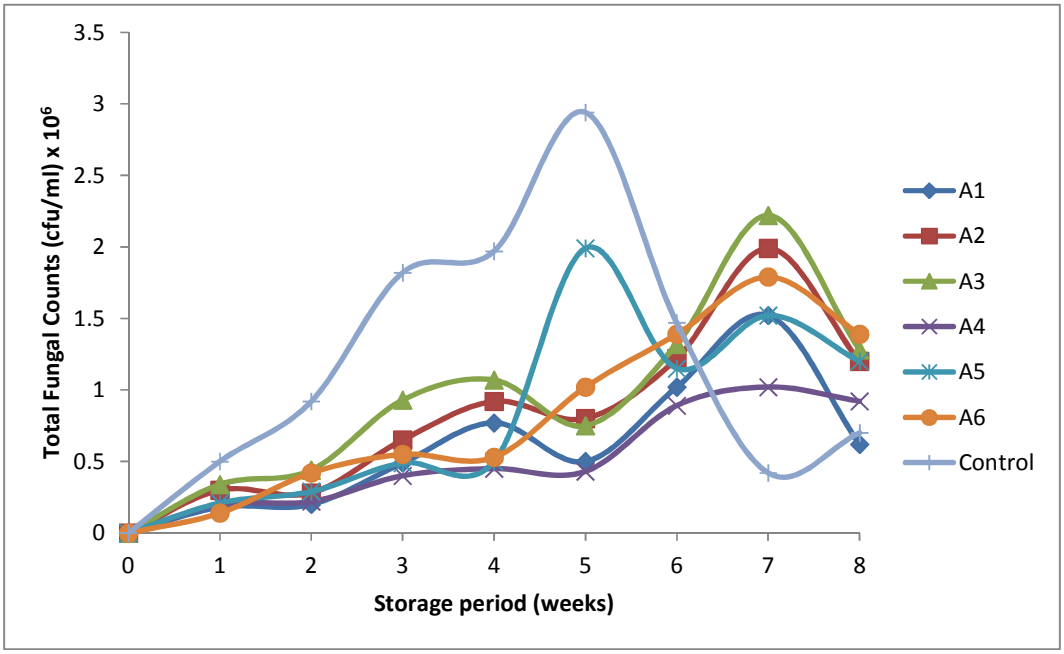


**Figure 2: Changes in viable fungal count of zobo samples treated with *X. aethiopica* fruit extract stored at room and refrigeration temperatures for eight weeks**

**Key:**
**A1** Zobo + 1% *X. aethiopica* (26±2°C), **A2** Zobo + 0.5 % *X. aethiopica* (26±2°C), **A3** Zobo + 0.25% X.aethiopica (26±2°C), **A4** Zobo + 1% *X. aethiopica* (5°C), **A5** Zobo + 0.5% *X.aethiopica* (5°C), **A6** Zobo + 0.25% *X. aethiopica* (5°C)

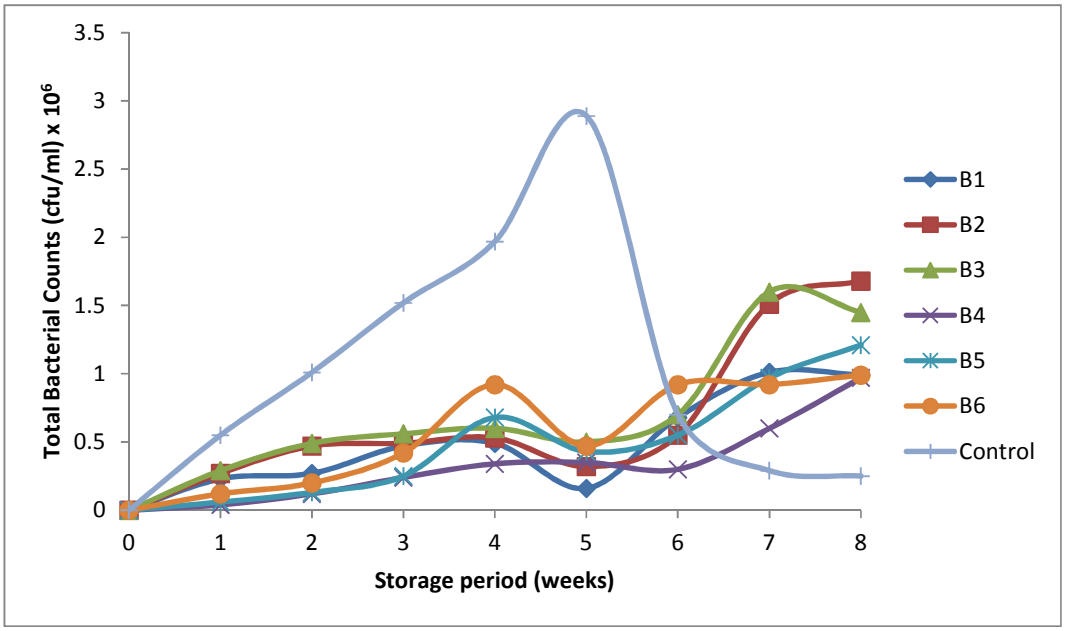


**Figure 3: Changes in viable bacteria count of zobo samples treated with benzoic acid stored at room and refrigeration temperatures for eight weeks**

**Key:**
**B1** Zobo + 1% Benzoic acid (26±2°C), **B2** Zobo + 0.5 % Benzoic acid (26±2°C), **B3** Zobo +0.25% Benzoic acid (26±2°C), **B4** Zobo + 1% Benzoic acid (5°C), **B5** Zobo + 0.5% Benzoic acid (5°C), **B6** Zobo + 0.25% Benzoic acid (5°C)

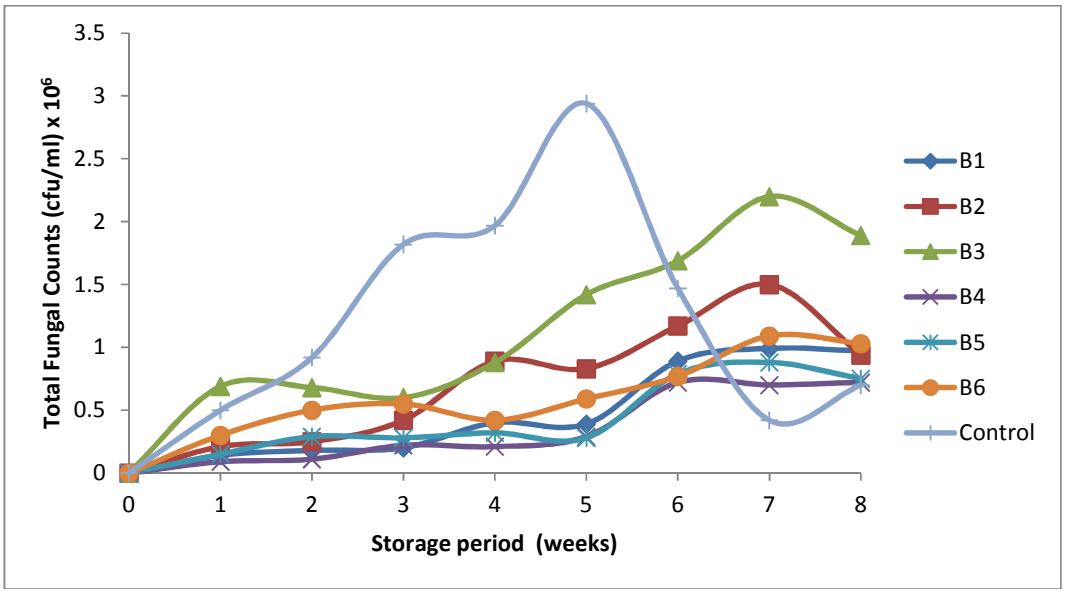


**Figure 4: Changes in viable fungal count of zobo samples treated with benzoic acid stored at room and refrigeration temperature for eight weeks**

**Key:**
**B1** Zobo + 1% Benzoic acid (26±2°C), **B2** Zobo + 0.5 % Benzoic acid (26±2°C), **B3** Zobo +0.25% Benzoic acid (26±2°C), **B4** Zobo + 1% Benzoic acid (5°C), **B5** Zobo + 0.5% Benzoic acid (5°C), **B6** Zobo + 0.25% Benzoic acid (5°C)

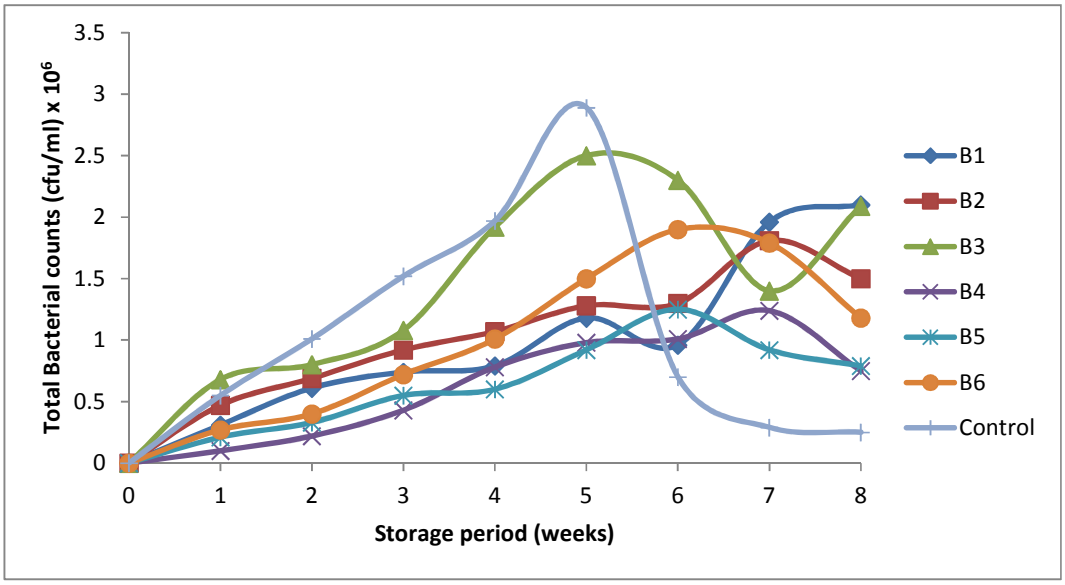


**Figure 5: Metabisulphite stored at room and refrigeration temperatures for eight weeks**

**Key:**
**C1** Zobo + 1% Sodium metabisulphite (26±2°C), **C2** Zobo + 0.5 % Sodium metabisulphite (26±2°C), **C3** Zobo +0.25% Sodium metabisulphite (26±2°C), **C4** Zobo + 1% Sodium metabisulphite (5°C), **C5** Zobo + 0.5% Sodium metabisulphite (5°C), **C6** Zobo + 0.25% Sodium metabisulphite (5°C)

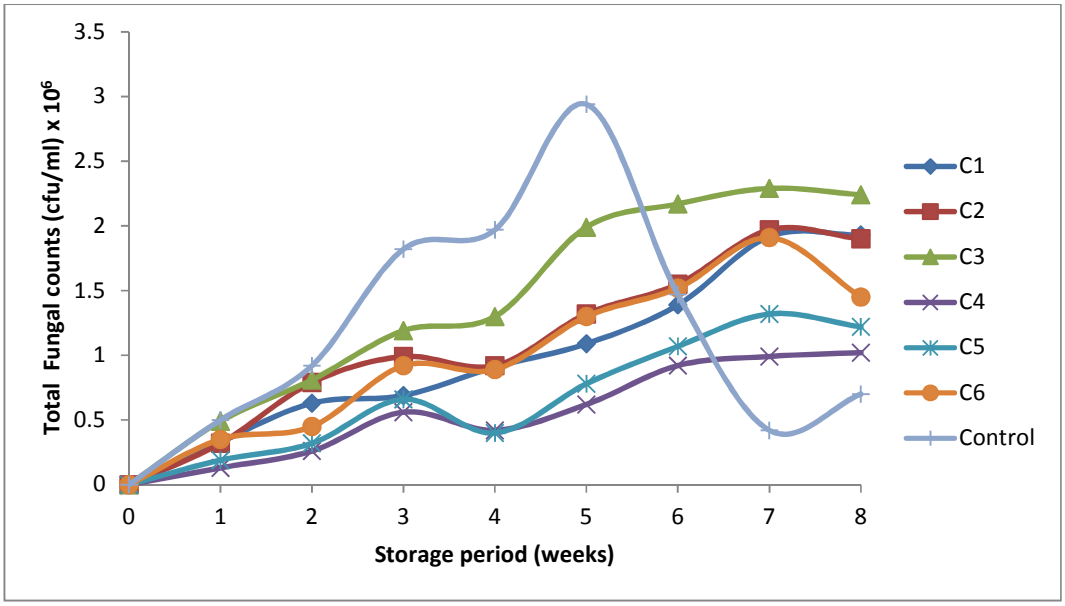


**Figure 6: Changes in viable fungal count of zobo samples treated with sodium metabisulphite stored at room and refrigeration temperatures for eight weeks**

**Key:**
**C1** Zobo + 1% Sodium metabisulphite (26±2°C), **C2** Zobo + 0.5 % Sodium metabisulphite (26±2°C), **C3** Zobo +0.25% Sodium metabisulphite (26±2°C), **C4** Zobo + 1% Sodium metabisulphite (5°C), **C5** Zobo + 0.5% Sodium metabisulphite (5°C), **C6** Zobo + 0.25% Sodium metabisulphite (5°C)

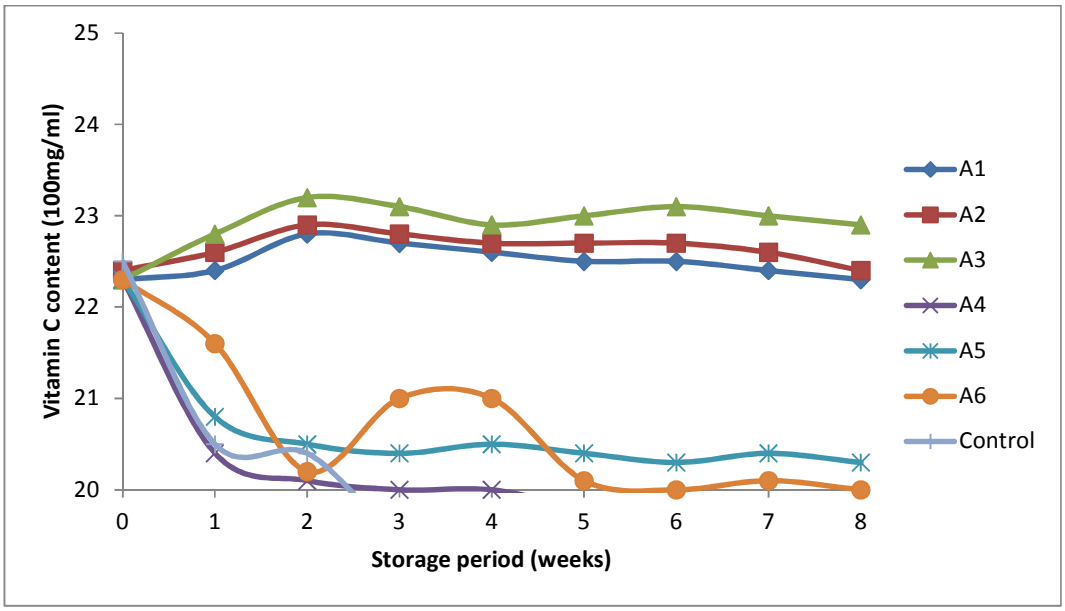


**Figure 7: Changes in vitamin C content of zobo samples treated with *X. aethiopica* fruit extract stored at room and refrigeration temperatures for eight weeks**

**Key:**
**A1** Zobo + 1% *X. aethiopica* (26±2°C), **A2** Zobo + 0.5 % *X. aethiopica* (26±2°C), **A3** Zobo +0.25% X. *aethiopica* (26±2°C), **A4** Zobo + 1% *X. aethiopica* (5°C), **A5**- Zobo + 0.5% *X.aethiopica* (5°C), **A6** Zobo + 0.25% *X.aethiopica* (5°C)

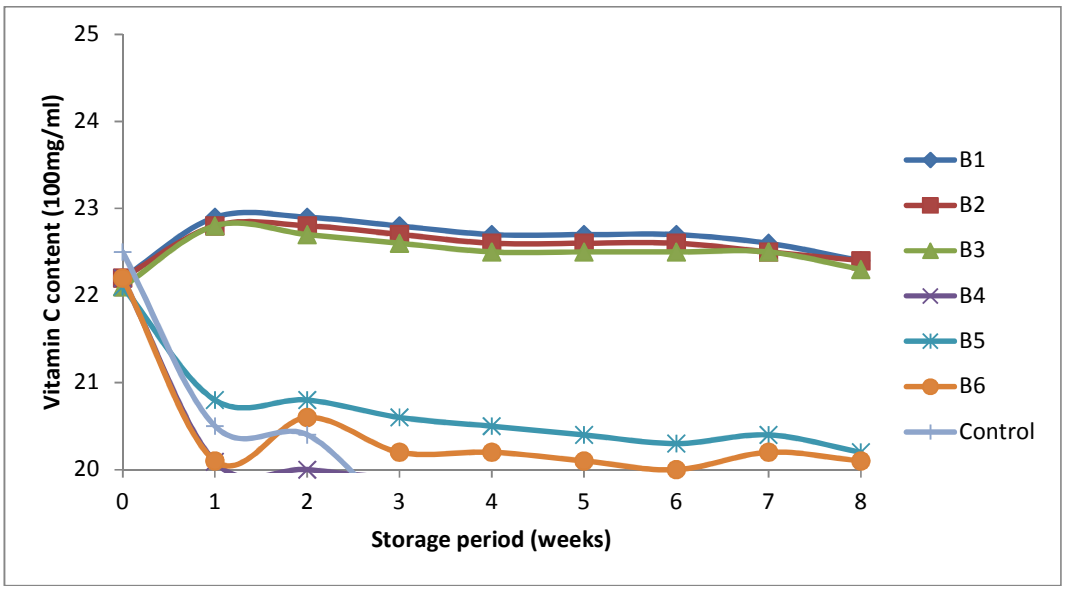


**Figure 8: Changes in vitamin C content of zobo samples treated with benzoic acid stored at room and refrigeration temperatures for eight weeks**

**Key:**
**B1** Zobo + 1% Benzoic acid (26±2°C), **B2** Zobo + 0.5 % Benzoic acid (26±2°C), **B3** Zobo +0.25% Benzoic acid (26±2°C), **B4** Zobo + 1% Benzoic acid (5°C), **B5** Zobo + 0.5% Benzoic acid (5°C), **B6**- Zobo + 0.25% Benzoic acid (5°C)

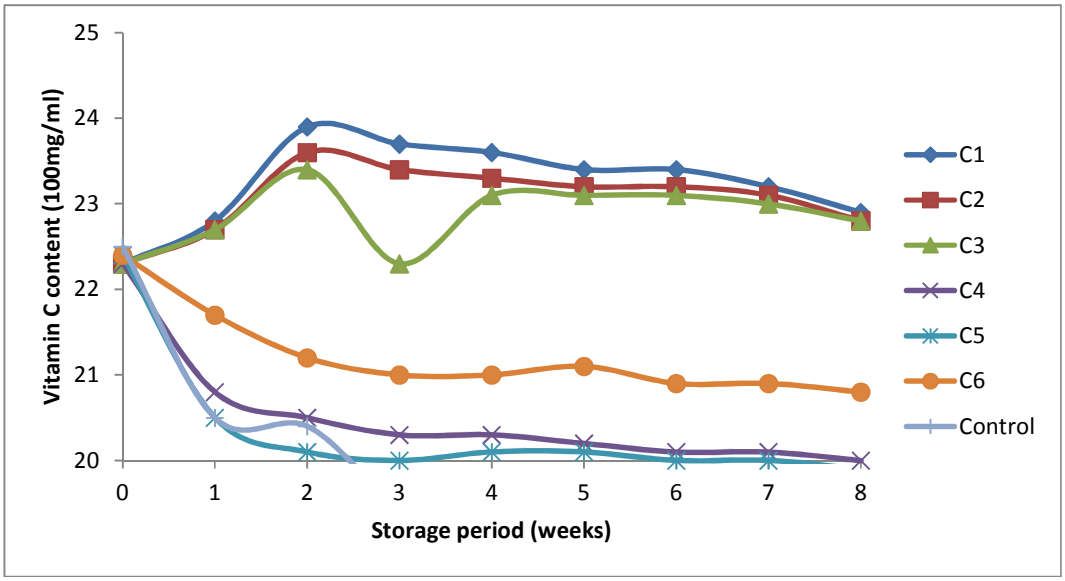


**Figure 9: Changes in vitamin C content of zobo samples treated with sodium metabisulphite stored at room and refrigeration temperatures for eight weeks**

**Key:**
**C1** Zobo + 1% Sodium metabisulphite (26±2°C), **C2** Zobo + 0.5 % Sodium metabisulphite (26±2°C), **C3** Zobo +0.25% Sodium metabisulphite (26±2°C), **C4** Zobo + 1% Sodium metabisulphite (5°C), **C5** Zobo + 0.5% Sodium metabisulphite (5°C), **C6** Zobo + 0.25% sodium metabisulphite (5°C).

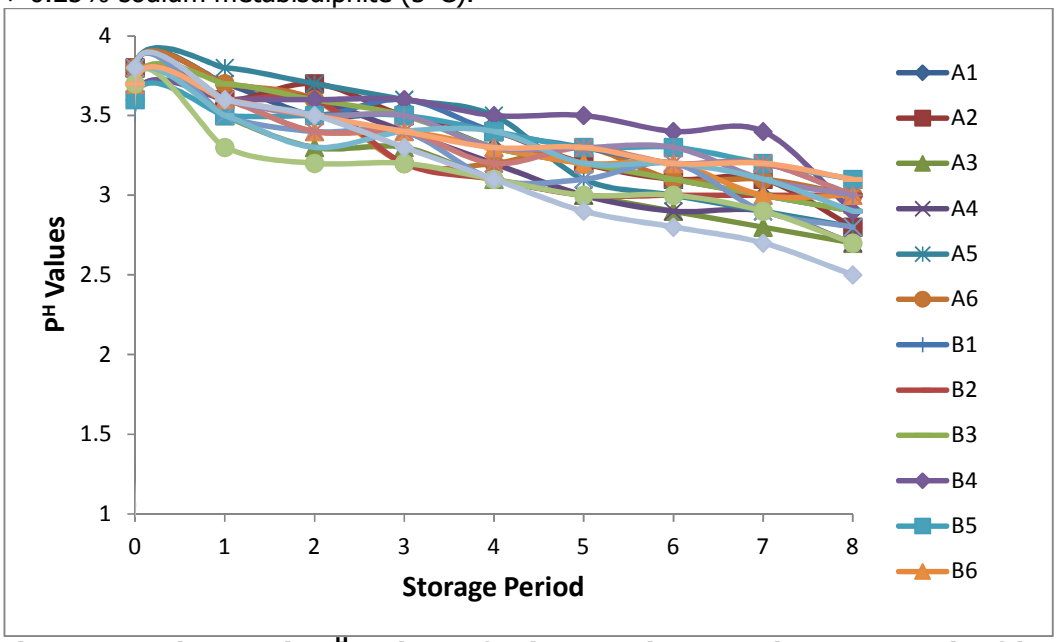


**Figure 10: Changes in $p^H$ values of zobo samples stored at room and refrigerated temperatures for eight weeks**

**Key:**
**A1** Zobo + 1% *X.aethiopica* (26±2°C), **A2** Zobo + 0.5 % *X.aethiopica* (26±2°C), **A3**- Zobo +0.25% X. aethiopica (26±2°C), **A4** Zobo + 1% *X. aethiopica* (5°C), **A5** Zobo + 0.5% *X. aethiopica* (5°C), **A6** Zobo + 0. 25% *X.aethiopica* (5°C)

**B1** Zobo + 1% Benzoic acid (26±2°C), **B2** Zobo + 0.5 % Benzoic acid (26±2°C), **B3** Zobo +0.25% Benzoic acid (26±2°C), **B4** Zobo + 1% Benzoic acid (5°C), **B5** Zobo + 0.5% Benzoic acid (5°C), **B6** Zobo + 0.25% Benzoic acid (5°C)

**C1** Zobo + 1% Sodium metabisulphite (26±2°C), **C2** Zobo + 0.5 % Sodium metabisulphite (26±2°C), **C3** Zobo +0.25% Sodium metabisulphite (26±2°C), **C4** Zobo + 1% Sodium metabisulphite (5°C), **C5** Zobo + 0.5% Sodium metabisulphite (5°C), **C6** Zobo + 0.25% Sodium metabisulphite (5°C)

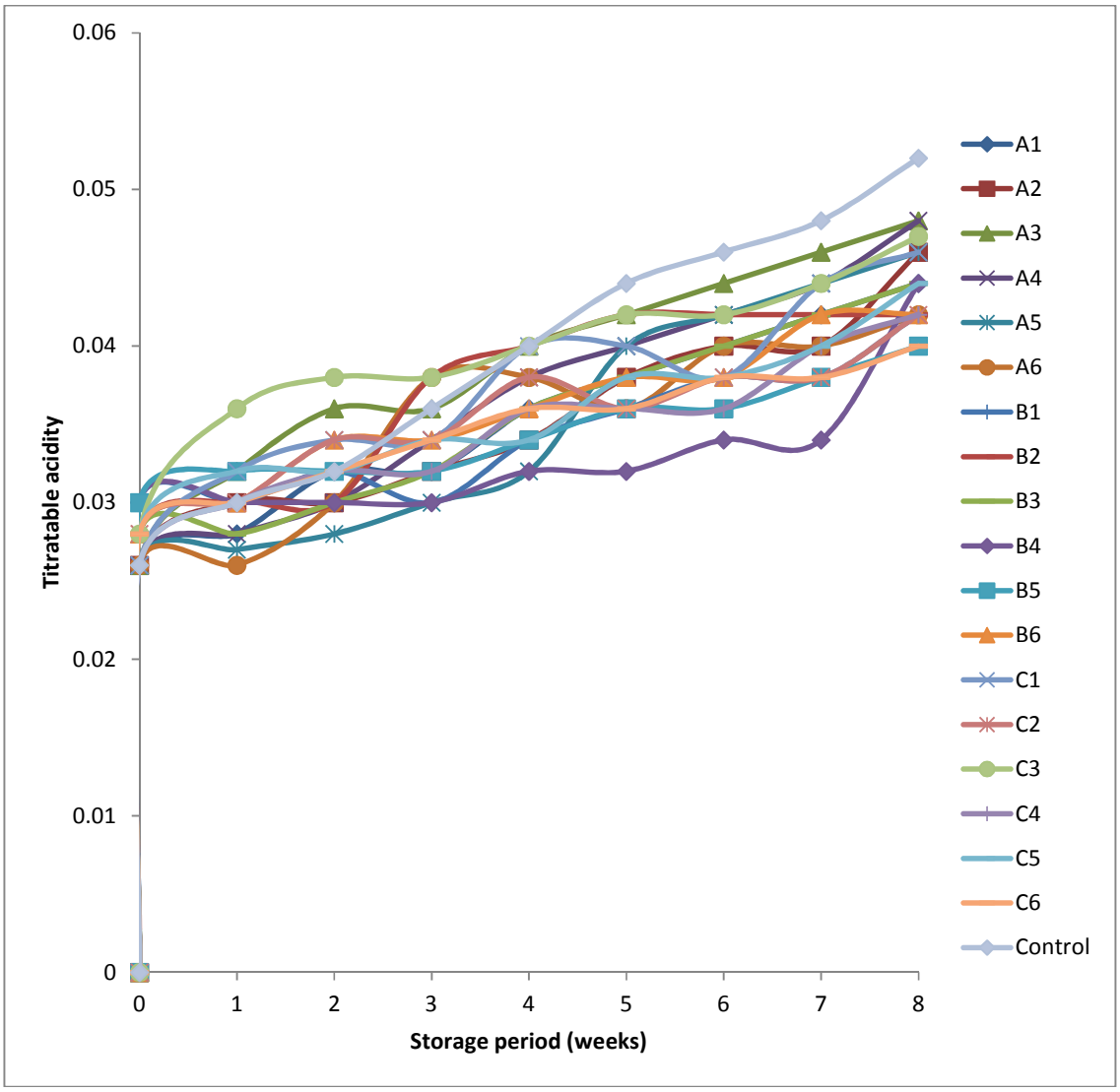


**Figure 11: Changes in titrable acidity of zobo samples stored at room and refrigeration temperatures for eight weeks**

**Key:**
**A1** Zobo + 1% *X. aethiopica* (26±2°C), **A2** Zobo + 0.5 % *X.aethiopica* (26±2°C), **A3** Zobo +0.25% X. aethiopica (26±2°C), **A4** Zobo + 1% *X.aethiopica* (5°C), **A5** Zobo + 0.5% *X. aethiopica* (5°C), **A6**-Zobo + 0.25% *X.aethiopica* (5°C)

**B1** Zobo + 1% Benzoic acid (26±2°C), **B2** Zobo + 0.5 % Benzoic acid (26±2°C), **B3** Zobo +0.25% Benzoic acid (26±2°C), **B4** Zobo + 1% Benzoic acid (5°C), **B5** Zobo + 0.5% Benzoic acid (5°C), **B6** Zobo + 0.25% Benzoic acid (5°C)

**C1**- Zobo + 1% Sodium metabisulphite (26±2°C), **C2**- Zobo + 0.5 % Sodium metabisulphite (26±2°C), **C3**- Zobo +0.25% Sodium metabisulphite (26±2°C), **C4**- Zobo + 1% Sodium metabisulphite (5°C), **C5**- Zobo + 0.5% Sodium metabisulphite (5°C), **C6-** Zobo + 0.25% Sodium metabisulphite (5°C)

**Table 3: Changes in Organoleptic acceptability/quality of zobo samples over the eight weeks period of storage**

| Sample | WEEKS | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 0 hr | 1wk | 2wks | 3wks | 4wks | 5wks | 6wks | 7wks | 8wks |
| **A1** | 4.4±0.32[a] | 2.8±0.48[b] | 2.4±0.32[a, b] | 1.8±0.48[a, b] | 1.4±0.52[b] | 1.4±0.52[b] | 1.2±0.26[b, c] | 1±0.00[b, c] | 1±0.00[b, c] |
| **A2** | 4.4±0.32[a] | 3.2±0.48[b] | 2.8±0.26[b] | 2.2±0.48[b] | 1.8±0.26[b] | 1.8±0.26[b, c] | 1±0.00[b, c] | 1±0.00[b, c] | 1±0.00[b, c] |
| **A3** | 4±0.00[a] | 3.4±0.32[a, b] | 2.8±0.26[b] | 2±0.41[b, c] | 1.6±0.52[b] | 1.2±0.26[b] | 1±0.00[b] | 1±0.00[b, c] | 1.2±0.00[b] |
| **A4** | 5±0.00[a] | 3.2±0.48[a] | 2.8±0.26[a, b] | 2±0.41[a, b] | 2.2±0.26[a, b] | 1.6±0.32[a, b] | 1.2±0.26[b] | 1±0.00[b, c] | 1.2±0.26[b] |
| **A5** | 4.8±0.26[a] | 3.8±0.26[a] | 3.2±0.48[a] | 2.4±0.32[b] | 1.8±0.26[c] | 1.2±0.26[c] | 1±0.00[b] | 1±0.00[c] | 1±0.00[c] |
| **A6** | 4.8±0.26[a] | 3.8±0.26[a] | 3.2±0.26[b] | 2.4±0.32[b] | 2±0.58[a, b] | 1.8±0.26[c] | 1.2±0.26[c] | 1±0.00[c] | 1±0.00[c] |
| **B1** | 4±0.00[a, b] | 3.4±0.52[a] | 3±0.41[a] | 2.2±0.48[a, b] | 2.2±0.48[b] | 1.8±0.63[b] | 1.4±0.32[a, b] | 1±0.00[b] | 1±0.00[b] |
| **B2** | 4.2±0.26[a] | 3.2±0.48[a] | 2.6±0.32[a, b] | 2.2±0.48[b] | 1.8±0.48[b] | 1.8±0.75[b] | 1.4±0.32[b] | 1.4±0.32[b] | 1±0.00[b] |
| **B3** | 4.6±0.32[a] | 3±0.58[a] | 2.8±0.26[a] | 3±0.41[a] | 2.2±0.48[a, b] | 1.8±0.48[a, b] | 1.4±0.32[b] | 1.4±0.32[b] | 1±0.00[b] |
| **B4** | 4.8±0.26[a] | 3.8±0.48[a] | 3.4±0.52[b] | 2.8±0.26[b, c] | 2.2±0.26[b] | 2.2±0.48[b] | 1.6±0.32[b, c] | 1.4±0.3[b, c] | 1±0.00[b, c] |
| **B5** | 4.8±0.26[a] | 3.8±0.26[a] | 3.6±0.32[a] | 3.2±0.26[b] | 2.4±0.32[b] | 1.6±0.32[b] | 1.6±0.32[c] | 1±0.00[c] | 1.2±0.26[b, c] |
| **B6** | 4.8±0.26[a] | 4±0.41[a] | 3.8±0.26[a] | 2.8±0.26[b] | 2.4±0.32[b] | 2.2±0.26[b] | 1.8±0.26[b] | 1.2±0.26[b, c] | 1±0.00[b] |
| **C1** | 4±0.00[a] | 3±0.41[a] | 2.4±0.32[a] | 2.2±0.48[a, b] | 1.4±0.32[b] | 1.4±0.32[a] | 1±0.00[b] | 1±0.00[b] | 1±0.00[b] |
| **C2** | 4±0.41[a] | 2.8±0.26[a] | 2.6±0.32[a, b] | 1.8±0.26[a, b] | 1.4±0.32[a, b] | 1±0.00[b] | 1±0.00[b] | 1.2±0.26[b] | 1±0.00[b] |
| **C3** | 3.8±0.26[a] | 2.4±0.32[a, b] | 2.6±0.32[a] | 2±0.41[a, b] | 1.4±0.52[a, b] | 1±0.00[b] | 1.2±0.26[b] | 1±0.00[b] | 1±0.00[b] |
| **C4** | 4.2±0.26[a] | 3.4±0.32[a] | 2.8±0.26[a, b] | 2±0.41[b] | 1.6±0.32[b] | 1±0.00[b] | 1.2±0.26[b] | 1.2±0.26[b] | 1.2±0.26[b] |
| **C5** | 4.2±0.48[a] | 3.2±0.48[b] | 2.6±0.52[b] | 2±0.41[b] | 1.8±0.48[b] | 1.6±0.52[b, c] | 1.4±0.32[c] | 1.2±0.26[c] | 1±0.00[c] |
| **C6** | 4.2±0.48[a] | 3.2±0.48[b] | 2.2±0.26[b] | 2±0.00[b] | 1.6±0.32[b, c] | 1.2±0.26[b,c] | 1.4±0.32[b,c] | 1±0.00[c] | 1±0.00[c] |
| **Control** | 4±0.00[a] | 2.2±0.26[b] | 1.6±0.32[b] | 1±0.00[c] | 1±0.00[c] | 1±0.00[c] | 1±0.00[c] | 1±0.00[c] | 1±0.00[c] |

**Table 4: Proximate Analysis of Zobo Samples**

| | Before storage % | | | After storage % | | |
|---|---|---|---|---|---|---|
| **Sample** | **Moisture** | **Protein** | **CHO** | **Moisture** | **Protein** | **CHO** |
| **A1** | 88.62 | 0.080 | 10.55 | 88.52 | 0.079 | 10.56 |
| **A2** | 88.60 | 0.079 | 10.56 | 88.49 | 0.080 | 10.54 |
| **A3** | 88.60 | 0.079 | 10.57 | 88.48 | 0.077 | 10.54 |
| **A4** | 88.40 | 0.080 | 10.77 | 88.36 | 0.078 | 10.67 |
| **A5** | 88.46 | 0.081 | 10.71 | 88.40 | 0.079 | 10.64 |
| **A6** | 88.49 | 0.081 | 10.68 | 88.41 | 0.079 | 10.62 |
| **B1** | 88.61 | 0.081 | 10.54 | 88.49 | 0.074 | 10.59 |
| **B2** | 88.60 | 0.080 | 10.55 | 88.50 | 0.078 | 10.52 |
| **B3** | 88.61 | 0.080 | 10.54 | 88.49 | 0.079 | 10.52 |
| **B4** | 88.44 | 0.081 | 10.73 | 88.40 | 0.079 | 10.65 |
| **B5** | 88.50 | 0.082 | 10.69 | 88.42 | 0.078 | 10.65 |
| **B6** | 88.55 | 0.082 | 10.59 | 88.48 | 0.078 | 10.55 |
| **C1** | 88.52 | 0.082 | 10.65 | 88.40 | 0.080 | 10.67 |
| **C2** | 88.50 | 0.081 | 10.69 | 88.37 | 0.081 | 10.69 |
| **C3** | 88.52 | 0.082 | 10.66 | 88.40 | 0.082 | 10.62 |
| **C4** | 88.42 | 0.081 | 10.75 | 88.37 | 0.080 | 10.68 |
| **C5** | 88.48 | 0.080 | 10.69 | 88.41 | 0.081 | 10.63 |
| **C6** | 88.53 | 0.082 | 10.64 | 88.45 | 0.081 | 10.57 |
| **CONTROL** | 88.68 | 0.082 | 10.45 | 88.60 | 0.078 | 10.43 |

**Key:**
**A1** Zobo + 1% *X.aethiopica* (26±2°C), **A2** Zobo + 0.5 % *X.aethiopica* (26±2°C), **A3** Zobo +0.25% X.aethiopica (26±2°C), **A4** Zobo + 1% *X.aethiopica* (5°C), **A5** Zobo + 0.5% *X.aethiopica* (5°C), **A6** Zobo + 0.25% *X.aethiopica* (5°C)

**B1** Zobo + 1% Benzoic acid (26±2°C), **B2** Zobo + 0.5 % Benzoic acid (26±2°C), **B3** Zobo +0.25% Benzoic acid (26±2°C), **B4** Zobo + 1% Benzoic acid (5°C), **B5** Zobo + 0.5% Benzoic acid (5°C), **B6** Zobo + 0.25% Benzoic acid (5°C)

**C1** Zobo + 1% Sodium metabisulphite (26±2°C), **C2**- Zobo + 0.05 % Sodium metabisulphite (26±2°C), **C3** Zobo +0.25% Sodium metabisulphite (26±2°C), **C4** Zobo + 1% Sodium metabisulphite (5°C), **C5** Zobo + 0.5% Sodium metabisulphite (5°C), **C6** Zobo + 0.25% Sodium metabisulphite (5°C)

**Table 5: Phytochemicals present in *Xylopia aethiopica* fruit extract**

| Phytochemicals | Crude Extracts |
|---|---|
| Alkaloids | ++ |
| Glycoside | + |
| Steroids | - |
| Terpenoids | - |
| Saponins | ++ |
| Flavonoids | ++ |
| Tannins | + |

**Key**:
(+): Present (-): Absent

**Discussion**

In this study, ten microorganisms were isolated comprising of six bacteria and they include: *Enterobacter cloacae* strain VCRC B519, *Enterobacter sp.*AAP4, *Enterobacter cloacae* strain HR-1, *Bacillus subtilis* strain YX3, *Klebsiella pneumonia* strain NF16 and *Salmonella enterica* subsp. *enterica* serovar *Typhimurium* strain SA40,while the remaining four were fungi which consists of: *Aspergillus niger, Aspergillus fumigatus, Acremonium strictum and Alternaria alternata.* The microorganisms that were found to be associated with the spoilage of zobo in this work is to a great extent in agreement with the work of Egbere *et al*. (2007) who reported the presence of *Bacillus* sp*, Lactobacillus sp, Sacharromyces cerevisiae, Mucor* sp and *Aspergillus* sp to be associated with the progressive spoilage of *zobo*. These organisms play important roles in the spoilage of food and some of them are pathogenic (Nwachukwu *et al.,* 2007; Obi, 2015). The organisms identified in this study are of great public health importance due to their ability to cause disease and might have found their way into the *zobo* drink as a result of the unhygenic handling of the *Hibiscus sabdariffa* in the market before purchase. This assertion is supported by the work of (Omemu *et al*.,2005) they found the isolation of *Bacillus subtilis, Aspergillus niger, Aspergilus flavus* as well as some other enteric organisms from dried Calyx of *Hibiscus sabdariffa* and *zobo* juice. The presence of this group of bacteria found in this study is an indication of contamination most likely from the *Hibiscus sabdariffa* calyx used in the production of the *zobo* juice as the water used in the preparation of the juice was sterilized before use.

Studies by several researchers have shown that *Bacillus, Aeromonas, Corynebacterium, Veilonella, Micrococcus, Pseudomonas, Streptococcus, Staphylococcus, Lactobacillus, Enterococccus, Escherichia, Proteus*, *Aspergillus, Penicillium,* and *Saccharomyces* are the genera of microbes that cause spoilage of *zobo* drink (Egbere *et al*., 2007; Braide *et al.,* 2012*.,* Seiyaboh *et al*., 2013; Ezearigo *et al*., 2014). Generally, the presences of these organisms in ready–to-eat foods and drinks may be potential source of various food borne disease conditions especially the ones associated with gastroenteritis in human (Bello *et al*., 2014).

One of the intended goals in the use of preservatives in food is the inhibition of growth and elimination of the undesirable activities of microorganisms thereby extending the shelf life under defined conditions (Egbere *et al*., 2007; Obioma, 2012). This study has revealed the effectiveness of different concentrations of *Xylopia aethiopica* extract, benzoic acids and sodium metabisulphite to inhibit the growth of spoilage microbes found in *zobo* drink at temperatures of $5^0$C and 26±2 $^0$C- for a period of eight weeks.

The bacterial counts during the 8 weeks storage period for the control sample ranged from $1.0\times10^4$ to $2.89 \times 10^6$ cfu/ml which was the highest among the different treatment conditions of the *zobo* samples. Benzoic acid as used in sample B4 *(zobo* + 1% Benzoic acid stored at 50 $^0$C) showed the greatest potency in inhibiting microbial growth with a viable bacteria and fungi count of zero after twelve hours of applying the treatment,a viable bacteria and fungal count of $4.0 \times 10^4$ cfu/ml and $9.0 \times 10^4$cfu/ml respectively at 7days of storage, and an eventual viable count of $9.7 \times 10^5$ and $7.2 \times 10^5$ for bacteria and fungi respectively at the end of the eight weeks storage period. The control sample however had viable counts of $3.0 \times 10^4$ and $2.3 \times 10^5$ for bacteria and fungi respectively after twelve hours which progressively increase to a peak of $2.89 \times 10^6$ and $2.94 \times 10^6$ for bacteria and fungi respective at week five and decrease in a similar retrogressive manner to $2.3 \times 10^5$ and $7.0 \times 10^5$ for bacteria and fungi respectively at the end of the eight week .The decline in proliferation of microbial growth in both fungi and bacteria observed after the 5$^{th}$ week might be associated with return of unfavourable micro-environmental conditions which enhances the undersecretion of metabolic enzymes and bring about homeostatic imbalance (Ogiehor *et al* 2008). It is also possible that the hurdles are acting at different targets (multi-target effect) in the microbial cells (Braide *et al*., 2012; Seiyaboh *et al*., 2013). These targets might be cell membrane, enzyme system, protein synthesis or DNA, thus distorting homeostasis in different respects which can make repair process difficult resulting in the death of the microorganisms (Leistner, 2000; Obioma, 2012). The steady increase in population observed from the beginning of the storage in the control samples till the 5$^{th}$ week may be attributed to presence of the organisms in suitable micro environment which makes it easy for the organisms to grow and proliferate.

The lesser growth recorded in the benzoic acid treated sample as well as other treated samples when compared to the control (untreated sample) might be an indication of the difficulty encountered by the vegetative cells present in the zobo samples to multiply due to the inhibitive nature of the treatments. 1% of *Xylopia aethiopica* with refrigeration temperature (A4) had strongest fungi inhibiting effect in *zobo* while sample A3 (*zobo* + 0.25% *Xylopia aethiopica* stored at 26±2$^0$C) sample had the lowest inhibiting effect on the fungi growths among the samples treated with *Xylopia aethiopica.*

The use of a chemical preservative (benzoic acid) in the preparation of zobo inhibited the proliferation of microbial contaminants in *zobo* when compared to control. Samples treated with the different concentrations of benzoic acid had relatively low microbial counts as indicated in Figures 7 and 8. Amongst the treated samples, Sample B4 (*zobo* + 1% benzoic acid stored at $5^0$C) had the fewest growth of viable count(bacteria and fungi) which increased gradually, while Sample B3 (*zobo* + 0.25% benzoic acid stored at 26±$^0$C) recorded the highest number of colony counts among the benzoic acid treated samples. This is suggestive that benzoic acid has significant effect in prolonging the shelf-life of *zobo*.

It was observed generally that microbial count of the control sample (without any treatment) increased rapidly from week 1 to week 5 before dropping drastically. This might be the result of the depletion of nutrient which resulted to the decrease in the bacteria counts with increase in days of storage (Seiyaboh *et al*., 2013). Other reports have been

documented that the presence of the preservatives might be responsible for extensive distortion of the homeostasis of the organisms leading to death as a result of their inability to overcome the resultant shock (Obi, 2015).

Samples treated with sodium metabisulphite showed the least growth inhibition compared to other treatments used in the study although, the controlled sample with no preservative had the highest number of microbial counts. Sample C4 (*zobo* + 1% sodium metabislphite + stored at $5^{o}$C) was the most effective in keeping the microbiological quality of the *zobo* with fewer colony counts as compared to other sodium metabisulphite treated samples. Higher concentrations of sodium metabisulphite showed greater growth inhibition of both bacteria and fungi counts. This implies that the effect of the sodium metabisulphite is not only concentration dependent, but was futher enhanced by refrigeration temperature. The microbial count of viable organisms was too high to be acceptable in a drink as found in the control sample.

Result from this studies revealed that, chemicals and natural preservative used were effective in elongating the shelf life of *zobo*. This is evident by the lower counts of bacteria and fungi over the first five weeks of the storage period, which contrasted to the case of the sample without preservatives. This conforms with the work of Dougheri *et al*. (2007) who tested the effect of sodium metabisulphite, sodium benzoate and benzoic acid in *zobo*. They found out that the *zobo* sample had an initial bacterial count of $1.13 \times 10^{7}$ cfu/ml, but the counts of the samples with preservatives was observed to drop retrogressively, while that of the samples without preservatives increased progressively over a period of eight days.

Babalola *et al*. (2001) reported the presence of ascorbic acid in different varieties of *H. sabdariffa*. On the level of vitamin C (Ascorbic acid) present in the drink, this work demonstrate that the ascorbic acid contents of z*obo* drinks supplemented with 0.25%, 0.5% and 1% *Xylopia aethiopica*, benzoic acid and sodium metabisulphite all had higher vitamin C content when compared to the control sample without treatment. This implies that the vitamin C status of *zobo* beverages can thus be conserved by treating the drink with *Xylopia aethiopica* and the two other chemical preservatives (benzoic acid and sodium metabisulphite) tested in this study. It was noted that the higher the concentration of the preservative used, the higher its corresponding ascorbic acid produced which might be as a result of the ability of all the preservatives tested to act in a concentration dependent manner on the *zobo* drink

By nature, *zobo* juice produced from the calyces of *Hibiscus sabdariffa* is always very acidic with low pH values and the high acidity of the juice might account for the low numbers and few types of organisms which are commonly acid producing organisms (Fasoyiro *et al*., 2005). As shown in Figures 14 and 15, the pH of the samples became lowered with a corresponding increase in titratable acidity over the eight weeks storage period. It was observed that the control sample possessed the highest level of acidity right during storage, followed by the room temperature treated samples and lastly the refrigerated treated sample. This account for the reason why *zobo* drink when left for two or three days at room temperature without any preservative turned sour (Fasoyiro *et al*., 2005; Builder *et al*., 2010) stated the pH of the fruit flavoured *zobo* drinks had a low value which ranged between pH 2.19 and 3.62 and this might be as a result of fermentation due to microbial action of acid producing organisms, this fermentation process may to lead to loss of taste and value, increased rate of browning and offensive odour, and perhaps presence of cloudy materials at the bottom of the container. Ajala *et al.* (2015) reported that the acidity of the *zobo* juice is attributed to the presence of naturally occuring organic acids such as malic,

citric and oxalic acids in the extract of *Hibiscus sabdariffa,* and as such*,* ulcer patients and people with other related problems are advised to desist from large consumption of the extract. Odebunmi *et al*. (2007) however suggested that with proper fortification with other less acidic fruit juices, it might be a good drink.

Titratable acidity of *zobo* samples treated with the different concentrations of the preservatives (0.25%, 0.5% & 1%) *Xylopia aethiopica*, benzoic acid and sodium metabisulphite, stored at both room and refrigerated temperatures were determined, There was a progressive increase over the eight weeks period of storage in the titre values of the zobo samples from 0.026 to 0.048 in zobo treated with *Xylopia aethipica*, 0.028 to 0.044 in benzoic acid treated *zobo* samples and 0.026 to 0.047 in *zobo* samples treated with sodium metabisulphite while the control (untreated zobo sample) had the highest increase in titratable acidity from 0.026 to 0.052 .

The result of organoleptic / acceptability as presented Table 4**,** indicated significant difference in the organoleptic properties of the different zobo samples at at $p < 0.05$.The organoleptic properties or sensory qualities of the samples were not really affected by the addition of preservatives and refrigeration as all the samples were found to have lost organoleptic wholesomeness as the storage period increased. Sample B6 (zobo + 0.25% benzoic acid stored at $5^0$C) was found to be the most effective in preserving the organoleptic quality of the *zobo* samples after two weeks of storage. The control sample lost acceptability faster than all other *zobo* samples with treatments after the first week while all other samples were found to have lost acceptability at the end of the $4^{th}$ week.

It was generally observed in the study that samples stored at refrigerated temperature of $5^0$C were found to have a more enhanced consumer acceptability, especially those with preservatives. This agrees with a study that reported that chilled beverage drinks, wine and juices taste better than unchilled ones, and as such, the most effective way of *zobo* drink preparation and preservation is to prepare it with the preservative, then pasteurize and refrigerate so as to achieve long uncontaminated storage life (Bankole *et al*., 2013). The proximate analysis of zobo was evaluated before and after production and the result as presented in Table 5 indicated that the carbohydrate, protein and fat value was almost the same throughout the storage period.

Preservative have been used to store food substances and they act by inhibiting, retarding or arresting the growth of microorganisms or of any such deterioration resulting from their presence or of masking the evidence of any such deterioration (Izah *et al*., 2015). To be in accordance with good manufacturing practice (GMP), the use of preservative should not adversely affect the nutritive value of foods or should not permit the growth of food poisoning organisms while suppressing the growth of others that would make spoilage evident (Onuoha and Fatokun, 2014).

**Conclusion**

This study has shown that *Xylopia aethiopica* and chemical preservatives (benzoic acid and sodium metabisulphite) had significant inhibitory effect in a concentration dependent manner on *zobo* with their effectiveness being futher aided by refrigeration. *Zobo* preservation through the use of preservatives was found to be most useful in elongating the shelf life of the beverage, being capable of either eliminating some bacteria or inhibiting the rapid acid, or alcohol production by the spoilage microbial agents. However there is urgent need for alternative preservative that is natural and human friendly, affordable and readily available which makes *Xylopia aethiopica* a promising choice.

The use of *Xylopia aethiopica* fruit extract, benzoic acid and sodium metabisulphite most especially under refrigeration condition elongated the shelf-life of *zobo* drink, such that fermentation was reduced and discoloration inhibited to a reasonable extent. Consequently, *zobo* drink can be produced on a large scale and preserved for longer periods and still retain its nutritive value such that the drink will attract acceptance. The cheapness of the raw materials, its preparation and the finished product makes the drink particularly a worthwhile venture and it large scale production feasible.

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