Original Research Article

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

ABSTRACT

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

Place and Duration of Study: Department of Microbiology at the 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 that combined the use of HACCP and Hurdle Technology for preservation and stored on the shelf for six months. The samples were categorized into Seven. Analyses were carried out on monthly basis with respect to Microbial quality, pH, TTA, TSS, Vitamin C content and Organoleptic properties of the beverage for six weeks. These parameters changed significantly ($p<0.05$) with respect to storage period. Zero microbial count was recorded for all the samples as at the time of production.

Results: The control deteriorated after one month, Pasteurization at 75^{o}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 Organoleptic properties of the drink. They recorded a significantly ($p<0.05$) the same overall acceptability, mouthfeel, flavour, colour and taste.

Conclusion: The sample that preserved best for these six months of shelf study is sample G_3

Keywords: Microbiological, Sensory properties, carbonation, Pasteurization

1. INTRODUCTION

Zobo drink is one of the numerous locally made Nigerian beverages, which are far more nutritious than most imported drinks, as most of these imported drinks have little or no food value. The beverage is known to be rich in Vitamin C, and its phytochemical properties reveals its richness in anthraquinones, glycosides, alkaloids, tannins, polyphenols and saponins. 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. 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 [3]. Sorrel drink has a limited shelf life of about 2-3 days and in a bid for the producers to overcome these challenges, resort to refrigeration as a means of prolonging their shelf life.

These efforts were however truncated by the near absence and epileptic public electricity power supply used to power home appliances including refrigerators, the preservation of such beverages in Nigeria has become horrendous and almost impossible beyond few days. This has propelled research into various methods by which these beverages can be preserved on the shelf with zero dependence on refrigeration as a means of preservation. The aim of this research was to evaluate the Evaluation of the Microbiological and Organoleptic Properties of Zobo Drink Preserved with Carbonation and Pasteurization.

2. MATERIAL 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. Processing, packaging and microbiological evaluation of the samples used for experimentation were 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] were carefully followed. Six hundred grams of the dried calyces was sorted out, washed in sterile water and boiled in water (15 litres) for five minutes. The liquid extracts were filtered immediately using a clean sterile muslin cloth. The filtrate was sweetened with sugar syrup, Flavoured with freshly prepared pineapple Juice (figure 1) and prepared for analyses as described in Table 1 below. The prepared samples were aseptically dispensed in sterile glass bottles, corked with sterile crown caps and stored on the shelf for six months.

2.3 Microbiological Analyses

Total Coliform Count (TCC) was determined using Most Probable Number (MPN) method and incubated at 37^{o}C for 24hrs as described by FSSAI [5] Escherichia coli Count (ECC) using Pour Plate method in Eosin methylene Blue agar incubated at 37^{o}C for 24hrs as described by FSSAI (2012);
Total Plate Count was determined using Spread Plate method (using appropriate serial dilutions in peptone water) on duplicate Plate Count Agar incubated at 37^{o}C for 24hrs as described by FSSAI [5]; Bacteria colonies with distinct characteristics were sub cultured in Nutrient Agar and identified using standard methods [6,7].

Table 1: Sample description:

SAMPLES	
Gcontrol	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

Total Fungal Count (TFC) were determined using Pour Plate method in acidified Malt Extract Agar and incubated at ambient temperature for 72hrs 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 Jenway model 302 pH meter after standardizing with phosphate buffer at pH 4 [9]. Titratable acidity (TA) was determined by titrating 0.10 M sodium hydroxide (NaOH) against 10 ml of zobo drink using phenolphthalein as indicator [9]. Titratable acidity was expressed as percentage lactic acid. Total carbohydrate content of zobo drink sample was determined according to Plummer [10] and Odibo *et al.* [11]. Protein content was estimated by the Lowry *et al*. [12] and Plummer [10] methods. Vitamin C content was estimated by titrating 2,6-dichlorophenolindophenol against 5 ml of zobo drink sample treated with glacial acetic acid [10, 9]. 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 [13].

2.5 Sensory evaluation

Organoleptic quality evaluation was carried out using the parameters of colour, Mouth-feel, taste, aroma and overall acceptability with a 9-point hedonic scale (1 - 9) according to Larmond [14]. A 10-member panel was used to score the various parameters.

2.6 Statistical Data Analysis

All experimental data will be subjected to statistical analysis of mean, standard error and analysis of variance (ANOVA) using the methods of Onwuka [15]. The significant values will be determined using the IBM Statistical Package for Social Science (SPSS) version 20 at the Degree of Freedom, $P < 0.05$. Statistical differences between means will be compared using paired Duncan HSD. Differences in means will be considered statistically significant at $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1 Microbial Quality Assessment

The results of the microbial analyses of Zobo beverages are shown in Tables 2 and 3. The microbial quality assessment showed that no microbial isolate was detected in all the seven samples after packaging. The results for the Coliform count (TCC) and that of the E. coli Count (EC) also showed no 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 which 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.*

Table 2: Total Bacterial Count (CFU/ml) on Sorrel Samples

	Month						
Samples	**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

	Month						
Samples	**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	$<1.0\times10^1$	$<1.0\times10^1$	$<1.0\times10^1$	$<1.0\times10^1$	$<1.0\times10^1$	$<1.0\times10^1$	$<1.0\times10^1$

3.2 Physicochemical Properties

The pH of the Zobo samples is presented in Table 4 below. 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.

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

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**
Gcontrol	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 ($p\leq0.05$).

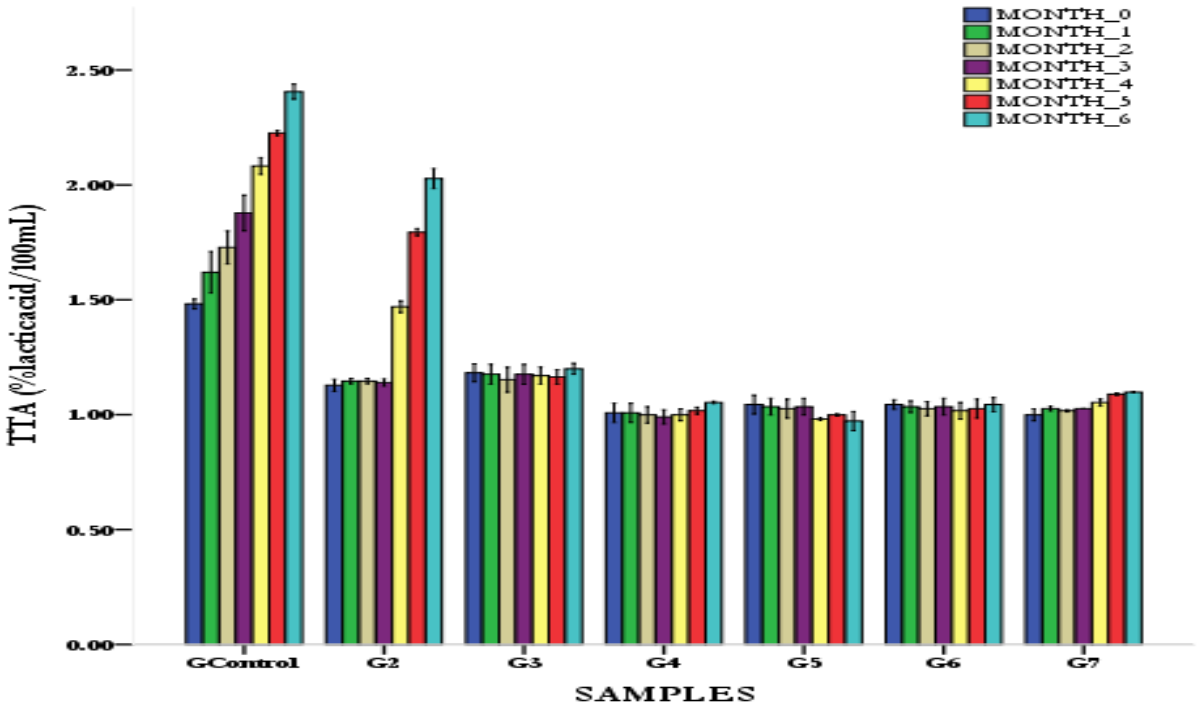
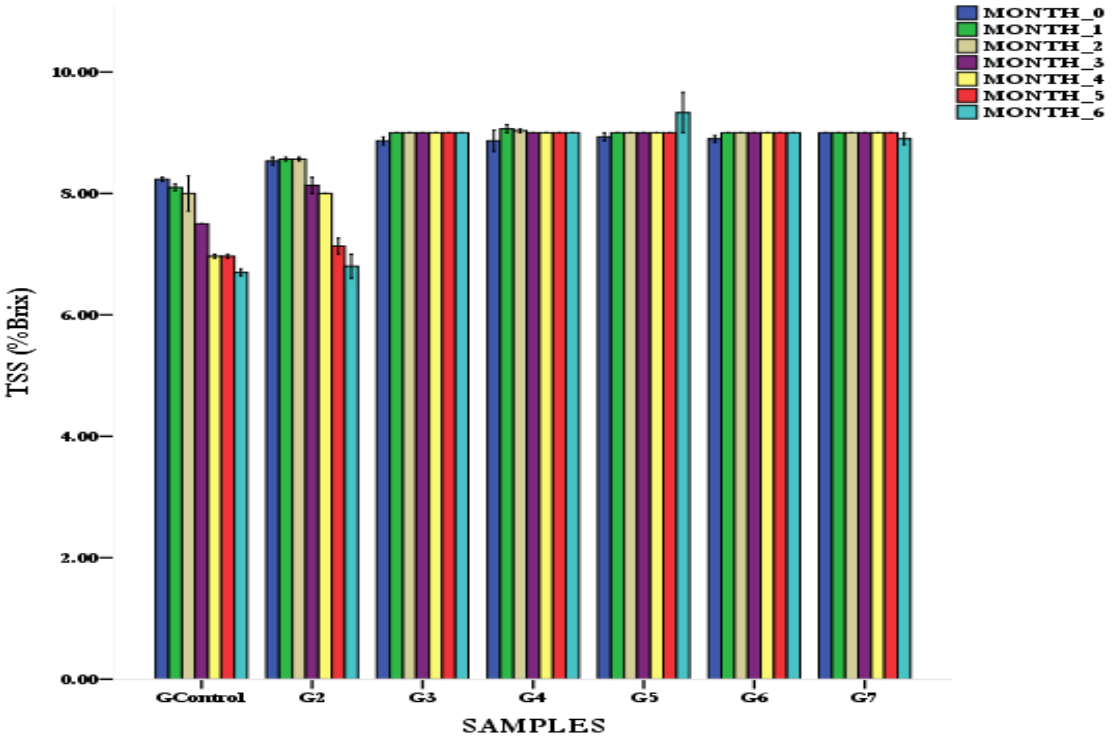


Figure 1: Total titratable acidity of the Zobo samples during shelf storage

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

The impart of the different preservation hurdles used on the Vitamin C content of the samples is shown in Figure 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.



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171
172 **Figure 2: Total Soluble Solids (°Brix) of the Zobo samples during shelf storage**
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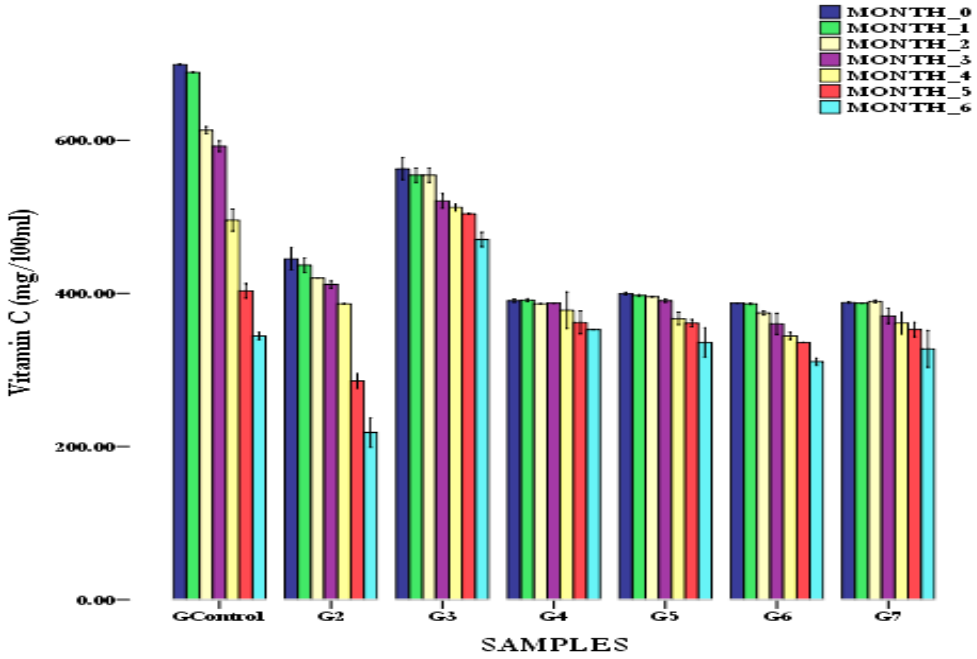

175
176 **Figure 3: Vitamin C content (mg/100ml) of the Zobo samples during shelf storage**

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 (Figure 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.

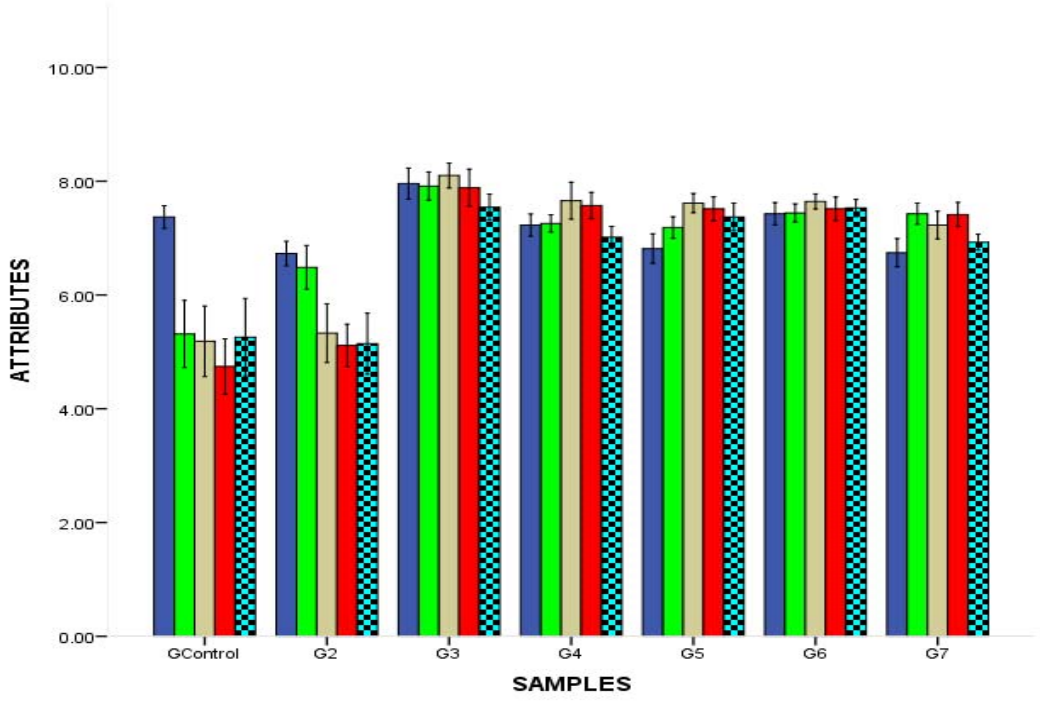

Figure 4: Overall Acceptability of the Zobo samples during shelf storage

4.0 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 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. In a similar report, Nwokocha *et al.,* [16] 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 exceeded the microbial border limit of 10^5 for ready to eat foods on the third and fifth months respectively [17]. 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, preventing the growth of spoilage organisms which are predominantly aerobic in nature Damisa *et al.,* [2].

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.* [2] 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.* [2], Nwafor and Ikenebomeh [18] 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 control revealed that these drops were as a result of the activities of the spoilage microorganisms as reported by Egbere *et al.,* [4].

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 the beverages are consumed. This special fizzy taste sensation was the main reason for the wide acceptability of all the carbonated beverages over non-carbonated ones.

5. CONCLUSION

The study revealed that carbonatation of Zobo drink enhances the shelf stability of Zobo by creating an anaerobic environment that prevents the proliferation of spoilage microorganisms which are predominantly aerobic. This study has also shown that the combination of different preservative hurdles such as Carbonation, Pasteurization and addition of preservatives at concentrations generally regarded as safe can prolong the shelf life of Zobo drink for a period of six months. Therefore, Zobo drink is can be preserved for six months with carbonation alone without imparting negatively on the nutritional and sensory properties of the beverage

COMPETING INTERESTS

Authors have declared that there are no competing interests exist regarding this work.

REFERENCES

1. Nwachukwu DC, Aneke E, Obika LFO and Nwachukwu NZ. Investigation of Antihypertensive Effectiveness and Tolerability of *Hibiscus Sabdariffa* in Mild to Moderate Hypertensive Subjects in Enugu, South-east, Nigeria. Ame J Phyt Clin Therapeutics. 2015;3(4):339-345

2. Damisa D, Ibrahim MB, Adewumi AA and Afiniki A. Effect of sodium benzoate and sodium metabisulphite on the shelf life of 'Zobo' drink. J Med Pharm Sci, 2007:3(1), 7 – 14.

3. Osuntogun B and Aboaba OO. Microbiological and physico-chemical evaluation of some non-alcoholic beverages. Pak J Nutr. 2004; 3(3):188-192.

4. Egbere OJ, Anuonye JC, Chollom PF and Okpara PV. Effects of some preservation techniques on the quality and storage stability of Zobo drink (A Nigerian, non-alcoholic beverage from Hibiscus sabdariffa). J Food Tech. 2007; 5 (3): 225-228.

5. Food Safety and Standards Authority of India. Microbiological methods. In: Manual on Methods of Analysis of Foods- Microbiological Testing, Laboratory Manual 14, FSSAI, Ministry of Health and Family Welfare. New Delhi, India. 2012.

6. Buchanan KE, Gibons NE. Bergey's manual of determinative bacteriology. (8th ed.) Williams and Williams company, Baltimoe. 1974.

7. Harigan WF, McCance ME. Laboratory methods in food and dairy microbiology. Academic Press London. 1976.

8. Barnett HL, Hunter BB. Illustrated general of imperfect fungi. (3rd edn). Burgress publishing company, New York; 1976.

9. AOAC. Official Method of Analysis. Association of Official Analytical Chemists (11th ed.). Washington DC; 2000.

10. Plummer DT. An introduction to practical Biochemistry. (2nd ed). McGraw-Hill Book Company, England; 1978.

11. Odibo FJC, Nwanunia E, Osigwe DI. Biochemical Changes during fermentation of Telfalria seeds for Ogi production. World J.Microbiol. Biotechnol. 1990;6: 425-427.

12. Lowry OH Nini JR, Farr AL. Protein measurement with Folinphenol reagent. J. Biological Chem. 1951; 193: 265-75.

13. Pearson D. The Chemical Analysis of Food (7th ed). Church-Hill Livingstone, New York. 1976.

14. Larmond EI. Laboratory methods for sensory evaluation of food.Food research institute, Ottawa, Canada Department of Agriculture; 1982.

15. Onwuka, GI. Sensory evaluation. In: Food Analysis: Instrumentation, Theory and Practice Lagos: Napthali prints; 2005.

16. Nwokocha JV, Okoronkwo NE, Eze SO, and Nwokocha NJ. Comparison of the preservative activity of alligator pepper and ginger extracts on Zobo Liquor during storage at ambient temperature; Acad Res Int. 2012; 2 (3):194 – 199.

17. Frazier WC and Westerhof, DC. Food Microbiology. New York, USA: McGraw Hill; 1988.

18. Nwafor OE, and Ikenebomeh MJ. (2009). Effect of different packaging materials on microbiological physicochemical and organoleptic quality of Zobo drink stored at room temperature. Afr J Bio. 2009; 8(12): 2848 – 2852.

19. Singh SP. Estimation of ascorbic acid (Vitamin C) in lemon juice. In Practical Manual of Biochemistry, 5th Edition New Delhi, India: CBS Publishers and Distributors; 2004.

20. Redondo N, Gomez-Martınez, S. and Marcos A. Sensory attributes of soft drinks and their influence on consumers' preferences. Food Fun. 2014; 5:1686–1694