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Comparative Effects of Some Preservative Hurdles on the Quality of Zobo Drink Stored at Ambient Temperature

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Authors' contributions

This work was carried out in collaboration among all authors. Author CVC designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors OSB and AJC managed the analyses of the study. Author BJD managed the literature searches. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aim: Comparative Effects of Some Preservative Hurdles on the Quality of Zobo Stored at Ambient Temperature were assessed.
Place and Duration of Study: Department of Microbiology Federal University of Technology, Minna, Nigeria, between June 218 and January 2019.
Methodology: Fresh zobo drink samples were prepared from *Hibiscus sabdariffa* using modified methods of HACCP and Hurdle technology for preservation and stored on the shelf for six months. The samples were divided into seven. Analyses were carried out on monthly basis with respect to microbial quality, pH, titratable acidity (TTA), total soluble solids (TSS), vitamin C content and sensory qualities of the beverage for six months. The parameters changed significantly ($p<0.05$)

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with respect to storage period. Zero microbial count was recorded for all the samples as at the time of production.
Results: The control sample deteriorated after one month, pasteurization at 75°C for 20 minutes successfully eradicated all coliforms and indicator organisms as none was isolated during the shelf study. *Bacillus subtilis, Lactobacillus fermentum, Aspergillus niger, penicillium sp, Saccharomyces cerevisiae* isolated from the both the control and pasteurized ($G_{control}$ and G_2) samples were responsible for the spoilage of the beverage after one month. G_3, G_4 G_5, G_6 and G_7 preserved beyond six months, without imparting negatively on the sensory qualities of the drink. They significantly ($p<0.05$) showed the same overall acceptability, mouthfeel, flavour, colour and taste.
Conclusion: Sample G_3 stored best after six months on the shelf.

Keywords: Microbiological; sensory properties; carbonation; pasteurization.

1. INTRODUCTION

'Zobo' drink also known as 'Sorrel' drink is a non-alcoholic beverage produced from the dried dark red calyces of the matured Hibiscus sabdariffa flower by boiling and filtration of dried calyces of the plant [1]. It is one of the numerous locally made Nigerian beverages, which are nutritious than most imported soft drinks. The beverage is known to be rich in vitamin C. Its phytochemical properties show that it is rich in anthraquinones, glycosides, alkaloids, tannins, polyphenols and saponins [1,2,3]. The drink is also of high medicinal value and has been used as antihypertensive, astringent, diuretic and purgative agents, which translates to its numerous health benefits [2]. In a study carried out by Nwachukwu et al. [1], Sorrel drink was observed to be more effective antihypertensive agent than the conventional hydrochlorothiazide (HCTZ), a diuretic widely used in the treatment of hypertension in mild to hypertensive Nigerians. Sorrel drink therapy showed a higher therapeutic effectiveness and longer duration of action without causing any electrolyte imbalance unlike the HCTZ [1].

However, Sorrel drink as well as other locally made beverages encounter similar challenges of abridged shelf lives, attributed to the crude and poor-sanitary methods of processing [2]. This deterioration might also be as a result of the additives used during preparation, such as sugar, sweeteners, flavourings or colourants [2,4]. Sorrel drink has a limited shelf life of about 2-3 days. Codina et al. [5] reported that Tigernut juice, a traditional beverage normally shows signs of deterioration within 24 hrs of production due to microbial proliferation. In a bid for the producers to overcome these challenges, local producers resort to refrigeration as a means of prolonging the shelf life.

These efforts are however adveserly affected by the near absence and epileptic public electricity power supply used to power home appliances including refrigerators. This had made the preservation of such beverages in Nigeria difficult limiting the shelf life of many of such beverages to just few days [2]. Research into the shelf-stability of these products without refrigeration has therefore intensified. The aim of this research was to compare the effects of some preservative hurdles on the quality of Zobo drink stored at ambient temperature.

2. MATERIALS AND METHODS

2.1 Sample Collection

Dried petals of Hibiscus sabdariffa, granulated sugar and fresh pineapple fruit were purchased from a local market (Kure Market, Minna) in Niger State. Preliminary microbial assessments processing, packaging though not reported here were carried out. Evaluation of the samples used for experimentation was carried out under strict and standard aseptic conditions in the microbiology laboratory of the Federal University of Technology, Minna, Nigeria.

2.2 Laboratory Preparation of Zobo Drink

Zobo drink was aseptically prepared according to the methods described by Egbere [4]. Six hundred grams of the dried calyces was sorted out, washed in sterile water and put into boiling water (15 litres) at 97± 3°C for five minutes. The liquid extracts were filtered using a clean sterile muslin cloth. The filtrate was sweetened with sugar syrup (200 g sugar in 200 ML boiled water). The beverage was flavored with freshly prepared pineapple Juice as shown in Fig. 1 and prepared for analyses as described in Table 1. The prepared samples were aseptically

dispensed in sterile glass bottles, corked with sterile crown caps and stored on the shelf at ambient temperature (30±3°C) for six months.

2.3 Microbiological Analyses

Total coliform count (TCC) was determined using the most probable number (MPN) method. The plates were incubated at 37°C for 24 hrs as described by FSSAI [6]. Escherichia coli count (ECC) using pour plate method in Eosin methylene blue agar (EMB) incubated at 37°C for 24 hrs as described by FSSAI [6].

Total plate count was determined using spread Plate method (using appropriate serial dilutions in peptone water) on duplicate plate count agar incubated at 37°C for 24 hrs as described by FSSAI [6]; Bacteria colonies with distinct characteristics were sub cultured in nutrient agar [7] and identified using standard methods [8,9].

Total fungal count (TFC) was determined using pour plate method in acidified malt extract agar and incubated at ambient temperature for 72 hrs. Growths were calculated and expressed as colony forming units per milliliter (cfu/ml). Discrete colonies were thereafter aseptically picked and stained with lactophenol cotton blue solution on a microscope slide and examined [7] and then identified [8].

2.4 Physicochemical Analysis

pH of Zobo drink was determined using pH meter (Jenway model 302) after standardizing with phosphate buffer at pH 4 [10]. Titratable acidity (TTA) was determined by titrating 0.10 M sodium hydroxide (NaOH) against Zobo drink (10 ml) using phenolphthalein as indicator [10]. Titratable acidity was expressed as percentage lactic acid. Total carbohydrate content of Zobo drink sample was determined according to AOAC [10]. Vitamin C content was estimated by titrating 2,6-dichlorophenolindophenol against Zobo drink (5 ml). Samples were treated with glacial acetic acid [11]. Total soluble solids content was determined at 29 ± 2°C using Abbe hand refractometer (Atago Co. Ltd, Japan). Percentage total soluble solids content was calculated as sucrose, using sucrose conversion Table corrected to 20°C [11].

2.5 Sensory Evaluation

Sensory quality evaluation was carried out using a 9-point hedonic scale (1 - 9). The parameters evaluated were colour, mouth-feel, taste, aroma and overall acceptability according to Onwuka [12]. A 10-member trained panel was used to evaluate the samples.

2.6 Statistical Data Analysis

All experiments were replicated thrice and data obtained were subjected to statistical analysis of mean, standard error and analysis of variance (ANOVA) using the methods of Onwuka [12]. The significant values were determined using the IBM Statistical Package for Social Science (SPSS) version 20 at the Degree of Freedom, $P < 0.05$. Statistical differences between means were compared using paired Duncan HSD. Differences in means were considered statistically significant at $p < 0.05$.

3. RESULTS

3.1 Microbial Quality Assessment

The results of the microbial analyses of Zobo beverages are shown in Tables 2 and 3. The results showed that no microbial isolate was detected in all the seven samples after packaging (zero day of storage). The results for the coliform count (TCC) and that of the E. coli count (EC) also showed no traces of microbial growth throughout the six months of storage. The results for the total plate count (Table 2) showed that samples G_3, G_4 G_5, G_6 and G_7 had no microbial growth throughout the six months of storage. However, microbial growths were observed in samples $G_{control}$ and G_2 on the first and third months respectively. The growths increased steadily till the last month of storage. Similar results were recorded for the total fungal count (Table 3). The Bacteria isolates identified from these two samples during the course of the study were *Bacillus subtilis, Lactobacillus fermentum, Aspergillus niger, penicillium sp, Saccharomyces cerevisiae.*

3.2 Physicochemical Properties

The pH of the Zobo samples is presented in Table 4. Samples $G_{control}$ and G_2 recorded the highest pH, which were significantly the same ($p<0.05$) but were significantly different from the rest of the samples. There were no significant differences ($p<0.05$) recorded for each of the samples G_3, G_4 G_5, G_6 and G_7 throughout the six months of storage. However, samples $G_{control}$ and G_2 recorded significant drop in their pH values as storage period progressed.

Table 1. Sample description

Samples	
$G_{control}$	Zobo without treatment (control)
G_2	Zobo + pasteurization only
G_3	Zobo + carbonation
G_4	Zobo + Pasteurization +carbonation
G_5	Zobo + Pasteurization +carbonation + Sodium benzoate
G_6	Zobo + Pasteurization +carbonation + Potassium sorbate
G_7	Zobo +Pasteurization + carbonation + Sodium benzoate + Potassium sorbate

Table 2. Total bacterial count (CFU/ml) on sorrel samples

Samples	Month						
	0	1	2	3	4	5	6
$G_{control}$	$<1.0\times10^1$	2.1×10^2	1.45×10^3	4.7×10^6	1.04×10^8	9.3×10^7	6.9×10^7
G_2	$<1.0\times10^1$	$<1.0\times10^1$	$<1.0\times10^1$	6.4×10^3	1.8×10^4	5.5×10^6	4.7×10^8
G_3	$<1.0\times10^1$	$<1.0\times10^1$	$<1.0\times10^1$	$<1.0\times10^1$	$<1.0\times10^1$	$<1.0\times10^1$	$<1.0\times10^1$
G_4	$<1.0\times10^1$	$<1.0\times10^1$	$<1.0\times10^1$	$<1.0\times10^1$	$<1.0\times10^1$	$<1.0\times10^1$	$<1.0\times10^1$
G_5	$<1.0\times10^1$	$<1.0\times10^1$	$<1.0\times10^1$	$<1.0\times10^1$	$<1.0\times10^1$	$<1.0\times10^1$	$<1.0\times10^1$
G_6	$<1.0\times10^1$	$<1.0\times10^1$	$<1.0\times10^1$	$<1.0\times10^1$	$<1.0\times10^1$	$<1.0\times10^1$	$<1.0\times10^1$
G_7	$<1.0\times10^1$	$<1.0\times10^1$	$<1.0\times10^1$	$<1.0\times10^1$	$<1.0\times10^1$	$<1.0\times10^1$	$<1.0\times10^1$

Table 3. Total fungal count (CFU/ml) on sorrel samples

Samples	Month						
	0	1	2	3	4	5	6
$G_{control}$	$<1.0\times10^1$	4.5×10^1	5.6×10^2	9.6×10^2	4.3×10^3	9.3×10^3	7.8×10^4
G_2	$<1.0\times10^1$	$<1.0\times10^1$	$<1.0\times10^1$	1.4×10^2	7.0×10^2	1.3×10^3	7.9×10^3
G_3	$<1.0\times10^1$	$<1.0\times10^1$	$<1.0\times10^1$	$<1.0\times10^1$	$<1.0\times10^1$	$<1.0\times10^1$	$<1.0\times10^1$
G_4	$<1.0\times10^1$	$<1.0\times10^1$	$<1.0\times10^1$	$<1.0\times10^1$	$<1.0\times10^1$	$<1.0\times10^1$	$<1.0\times10^1$
G_5	$<1.0\times10^1$	$<1.0\times10^1$	$<1.0\times10^1$	$<1.0\times10^1$	$<1.0\times10^1$	$<1.0\times10^1$	$<1.0\times10^1$
G_6	$<1.0\times10^1$	$<1.0\times10^1$	$<1.0\times10^1$	$<1.0\times10^1$	$<1.0\times10^1$	$<1.0\times10^1$	$<1.0\times10^1$
G_7	$<1.0\times10^1$	$<1.0\times10^1$	$<1.0\times10^1$	$<1.0\times10^1$	$<1.0\times10^1$	$<1.0\times10^1$	$<1.0\times10^1$

Table 4. Effects of the combined hurdles on the Hydrogen ion Concentration (pH) of Sorrel Drink

pH	Month						
	0	1	2	3	4	5	6
$G_{control}$	3.5 ± 0.07^c	3.5 ± 0.06^c	3.4 ± 0.03^c	3.3 ± 0.18^c	2.8 ± 0.12^b	2.3 ± 0.15^a	2.0 ± 0.18^a
G_2	3.5 ± 0.06^c	3.4 ± 0.03^c	3.4 ± 0.06^c	3.3 ± 0.09^c	2.9 ± 0.07^b	2.7 ± 0.06^b	2.2 ± 0.27^a
G_3	3.2 ± 0.03^a	3.1 ± 0.03^a	3.1 ± 0.00^a	3.0 ± 0.03^a	3.1 ± 0.03^a	3.0 ± 0.12^a	2.7 ± 0.53^a
G_4	3.2 ± 0.03^a	3.1 ± 0.06^a	3.1 ± 0.06^a	3.1 ± 0.12^a	3.0 ± 0.06^a	2.9 ± 0.03^a	3.0 ± 0.15^a
G_5	3.0 ± 0.12^a	3.1 ± 0.06^a	3.1 ± 0.21^a	3.0 ± 0.06^a	3.1 ± 0.12^a	3.0 ± 0.07^a	3.1 ± 0.06^a
G_6	3.1 ± 0.06^a	3.1 ± 0.03^a	3.1 ± 0.06^a	3.1 ± 0.00^a	3.1 ± 0.12^a	3.1 ± 0.06^a	$3.10\pm.10^a$
G_7	3.2 ± 0.06^a	3.2 ± 0.03^a	3.2 ± 0.06^a	3.2 ± 0.07^a	3.2 ± 0.09^a	3.1 ± 0.06^a	3.1 ± 0.03^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≤0.05)*

On the contrary, the TTA for samples $G_{control}$ and G_2 recorded significant increases from the first and third months respectively (Fig. 1) while the TTA for the rest of the samples remained significantly the same ($p<0.05$) throughout the storage period.

The result for the total soluble solids (TSS) is shown in Fig. 2. The results show that samples $G_{control}$ and G_2 had the least TTS. These were significantly ($p<0.05$) different from the rest of the samples (Fig. 2). Like the pH, samples $G_{control}$ and G_2 recorded significant drops in their TTS values as storage period progressed.

The impact of the different preservation hurdles used on the vitamin C content of the samples is shown in Fig. 3. There were significant differences in the vitamin C content of the samples analysed. The drop in the vitamin C content of samples $G_{control}$ and G_2 were more pronounced than those of the other samples. Sample $G_{control}$ had the highest vitamin C content as at the time of production which dropped significantly as the storage period progressed.

3.3 Sensory Evaluation

The sensory attributes of all the seven samples assessed showed that the sample G_3 scored the highest in colour, appearance, flavour, taste and in consistency (Fig. 4). However, the control sample ($G_{control}$) scored the least in the overall acceptability of all the samples at the end of the sixth month.

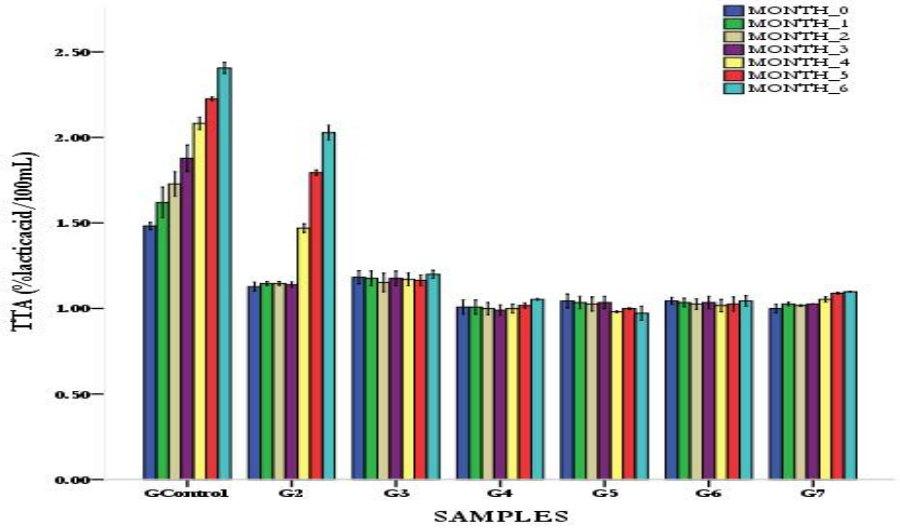

Fig. 1. Total titratable acidity of the Zobo samples during shelf storage

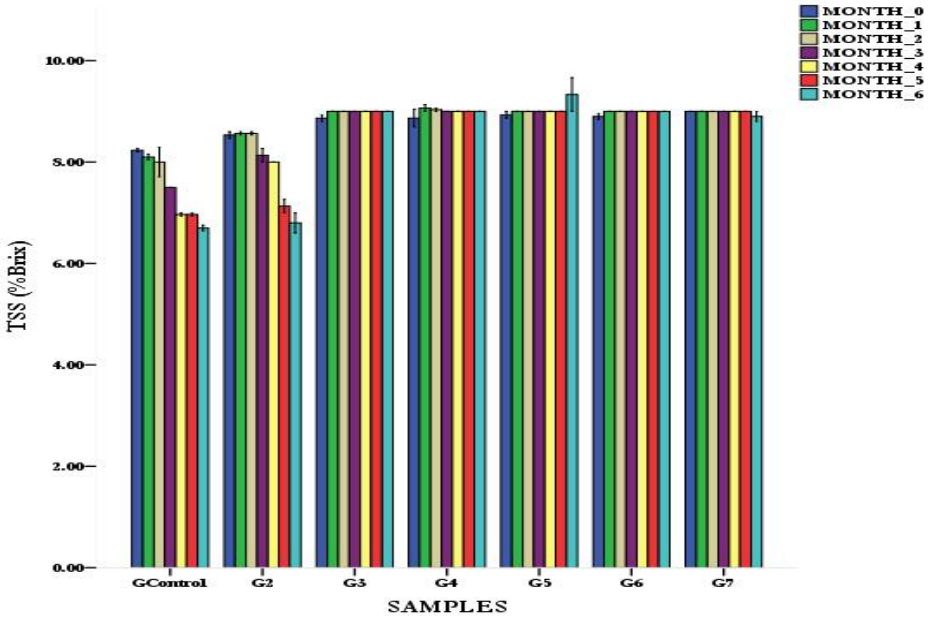

Fig. 2. Total Soluble Solids (^{o}Brix) of the Zobo samples during shelf storage

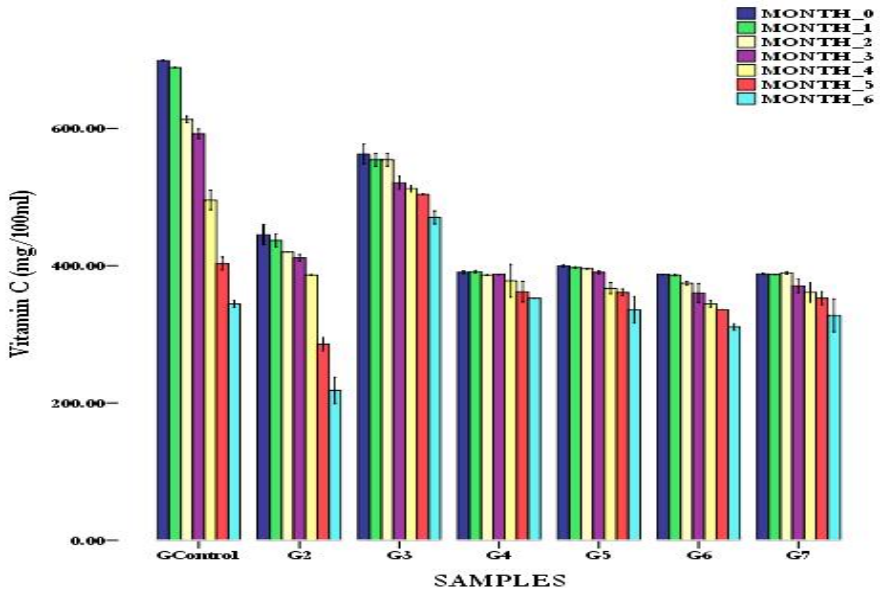

Fig. 3. Vitamin C content (mg/100ml) of the Zobo samples during shelf storage

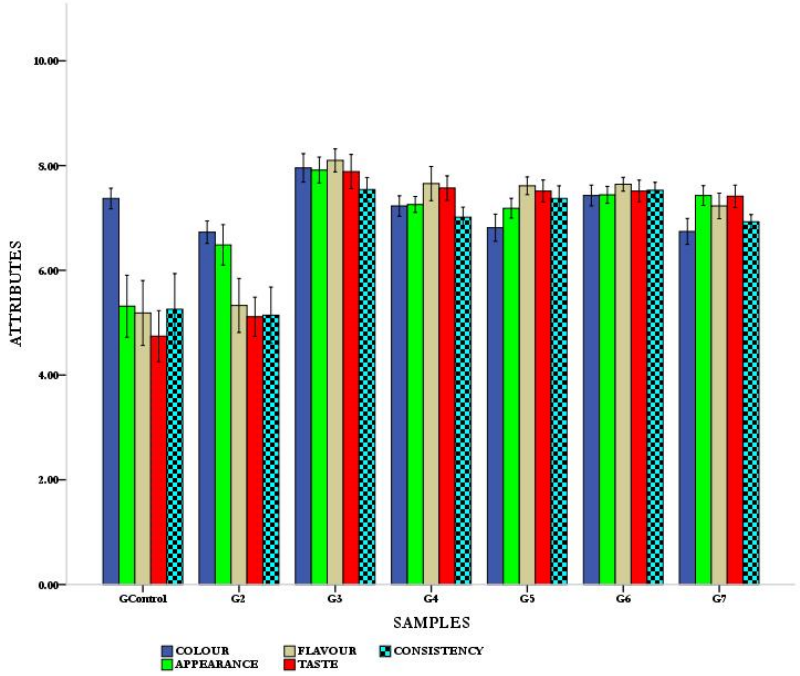

Fig. 4. Overall acceptability of the Zobo drink samples during shelf storage

4. DISCUSSION

The result of the microbial assessment of the samples emphasised the application of Hazards Analysis and Critical Control Point (HACCP) in food processing in order to prevent all forms of food contamination before, during and after production. HACCP application alongside the combination of other hurdles such as modified atmosphere packaging (Carbonation) added preservatives and heat treatment successfully eliminated all microorganisms present as at the

time of packaging and as well ensured the shelf stability of all the samples after the first month (Tables 2 and 3). In a similar report, Nwokocha et al. [13] attributed the zero microbial count observed to the combination of sanitary procedures used during the preparation of the Zobo beverage, the incorporation of natural plant extracts and their consequent pasteurization. The microbial load of the control sample and that of sample G_2 as seen in Tables 2 and 3, exceeded the microbial border limit of 10^5 for ready to eat foods on the third and fifth months respectively [14]. However, the zero microbial count recorded for all the carbonated samples showed the efficacy of the anaerobic condition created due to the modified atmosphere packaging and the inability of the spoilage organisms to withstand it, preventing their growth and proliferation in the beverage; this corresponds with the report of Juvonen [15], who stated that the spoilage organism present in the beverage must possess the ability to withstand the CO_2 present in the beverage.

The drop in pH recorded in the control and sample G_2 revealed the presence of acid producing organisms such as *Lactobacillus fermentum* and *Saccharomyces cerevisiae* responsible for the deterioration and the production of acid cum the alcoholic odour perceived from the spoilt samples. The findings were similar to that of Egbere et al. [4], who reported that the pattern was obviously due to the acid producing activities of spoilage bacteria isolated from deteriorating Zobo drink. In a similar study, Damisa et al. [16] attributed the significant decreases in the pH of the beverage during storage to the actions of various microorganisms, which might have survived the preservation hurdles.

The significant drop in TTA values recorded in the control as well as in sample G_2 showed the presence of acid producing organisms such as *Lactobacillus fermentum* and *Saccharomyces cerevisiae.* They were responsible for the deterioration and acid production cum alcoholic odour perceived from the spoilt samples. Similar findings have been reported by Damisa et al. [16], Nwafor and Ikenebomeh [17] and Egbere et al. [4], who attributed the lactic acid production and increase in TTA of the Zobo beverage as the storage period increased to the acid producing potentials of Zobo spoilage microorganisms present in the drink. Similarly, the steady decrease in TSS observed in the samples G_2 and the $G_{control}$ revealed that these drops were as activities of the spoilage microorganism such as *Lactobacillus fermentum* and *Saccharomyces cerevisiae.* These organisms utilize the sugars present in the beverage to carry out their alcoholic fermentation resulting to the decrease in the Brix content of the two samples. This result is similar to the findings of Kocher et al. [18], who reported that alcoholic fermentation carried out with *S. cerevisiae* strain revealed the decrease in Brix with time which was accompanied with an increase in ethanol content due to the consumption of sugar during the preparation and evaluation of red wine from Punjab purple variety of grapes.

The sharp decreases in the vitamin C content observed for all the seven samples were different from the ones observed initially at the beginning of product storage; this could be as a result of the combined activities of both the preservatives and the microbial flora isolated from the drink. The impact of the combined hurdles on vitamin C content was seen in all the samples and this went further to reveal that these hurdle treatments had negative effects on the vitamin C content of all samples with various preservative hurdles. Vitamin C content are easily denatured by the slightest stress encountered [19]. This was evidenced by the fact that microorganisms were totally absent in all these samples throughout the storage period yet, loss in vitamin C was recorded. This was similar to the observed decrease in vitamin C content as a result of the addition of organic acid preservatives in Zobo drink samples by Egbere et al. [4].

The sensory evaluation of the samples revealed that the carbonated samples had higher acceptability than the non-carbonated Zobo samples. The assessors observed that the carbonation of these beverages positively improved the taste and flavour of the beverages by imparting the fizzy taste on them. Similar results were obtained by Redondo et al. [20], who pointed out that carbonated carrot juice maintained a better taste by the impartation of a 'fizzy' taste to the juice. Redondo et al. [20] also pointed out that one of the sensory attributes of soft drinks is the impartation of a fizzy taste sensation when these beverages are consumed. This special fizzy taste sensation was the main reason for the wide acceptability of carbonated beverages over non-carbonated ones.

5. CONCLUSION

The study revealed that carbonation of Zobo drink enhanced the shelf stability of Zobo by

creating an anaerobic environment that prevented the proliferation of spoilage microorganisms which are predominantly aerobic. This study had also shown that the combination of different preservative hurdles such as carbonation, pasteurization and addition of preservatives at concentrations generally regarded as safe could prolong the shelf life of Zobo drink for a period of six months. Therefore, Zobo drink could be preserved for six months with carbonation alone without imparting negatively on the nutritional and sensory properties of the beverage.

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COMPETING INTERESTS

There are no competing interests exist regarding this work. This article solely belongs to the authors.

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