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Shelf Stability, Microbiological and Physicochemical Studies of '*Zobo*' Drink Pasteurized and Treated with Preservative

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ABSTRACT

Background: The combined effect of pasteurization, improved hygiene and varied concentrations of sodium benzoate on the shelf stability and physicochemical attributes of *'Zobo'* drink, a nutritious non-alcoholic indigenous beverage was evaluated.
Objectives: To evaluate the combined effects of pasteurization, improved hygiene and food preservatives on the shelf stability of '*Zobo*' drink.
Methods: Freshly prepared '*Zobo*' samples were aseptically dispensed in 100 mL capacity Polyethylene terephthalate (PET) bottles containing various concentrations of sodium benzoate (0.05%, 0.25%, 0.50%, 0.75% and 1.00%). Six '*Zobo*' samples were produced from the fresh '*Zobo'* using a modified method that combined Hurdle Technology and Hazard Analysis and Critical Control Points (HACCP) for preservation and stored for eight weeks on the shelf. Analyses were carried out weekly on loss in vitamin C content, microbial quality and physicochemical properties of the beverage for eight weeks.
Results: Parameters analysed significantly ($p<0.05$) changed considering the methods of treatment and storage period. Pasteurization at 68^{o}C for 20 minutes eliminated successfully all pathogenic organisms and coliforms as none was isolated from the drink throughout the eight weeks of shelf study. *Saccharomyces cerevisiae* and *Lactobacillus fermentum* isolated from the samples during storage were responsible for the deterioration and spoilage of the beverage.
Conclusion: Although the five different concentrations of the preservative used in combination with pasteurization were effective in extending the shelf life of the beverage, sample Bz 0.05% preserved better than all others without impacting negatively on the vitamin C content and other physicochemical properties of the drink.

Keywords: *Zobo*, *Hibiscus sabdariffa*, Sodium Benzoate, Pasteurization, shelf life

INTRODUCTION

Nigeria is home to many indigenous produced beverages which are by far more nutritious than most factory processed soft drinks (Chukwu *et al*, 2019). These indigenous beverages are mainly drunk after meals or as thirst quencher in many homes and restaurants. Some of them are alcoholic e.g. *Burukutu Pito* and palm wine; while others are non-alcoholic e.g. *Kunun zaki, Zobo*, soymilk, tigernut milk and ginger drink. The non-alcoholic beverages, however, are widely accepted than their alcoholic counterparts and are more consumed due to increased religious and health campaigns against such beverages. *Zobo* or sorrel drink is one of such non-alcoholic beverages made from the hot water extracts of the petals of *Hibiscus sabdariffa* (Nwokocha *et al*., 2012). It is rich in Ascorbic acid and in contains phytochemicals such as alkaloids, anthraquinones, tannins, glycosides, saponins and polyphenols (Osuntogun and Aboaba, 2004). Its medicinal value is high and mostly used by locals as antihypertensive, astringent, diuretic and purgative agents. It has been indicated that the herb may have activity in the remedy for fever, cough, constipation and abscesses (Egbere *et al.,* 2007). Despite their nutritional characteristics, these beverages have short shelf lives of about 3 days, ascribed to the poor -sanitary and crude processing methods (Damisa *et al*., 2007). The deterioration may also be likened to contamination originating from the additives (such as sugar, flavouring and colourants) used during processing. Nwafor and Ikenebomeh (2009) reported that contamination arising from packaging materials and packaging methods are bane and could lead to the deterioration of the product.

In a bid to overcome this challenge of abridge shelf life, the drink is often refrigerated to prolong the shelf quality. The epileptic public power supply in Nigeria however, hampers the efficient use of refrigerators for keeping this drink beyond a few days. This has propelled research into various methods by which these beverages can be preserved on the shelf with zero dependence on refrigeration. Onyeagba *et al*. (2004) reported the preservation of *Zobo* using natural preservatives as ginger, lime and trona. Damisa *et al*., (2007) also compared the efficacies of pasteurization compared with Sodium benzoate and Sodium metabisulphite (organic acids) on the shelf stability of *Zobo*; inferring that the use of organic acid preservatives remarkably extended the shelf life of the beverage for 15 days longer than the ones without preservatives. Similar findings by Chukwu *et al*. (2019) revealed that the combination of different preservative hurdles like carbonation, pasteurization, different chemical preservatives applied at concentrations generally regarded as safe prolonged the shelf stability of *Zobo*. This research was therefore aimed at evaluating the combined effects of pasteurization, improved hygiene and sodium benzoate at varied concentrations on the physicochemical properties and shelf stability of '*Zobo*' drink.

MATERIALS AND METHODS

Collection of samples

Fresh pineapple fruit, granulated sugar and dried calyxes of red *Hibiscus sabdariffa*, were purchased from a nearby local market bordering the Federal University of Technology, Minna in Niger State. The packaging containers (100 millilitre capacity sterile plastic bottles) were purchased from Kano State. Processing, packaging and microbiological evaluation of the samples were carried out under strict and standard aseptic conditions in the microbiology laboratory of the university.

Processing of *Zobo* Drink

Six hundred grams of the dried calyces were sorted out, washed in sterile water and was boiled in fifteen (15) litres of water for five minutes. The liquid extract was filtered immediately using a clean pre-sterilized muslin cloth. The filtrate was sweetened with sugar syrup (200 grams) and flavoured with pre-pasteurized fresh pineapple juice according to the procedure of Maggi Family Menu Cook Book (1996) (Figure 1).

Sample preparation for analyses

The bulk of the prepared '*Zobo*' drink was divided into two batches: designated as control (pasteurized samples only), while the second batch of samples contained five different concentrations of Sodium benzoate designated as Bz 0.05 %, 0.25 %, 0.5 %, 0.75 % and 1.0 % respectively.

Packaging

The different concentrations of sodium benzoate as described above were aseptically weighed into the one hundred (100) millilitre capacity sterile plastic bottles, then one hundred (100) millilitres of '*Zobo*' drink were aseptically dispensed into the bottles, corked and labelled appropriately.

Pasteurization

All the samples including the control were pasteurized at 68 °C for 20 minutes. The samples were allowed to cool at room temperature and thereafter arranged on the shelf for storage at ambient temperature. These samples were monitored weekly for eight weeks on the changes in their microbial, physicochemical and vitamin C contents.

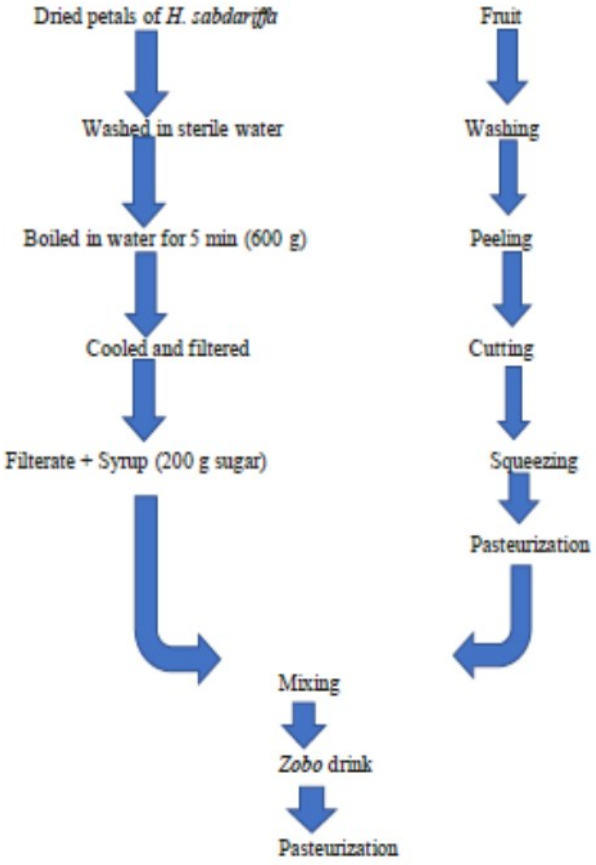

Figure 1: Steps in the preparation of *Zobo* drink

Microbiological Analysis

Microbial analyses on the products were performed weekly for eight weeks pipetting 1 mL of the serially diluted liquid homogenate into appropriately labelled petri dishes. Enumeration of total bacteria count was carried out using the Pour Plate Method according to the procedure of FSSAI (2012). Most Probable Number method was used for the enumeration of coliform bacteria; while fungal counts were carried out on Saboraud Dextrose Agar (SDA) supplemented with 0.1% w/v chloramphenicol solution.

The unknown bacteria were identified using Bergey's Manual of Determinative Bacteriology (Buchanan and Gibons, 1974); while that of the molds and yeasts were identified using the methods described by Barnett *et al*. (1990).

Physicochemical Analyses of the *Zobo* Drink Samples

The pH of the samples was determined weekly throughout shelf storage using Jenway pH meter (Model 3505 Cumlab, UK) previously standardized using buffer solutions at pH 7 (AOAC, 2010).

The Total Titratable Acidity (TTA), calculated as lactic acid was determined by titrating 0.1M sodium hydroxide against five millilitres of the *Zobo* using phenolphthalein as an indicator (AOAC, 2010); while the Total Soluble Solids (TSS) was determined using a handheld analogue refractometer as described by Onwuka (2005) and were recorded in ^{o}Brix.

The vitamin C content in each of the samples was determined by the titrimetric method described by Singh (2004), which involved the use 2, 6—dichloro-indophenol dye.

Statistical Data Analysis

All experimental data were subjected to statistical analysis of mean, standard error and analysis of variance (ANOVA) using IBM SPSS version 19 at $P < 0.05$.

RESULTS AND DISCUSSION

Microbiological Quality Assessment

The microbial quality assessment during the shelf study revealed that no microbial isolate was detected in all the eleven samples after packaging and during the first five weeks of the shelf study (Tables 1 – 2). This result is similar to the findings of Nwokocha et al. (2012), who observed that the Zobo drink samples preserved with alligator pepper and ginger recorded no microbial growth for two weeks from the day of production. The chemical constituents of the spices exhibited antimicrobial properties. The procedure emphasized the adoption of HACCP in food production and processing, which is useful in checking all forms of contamination that may arise before, during and after production. Application of HACCP and hurdle technology (combination of other hurdles such as the low pH, suppression of water activity by solute addition, added preservatives, and heat treatment) successfully eradicated all microorganisms present as at the time of packaging and as well ensured the shelf stability of all the samples for first five weeks.

Table 1 shows the Total Bacterial Count of samples preserved using Sodium Benzoate and Sodium Citrate at varied concentrations while Tables 2 and 3 show the fungal count and coliform count for all the eleven samples respectively. These results, therefore, reveal that the combination of pasteurization and sodium benzoate was effective in lowering the microbial load; thus, extending the shelf life of all the samples preserved with benzoate (Bz) beyond eight weeks. The control sample consisting of only the pasteurized samples, on the other hand, preserved the '*Zobo*' drink and extended its shelf life to five weeks before they began to deteriorate at week six (Table 1). Similar results were also obtained for the total fungal count in which the control sample recorded its first microbial count on the sixth week, increasing steadily through the seventh week till week eight. However, there were no coliforms detected from the control samples throughout the eight weeks of shelf study.

This result confirms that pasteurization alone can extend the shelf life of the drink, thus preserving it for about five weeks without the addition of chemical preservatives, whose consequence impart negative tastes, flavour and very often alters the organoleptic properties of the drink. The result reveals that pasteurization at 68 ^{o}C for 20 minutes successfully eradicated all coliforms and pathogenic organisms throughout the eight weeks of shelf study. The microorganisms isolated from the spoiled samples were *Lactobacillus fermentum* and *Saccharomyces cerevisiae*. The isolation of these two microorganisms connote their ability to withstand the hurdles when others are eliminated.

Physicochemical Properties of the *Zobo* Drink

Hydrogen Ion Concentration (pH): The pH values for all the freshly prepared samples ranged from 2.4 to 3.5 (Figure 2).

Table 1: Total Bacterial count (cfu/ml) on *Zobo* samples preserved using Sodium benzoate

	WEEK								
SAMPLES (%)	Fresh	1	2	3	4	5	6	7	8
Bz 0.05	ND	ND	ND	ND	ND	ND	ND	ND	ND
Bz 0.25	ND	ND	ND	ND	ND	ND	ND	ND	ND
Bz 0.50	ND	ND	ND	ND	ND	ND	ND	ND	ND
Bz 0.75	ND	ND	ND	ND	ND	ND	ND	ND	ND
Bz 1.00	ND	ND	ND	ND	ND	ND	ND	ND	ND
CONTROL	ND	ND	ND	ND	ND	ND	1.8×10^2	4.55×10^3	7.1×10^3

ND = Not Detected

Table 2: Total Fungal count (cfu/ml) on '*Zobo*' samples preserved using Sodium benzoate

	WEEK								
SAMPLES (%)	Fresh	1	2	3	4	5	6	7	8
Bz 0.05	ND	ND	ND	ND	ND	ND	ND	ND	ND
Bz 0.25	ND	ND	ND	ND	ND	ND	ND	ND	ND
Bz 0.50	ND	ND	ND	ND	ND	ND	ND	ND	ND
Bz 0.75	ND	ND	ND	ND	ND	ND	ND	ND	ND
Bz 1.00	ND	ND	ND	ND	ND	ND	ND	ND	ND
CONTROL	ND	ND	ND	ND	ND	ND	1.1×10^2	5.867×10^3	7.86×10^3

This implies that the drink belongs to the class of high acid foods and the advantage of such food is that they do not support the proliferation of most pathogenic microorganisms most especially coliforms, which explains why they were not detected but permits only aciduric microbes to thrive. This explains the domination of *Lactobacillus fermentum* and *S. cerevisiae* in all the spoilt samples. Reduction in pH observed in the control sample unveiled the presence of acid-producing microorganisms like *Saccharomyces cerevisiae* and *Lactobacillus fermentum;* they were responsible for the production of acids with perceived alcoholic odour from the spoilt samples. The results are in agreement with the work of Egbere *et al.* (2007) who noted that the similar pH pattern observed was due to the activities of acid -producing spoilage bacteria isolated from beverage.

Total Titratable Acidity

The result obtained for the Total Titratable Acidity (TTA) of all the six samples ranged from 1.1% to 3.2% Lactic Acid/100 mL. It similar to 2.3 % Lactic Acid/100 mL obtained by Osuntogun and Aboaba (2004). The significant increase in TTA values recorded in the control (Table 3) may have been occasioned and exacerbated by the presence of acid-producing microorganisms pisolated from the beverage .

Despite the increase in TTA (Table 3), the microbial population was on the increase even with an increase in storage period (Tables 1 and 2). The slow pace of growth may however be attributed to the fact that the surviving microorganisms possibly might have been injured by either of the hurdles whether combined or singly (temperature or reduced water activity or

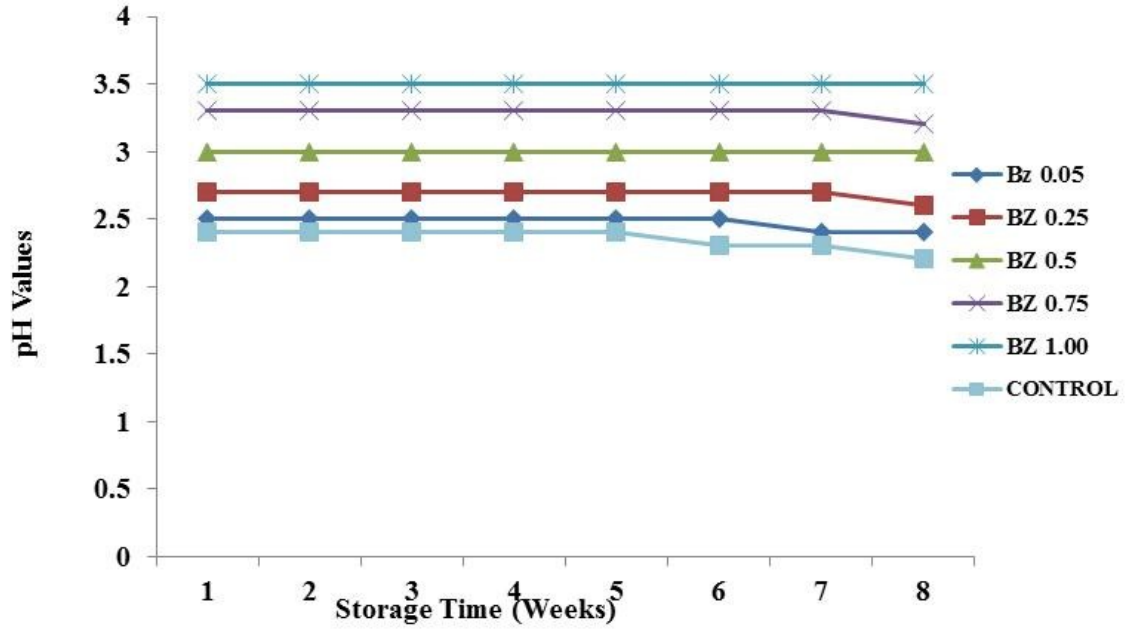

Figure 2: pH values of stored '*Zobo*' drink with respect to Storage time (weeks)

Table 3: Total Titratable Acidity (% lactic acid/100ml) of *Zobo* samples

	WEEK								
SAMPLES (%)	0	1	2	3	4	5	6	7	8
BZ 0.05	2.6±0.40^{a}	2.6±0.40^{a}	2.6±0.40^{a}	2.6±0.40^{a}	2.6±0.40^{a}	2.6±0.40^{a}	2.6±0.40^{a}	2.9±0.15^{a}	3.15±0.00^{a}
BZ 0.25	1.8±0.00^{a}	2.0±0.15^{a}	2.0±0.15^{a}	2.0±0.15^{a}	2.0±01.5^{a}	2.0±0.15^{a}	1.8±0.42^{a}	2.3±0.00^{a}	2.4±0.15^{a}
BZ 0.50	1.5±0.15^{a}	1.5±0.15^{a}	1.5±0.15^{a}	1.5±0.15^{a}	1.5±0.15^{a}	1.5±0.15^{a}	1.8±0.00^{a}	1.8±0.00^{a}	1.8±0.00^{a}
BZ 0.75	1.4±0.00^{a}	1.4±0.00^{a}	1.4±0.00^{a}	1.4±0.00^{a}	1.4±0.00^{a}	1.4±0.00^{a}	1.5±0.15^{a}	1.5±0.15^{a}	1.5±0.15^{a}
BZ 1.00	1.1±0.15^{a}	1.1±0.15^{a}	1.1±0.15^{a}	1.1±0.15^{a}	1.1±0.15^{a}	1.1±0.15^{a}	1.1±0.15^{a}	1.1±0.15^{a}	1.1±0.15^{a}
CONTROL	3.2±0.00^{a}	3.2±0.00^{a}	3.2±0.00^{a}	3.2±0.00^{a}	3.2±0.00^{a}	3.3±0.15^{a,b}	3.3±0.15^{a,b}	3.5±0.15^{b,c}	3.6±0.00^{b,c}

*Results represent Mean ± Standard Error Mean of triplicate determinations. Results with the same superscript on the same row are not significantly different

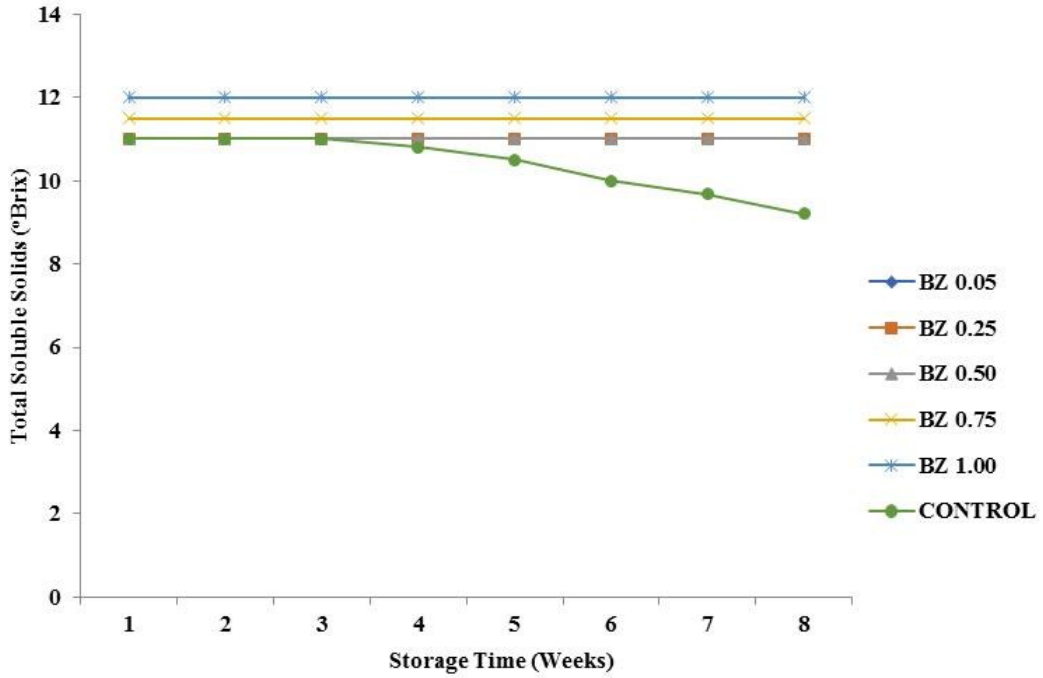

Figure 3: Effects of the preservatives on the Total Soluble Solids (^{o}Brix) of the drink

Table 4: The Effect of Sodium Benzoate on Vitamin C (mg/100ml) Content of '*Zobo*' Drink samples during storage

	SAMPLES					
WEEK	Bz 0.05	Bz 0.25	Bz 0.50	Bz 0.75	Bz 1.00	CONTROL
0	640.43±42.73^{b}	615.77±24.67^{c}	591.10±0.00^{b}	615.77±24.67^{b}	615.76±24.67^{b}	739.20±0.00^{c}
1	591.10±0.00^{b}	591.10±0.00^{c}	591.10±0.00^{b}	591.10±0.00^{b}	591.10±0.00^{b}	739.20±0.00^{c}
2	591.10±0.00^{b}	591.10±0.00^{c}	591.10±0.00^{b}	615.77±24.67^{b}	615.77±24.67^{b}	689.83±49.37^{c}
3	591.10±0.00^{b}	591.10±0.00^{c}	591.10±0.00^{b}	591.10±0.00^{b}	591.10±0.00^{b}	615.77±24.67^{b}
4	615.77±24.67^{b}	591.10±0.00^{c}	591.10±0.00^{b}	615.77±24.67^{b}	591.10±0.00^{b}	615.77±24.67^{b}
5	615.77±24.67^{b}	591.10±0.00^{c}	591.10±0.00^{b}	591.10±0.00^{b}	591.10±0.00^{b}	591.10±0.00^{b}
6	492.40±24.70^{a}	443.00±0.00^{b}	368.90±0.00^{a}	323.97±24.93^{a}	343.97±24.93^{a}	294.10±0.00^{a}
7	467.70±24.70^{a}	443.00±0.00^{b}	368.90±0.00^{a}	343.97±24.93^{a}	319.03±24.93^{a}	269.63±24.47^{a}
8	467.70±24.70^{a}	393.30±24.85^{a}	368.90±0.00^{a}	343.97±24.93^{a}	294.10±0.00^{a}	294.10±0.00^{a}

*Results represent Mean ± Standard Error Mean of triplicate determinations. Results with the same superscript on the same column are not significantly different at ($p \leq 0.05$)

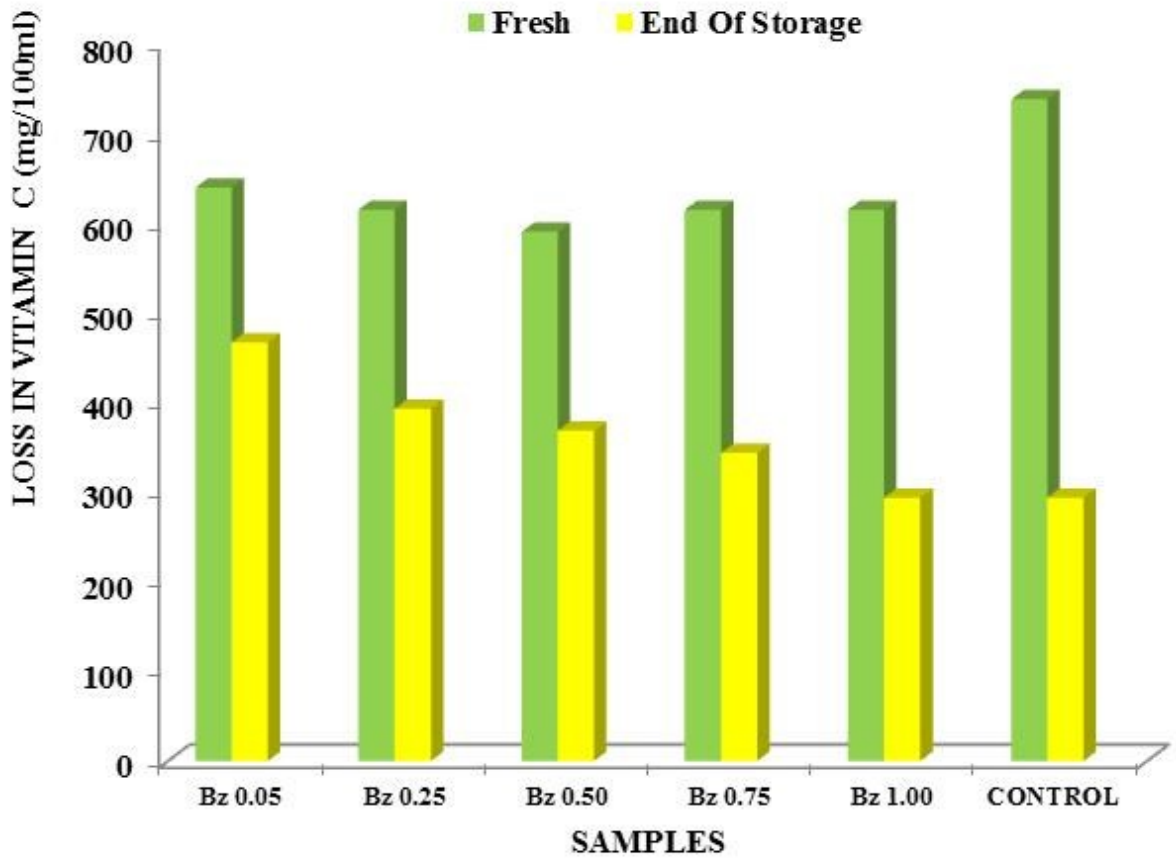

Figure 4: Loss in the Vitamin C Content (mg/100ml) of '*Zobo*' Drink Samples with Increase in Storage Period

pH or the preservative); and as such took some time to recover; or that they were still in their exponential phases (Willey *et al.,* 2011).

Total Soluble Solids (TSS)

The Total Soluble Solids (TSS) obtained from the six '*Zobo*' samples during the eight weeks of shelf study is presented in Figure 3. All samples preserved with sodium benzoate recorded no significant increase or decrease in their TSS. This could be due to the absence of microorganisms, which would have utilized these sugars (Egbere *et al*., 2007). However, the steady decrease in TSS observed in the control revealed that the drop was significantly different ($p<0.05$) from the initial 11.0 ^{o}Brix obtained at week zero. The drop may be ascribed to the activities of the spoilage microorganisms (*Lactobacillus fermentum* and *Saccharomyces cerevisiae*) present in the drink. This agrees with the findings by Damisa *et al.* (2007), whose work revealed that the decrease in the TSS with an increase in storage period in *Zobo* drink samples analysed was because sugars are utilized by microorganisms via fermentation for acid production (Egbere *et al*., 2007).

Vitamin C Content

The impact of combination of pasteurization and sodium benzoate on the vitamin C content of *Zobo* drink at varied concentration is presented in Table 4. The vitamin C content of the beverage ranged from 739.20 (control) to 591.10 mg/100 mL**.** The sharp decrease in the vitamin C content observed for all the six samples from the sixth week was significantly different ($p<0.05$) from the ones observed at the beginning of product storage (Table 4). The impact of the preservative on the vitamin C content of the drink was obvious in all the samples preserved with sodium benzoate. This was because microorganisms were absent in all the samples throughout the storage period.

The decrease in the vitamin C content observed in the control sample may be attributable to the presence of microorganisms whose metabolic activities depleted it. Vitamin C breaks down in the presence of oxygen (Lima *et al*., 2009). This was evidenced by the fact that microorganisms were isolated from these samples on the same week the vitamin C content of these samples recorded a significant decrease ($p<0.05$). The decrease recorded was however more pronounced when compared with the vitamin C decrease due to the stress from the preservatives.

The loss in the vitamin C content of the beverage as the storage period progressed is presented in Figure 3. This reveals that organic salt preservatives significantly reduced the vitamin C content of the beverage after preparation by 10% to 20% compared to that of the control (pasteurized) samples (Figure 4). However, sample Bz 0.05 was highest in ascorbic acid content at the end of the study. This loss underpins the fact that vitamin C content of any sample can easily be lost during such processes as grinding, cooking, contact with heat, chemicals, oxygen and other production processes (Singh, 2004; Wardlaw and Kessel, 2002). Despite the losses encountered, they still met the recommended Daily Intake (RDI) of between 40mg/100ml for toddlers and 90mg/100ml for adult men.

CONCLUSION

This result has shown that pasteurization at 68 ^{o}C for 20 minutes alone can extend the shelf life of '*Zobo*' drink, on the shelf for five weeks without the addition of synthetic preservatives. Preservatives impart negative taste, flavour and very often alters the physicochemical properties of the drink but successfully eradicated all pathogenic microorganisms present. Although the five different concentrations of the preservative used in combination with pasteurization were effective in extending the shelf life of the beverage, sample Bz 0.05 preserved better than the other four concentrations without impacting negatively on the vitamin C content and other physicochemical properties of the drink. This result therefore confirms that sodium benzoate may be used to preserve *Zobo* at concentrations lower than 0.1%.

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