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COMPARISM OF THE PRESERVATIVE ACTIVITY OF ALLIGATOR PEPPER AND GINGER EXTRACTS ON ZOBO LIQUOR DURING STORAGE AT AMBIENT TEMPERATURE

Nwokocha, J. V.
Abia State University
NIGERIA
victorjohn0@gmail.com

Okoronkwo, N.E.
Abia State University
NIGERIA
nnennaejijeokorkonkwo@yahoo.com

Eze, S. O.
Abia State University
NIGERIA
sundayoeze@yahoo.com

Nwokocha, N. J.
Abia State Polytechnic
NIGERIA
joy_nwokocha@yahoo.com

ABSTRACT

The preservative effect of the ethanol extracts alligator pepper and ginger on the shelf life duration of H. sabdariffa calyx liquor commonly known as 'zobo' was compared by introducing different concentrations of extracts into laboratory prepared zobo in order to arrest deterioration in physico-chemical and organoleptic quality due to microbial proliferation. The physico-chemical and microbial parameters were monitored during storage at ambient temperatures (27±3^{o}C) for four weeks. End-of-storage results showed that pH values ranged from 2.3 to 3.46. %TTA ranged from 0.0616 to 0.506%. Vitamin C values ranged from 2.93mg/100g to 19.36mg/100g. TVC (bacterial) values ranged from 2.59±0.19 log_{10}cfu/ml to 6.3±0.19 log_{10}cfu/ml. TVC (fungal values) ranged from 2.8log_{10}cfu/ml to 4.51log_{10}cfu/ml. The results also indicated the preservative activity of the incorporated extracts when compared to the control sample. There was, with respect to concentration of extract added, little significant difference in preservative effect between samples preserved with ginger and alligator pepper extracts.

Keywords: Zobo, H. sabdariffa, ginger, alligator pepper, food preservation

INTRODUCTION

In Nigeria's quest toward zero import dependence 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*. *Zobo* is the hot water extract of *H. sabdariffa* calyx and has been shown to possess medicinal/pharmaceutical properties including anti-diabetic, anti-hypertensive and anti-inflammatory properties (Osueke and Ehirim, 2004). Despite these obvious advantages, the leap from locally marketed product to commercial product 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.

A lot of research has been conducted using both natural (lime, ginger and trona) and synthetic preservatives (sodium benzoate) in the preservation of *zobo* liquor (Nwachukwu*et al.*, 2007; Ogiehor*et al.*, 2008; Onyeagba*et al*., 2004). Ginger extract (ethanol) has been shown to elongate the shelf life of *zobo* liquor to up to two weeks (Ogiehor*et al*., 2008) while lime has been shown to be active against a littany of bacteria present in *zobo* liquor (Nwachukwu*et al*., 2007). Alligator pepper (*Afromomummelegueta*Roscoe) is a member of the family zingiberaceae, to which ginger (*Zingiberofficinale)* belongs. According to Doherty *et al*. (2010), alligator pepper has been shown to possess anti-tumor, anti-proliferative, amphitensive, larvicidal and nematicidal properties due to its 6-gingerol, 6-paradol, shagaols and zingiberene content. It is also anti-inflammatory and cancer-inhibiting. Although it has been shown to possess antimicrobial activity against food microbes, there are relatively few literatures on its use in food preservation.

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The aim of this research therefore, is to evaluate the preservative efficacy of alligator pepper seed extract in comparism to that of ginger extract.

MATERIALS AND METHODS

Dried *H. sabdariffa* calyces were obtained from New Market in Aba, Abia State, Nigeria. Also ripe lime fruits and mature alligator pepper pods were obtained from the same market and identified. The calyxes were then inspected and sorted to remove sticks and stones which are commonly associated with the calyxes. The calyxes were then dried in a laboratory oven at 60^{o}C. After drying, they were ground in a laboratory grinder until a smooth powder was obtained in order to ensure high surface area for increased diffusion. 200g of the calyx powder was then added to one liter of hot boiling water and covered for 20 minutes. The filterate was obtained via sieving with a clean hand sieve while the residue was discarded. To the filterate, 100g of granulated sugar was added and then stirred to hasten dissolution. The resulting liquor was then filtered again to remove undissolved sugar crystals and particulate matter which had not been earlier removed. The filterate was then dispensed into factory sterilized 15ml plastic bottles.

The extracts were prepared according to the method prescribed by AOAC (1980). The alligator pepper pods were opened and the seeds removed and separated from the lint-like material in the pod. The seeds were then ground to a fine powder in a clean laboratory blender. Also, the ginger rhizomes were peeled, washed and dried in a laboratory oven at 85^{o}C for 10 hours. The product was then ground to a fine powder. 20g of the resulting powder was then weighed out and wrapped in five Whatman No.1 filter papers. Soxhlet extraction of the samples was accomplished and the products concentrated by evaporation over a monotherm at 65^{o}C for 10 minutes and filtered.

The 15 ml plastic bottles containing *zobo* liquor were divided into three main groups thus

a. Control (no treatment)
b. Zobo liquor + Ginger extract
c. Zobo liquor + Alligator pepper extract

Each group had subgroups each of 1%, 2% and 3% extract concentration.

The extracts were added by replacing the required volume of *zobo* liquor with the required volume of extract such that the volume of all the samples remained the same before and after extract addition. Eight samples were prepared per subgroup in order to serve the required analyses and maintain a reasonable margin for error.

After the various treatments, all the samples were pasteurized in a water bath set at 75^{o}C for 20 minutes. The samples were then stored at ambient temperatures (31+2^{o}C) for 42 days.

Chemical Analysis

The following analyses were carried out on the beverages already prepared on a weekly basis beginning from the time of preparation (Wk 0). The pH values of the samples were obtained using a pH meter (Hanna, England). The %total titrable acidity (expressed as lactic acid) was determined via the method of AOAC, (1980). Vitamin C content expressed in mg/100g and %Total Soluble Solids were determined via the method of AOAC (1984).

Microbial Analysis

The sample bottles were aseptically opened and 1ml aliquot collected by means of a micropipette and transferred into 9ml of sterile peptone water. Ten-fold serial dilution was accomplished and the appropriate dilution plated aseptically via spread plate technique on Mac-Conkey Agar (bacteria) and Saboraud Dextrose Agar (Fungi). The media were prepared, autoclaved and incubated strictly according to the manufacturer's instructions, after which the number of viable bacteria and fungi was counted, calculated and expressed as $\log_{10}$ colony forming units per ml (cfu/ml).

Data from the analyses above were analyzed using single variable analysis of variance (ANOVA) using Microsoft Excel 2007.

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RESULTS AND DISCUSSION

The following are the results of analyses carried out during the storage period. Figure1 shows the results of vitamin C analysis carried out during the period of the research. It shows the significant drop in Vitamin C content during storage in all samples especially in the control sample which has a more drastic rate of reduction than any of the other samples. The results also show that there was no significant difference between samples preserved with either ginger extracts or alligator pepper extracts. The total soluble solids and pH values also reduced during storage with the control value having the highest rate of reduction, with no significant difference observed between samples preserved with either of the two extracts. However, %Total titrable acidity increased during the period of storage again with control having the highest rate of increase, with no significant difference between samples preserved with the two extracts.

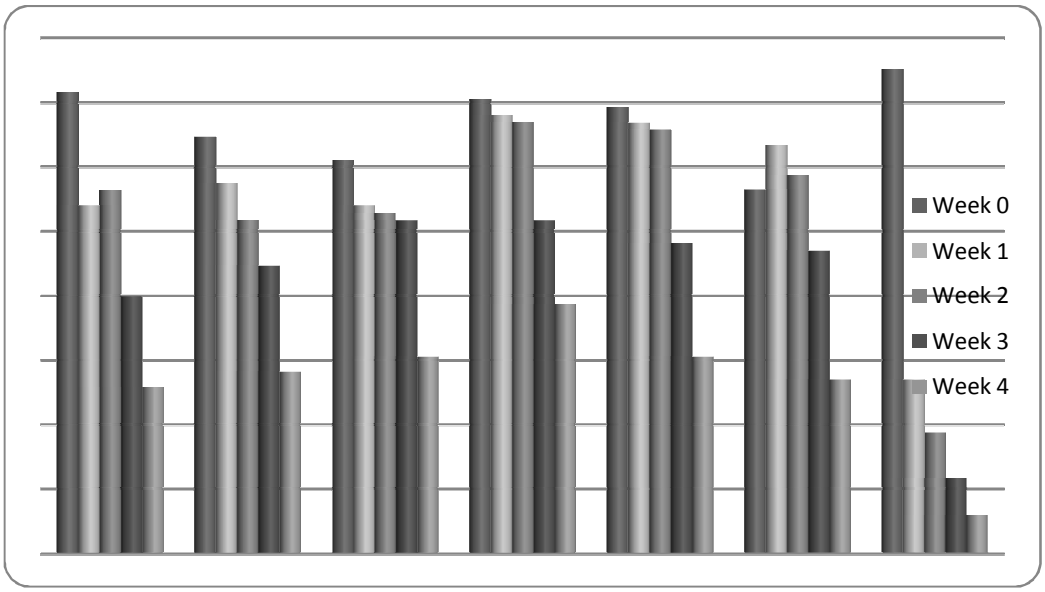

Figure 1.Variation of Vitamin C values during the storage period

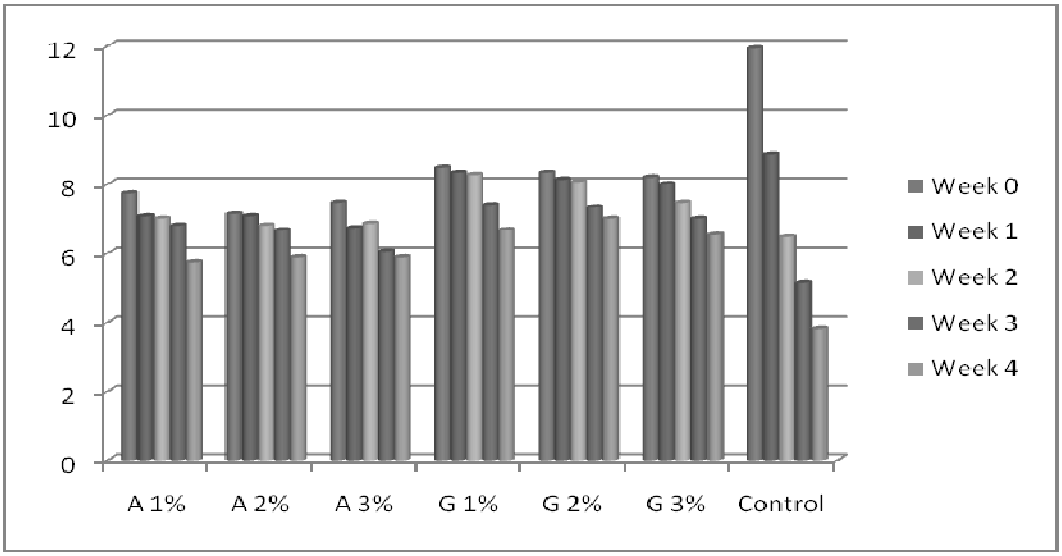

Figure 2.Variation of %Total Soluble Solids content during storage

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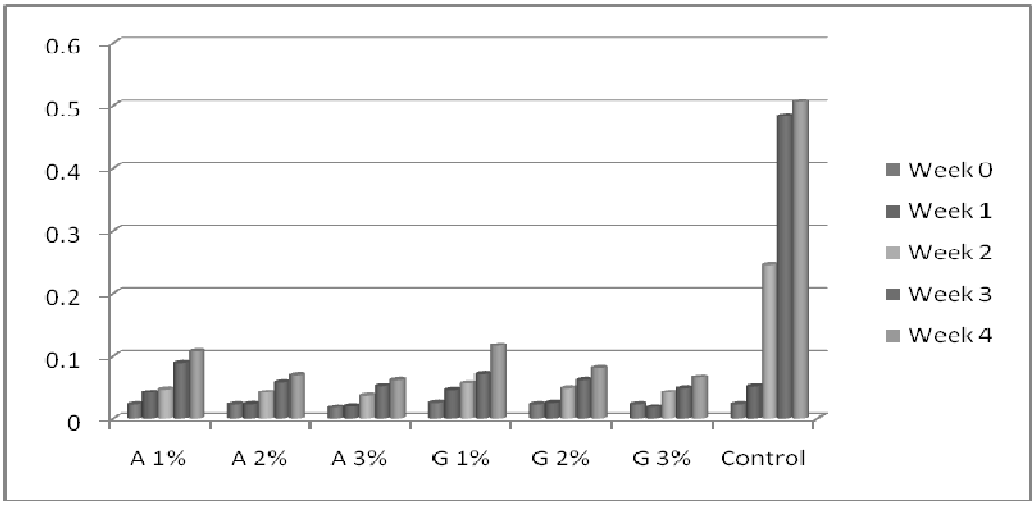

Figure 3.Variation of %TTA values for samples during storage

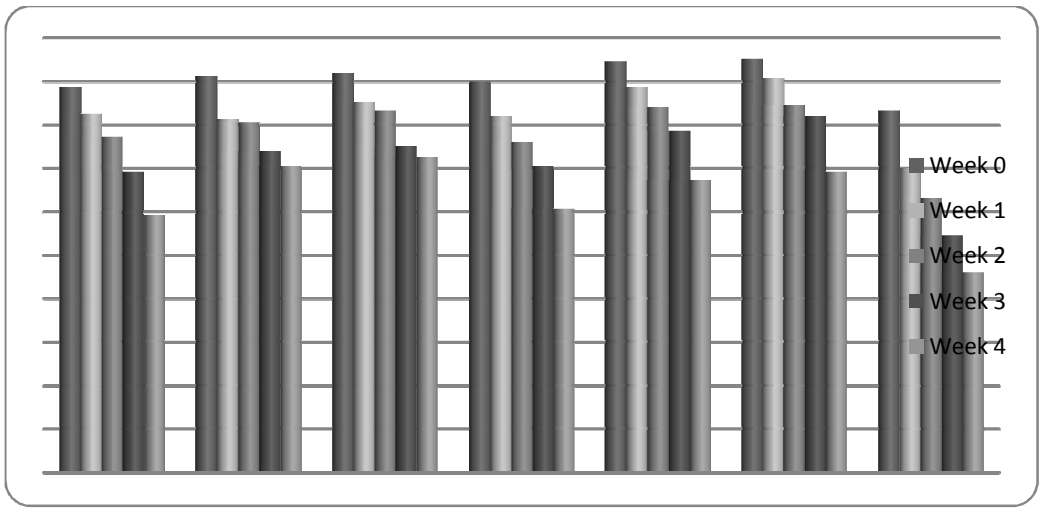

Figure 4.Variation of pH values in samples during the storage period

Microbial analysis, however, showed that for the first two weeks of storage at ambient temperature, no viable count was observed. This may be attributed to the combination of sanitary procedures used in the preparation of the *zobo* liquor, the incorporation of the extracts which served as preservatives and the consequent pasteurization at 75°C for 20 minutes. The microbes, could not overcome the hurdles or obstacles to microbial proliferation until the third week of storage when both bacterial and fungal counts increased in samples preserved with 1% and 2% concentration of extracts respectively while those with 3% extracts showed no growth. In the fourth week, a logarithmic increase in microbial count was observed.

Only the control sample and 1% alligator pepper treated sample significantly went beyond the FAO critical microbial count for ready to eat foods (10^4 cfu/ml).

From Tables 1 and 2, the results showed a significant difference in parameters from the first week of storage between the control and samples preserved with the extracts. The %TTA values showed no significant difference was observed between samples preserved with alligator pepper extract and ginger extract regardless of concentration although there was a significant increase during storage. This shows that the efficacy of the extracts in preventing acidification of the substrate due to microbial proliferation (eg*Lactobacillus acidophilus*) is similar. The pH values of samples preserved with similar quantities of the extracts were also similar although there was a significant decrease with respect to the Week 0 value. This observation agrees with research that %TTA and pH are inversely

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related (Nwafor and Ikenebomeh, 2009a,b; Egbere*et al.*, 2007). The %TSS values show a controlled reduction in preserved samples, as the control sample reduced drastically. This shows that the extracts were effective at preventing microbial decomposition of sugar crystals as %TSS can also be used a measure of the quantity of sugar present in a beverage. Also, there was no significant difference in samples preserved with either alligator pepper or ginger extracts. Vitamin C values are also similar and no significant difference occurs between samples preserved with either extract. However, the preserved samples show significant difference from the control sample as the reduction in vitamin C is not as drastic.

Table 1.TVC (bacteria) count (log_{10}cfu/ml)of samples during storage

Sample	*Week 0*	*Week 1*	*Week 2*	*Week 3*	*Week 4*
A 1%	NG	NG	NG	1.43±0.20[a]	3.63±0.10[a]
A 2%	NG	NG	NG	1.9±0.08[a]	3±0.10[a]
A 3%	NG	NG	NG	NG	2.9±0.13[a]
G 1%	NG	NG	NG	1.62±0.3	3.43±0.13[a]
G 2%	NG	NG	NG	NG	2.84±0.22[a]
G 3%	NG	NG	NG	NG	2.32±0.20[a]
Control	2±0.09	4.28±0.15	5.70±0.24	6.85±0.12[b]	6.3±0.19[b]

Values are means of three readings ± standard deviation
Means in same column not followed by the same letter are different at 5% level of significance

Table 2: TVC (fungal) count (log_{10}cfu/ml)of samples during storage

Sample	*Week 0*	*Week 1*	*Week 2*	*Week 3*	*Week 4*
A 1%	NG	NG	NG	2±0.09[a]	4.36±0.20[b]
A 2%	NG	NG	NG	2.3±0.12[a]	4.2±0.15[ab]
A 3%	NG	NG	NG	NG	3.72±0.12[ab]
G 1%	NG	NG	NG	2.3±0.20[a]	3.99±0.2[ab]
G 2%	NG	NG	NG	NG	2.8±0.2[a]
G 3%	NG	NG	NG	NG	2.82±0.2[a]
Control	1.6±0.18	3.95±0.39	5.63±0.22	5.90±0.13[b]	5.48±0.18[b]

Values are means of three readings ± standard deviation
Means in same column not followed by the same letter are different at 5% level of significance

CONCLUSION

The similarity between samples preserved with either of the two extracts can be traced to similarities in phytochemical constituents even though present in differing amounts. These phytochemicals include zingiberene, 6-gingerol, 6-paradol and shagaols. This means that preservation using alligator pepper and ginger extracts show similar effects. However, further research is necessary to investigate the possibility of a synergistic effect in preservation using a combination of the extracts. Also, further research is necessary on the effect of the above extracts on the organoleptic quality of *zobo* liquor.

REFERENCES

Aboaba O. O., Smith S. I. &Olude F. O. (2006). Antibacterial effect of edible plant extract on *Escherichia coli* O157:H7. *Pakistan Journal of Nutrition*, Volume 5 No. 4 pp 325 – 320.

Anosike C.A, Obidoa O., Ezeanyika L.U, &Nwuba M.M. (2009). Anti-inflammatory and anti-ulcerogenic activity of the ethanol extract of ginger (*Zingiberofficinale). African Journal of Biochemistry. Research* Volume 3, No.1 pp 379 – 384

AOAC (1980). *Official Methods of Food Analysis.Association of Official Analytical Chemists* (10th edition). Washington D.C.

AOAC (1984). *Determination of the quantity of soluble solids.Association of Official Analytical Chemists* (14th edition). Washington D.C.

Doherty V.F., Olaniran O.O &Kanife U.C. (2010). Antimicrobial activities of *Afromomummelegueta* (Alligator pepper). *International Journal of Biology*. Volume 2 No.2 pp 126 – 131

Egbere O.J, Anuonye J.C., Chollom P.F. &Okpara P.V. (2007). Effects of some preservation techniques on the quality and storage stability of zobo drink (A Nigerian, non-alcoholic beverage from (*Hibiscus sabdariffa*) Journal of Food Technology Volume 5 No.3 pp 225 – 228

Food and Agricultural Organization (1979). Manuals of Food Quality Control. FAO Food and Nutrition Paper, United Nations, Rome. Volume 14, No.4 A1 – F10

Nwachukwu, E., Onovo, O.M. &Ezema, C.F. (2007). Effect of lime juice on the bacterial quality of zobo drinks locally produced in Nigeria. *Res. J. Micr*. 2(10) 787-790

Nwafor, O. E. &Ikenebomeh, M. J. (2009a). Effect of Sodium Benzoate on the Growth and Enzyme Activity of *Aspergillusniger* and *Penicilliumcitrunum* on Zobo Drink During Storage at 30±2°C. *African Journal of Health Technology*.Volume 8.No. 12.pp 2843 – 2847.

Nwafor, O. E. &Ikenebomeh, M. J. (2009b). Effect of Different Packaging Materials on Microbiological Physicochemical and Organoleptic Quality of Zobo Drink Stored at Room Temperature. *African Journal of Health Technology*.Volume 8.No. 12.pp 2848 – 2852.

Ogiehor, S. I., Nwafor, O. E. &Owhe-Ureghe, U. B. (2008). Changes in the quality of zobo beverages produced from *Hibiscus sabdariffa*(Linn roselle) and the effects of extract of ginger alone or in combination with refrigeration. *African Journal of Biotechnology* Volume 7, No. 8 pp 1176 – 1180.

Okoli, C. O., Akah, S. V., Ihemelandu, U. U. &Amadife, C. (2007). Anti-inflammatory activity of seed extracts of *Afromomummelegueta*. *Journal of Herbs, Spices and Medicinal Plants.*Volume 13 Issue 1.

Omemu, A., Edema, M., Atayese, A &Obadina, A. (2006). A survey of the microflora of *Hibiscus sabdariffa* (Roselle) and the resulting zobo juice.*African Journal of Biotechnology*. Volume 5 No. 3 pp 254 – 259.

Onyeagba, R. A., Ugbogu, O. C. &Iroakazi, O. (2004). Studies on the antimicrobial effects of garlic (*Allium sativum*Linn), ginger (*Zingiberofficinale*Roscoe) and lime (*Citrus aurantifolia*Linn). *African Journal of Biotechnology*. Volume 5 No.3 pp 254 – 259.

Osueke , J. C. &Ehirim, F. N. (2004). Chemical, Nutritional and Sensory analysis of zobo drink (varsabdariffa) and selected soft drinks. *Journal of Agriculture and Food Science*. Volume 2, No. 1 pp 33 – 37.

Paine, F. A. (1992) .What is Shelf Life? *Food Technology International* Volume 21, No. 2 pp 234 – 235.