Journal of Food Technology 5 (3): 225-228, 2007

Effects of Some Preservation Techniques on the Quality and Storage Stability of Zobo Drink (A Nigerian, Non Alcoholic Beverage from *Hibiscus sabdariffa*)

[1]O.J. Egbere, [2]J.C. Anuonye, [1]P.F. Chollom and [1]P.V. Okpara
[1]Department of Microbiology, University of Jos PMB 2084, Jos Plateau State, Nigeria
[2]National Cereals Research Institute Badeggi, P.M.B. 8 Bida, Niger State, Nigeria

Abstract: The microbial and nutritional quality of laboratory produced Zobo drink (obtained from aqueous extract of dried calyces of *Hibiscus sabdariffa*) was investigated. The samples were subjected to pasteurization (at 70°C for 25 min) alone and with sodium benzoate (0.1%) in combination with pasteurization. Storage stability at room temperature (28±2°C) of aseptically packaged sachets of the beverage in 50 mL was assessed using physico-chemical parameters (loss in vitamin C content, changes in pH and titratable acidity) and microbial changes. The proximate composition of Zobo drink was 88.88% water, 0.046% protein, 0.15% fat, 0.61% crude fibre, 0.21% ash and 10.64% carbohydrate. These values did not change significantly ($p>0.05$) with respect to treatment methods nor storage time. However, vitamin C content of the beverage 1,105.88 mg 100 mL in the control samples decreased by 53.4 and 61.46% due to pasteurization and sodium-benzoate plus pasteurization respectively. The total plate count of the unpreserved beverage (control) decreased from 1.2×10^5 cfu mL^{-1} to 5.0×10^3 and 1.0×10^3 cfu mL^{-1} when subjected to pasteurization and to a combination of sodium benzoate plus pasteurization, respectively. The two methods were both effective in eliminating *E. coli* and *Staplyloccus aureus* in the beverage. *Bacillus* sp., *Lactobacillus* sp., *Saccharomyces*, *cerevisiae*, *Mucor* sp. and *Aspergillus niger* were found in association with progressive spoilage [detected in acid- and alcohol production] in the beverage. The shelf life of the beverage on storage was 4, 14 and above 40 days for the control, pasteurized only and sodium-benzoate plus pasteurized samples, respectively.

Key words: Zobo drink, stability, pasteurization, beverage, sodium benzoate

INTRODUCTION

The simplicity in the production, availability of raw plant materials and abject poverty in many rural communities as well as the new economy-revamping policies of the government has resulted in increased consumption and merchandise of many traditional foods at cottage levels in Nigeria. The consumption of alcoholic beverages could also be on the decrease in certain areas due to increased religious and health campaigns against such beverages. This has made Zobo drink a potential, ready local alternative to, alcoholic beverages in particular and imported red wines in general.

Zobo or *Yakwua* (Hausa) is an aqueous extract of the of the dried reddish-brown petals (calyces) of *Hibiscus sabdariffa* which is normally sweetened to taste with sugar and sometimes flavoured with spices like ginger hot pepper etc along with natural flavourants such as pineapple juice or lime juice or artificial flavourings like strawberry vanilla etc, depending on individual taste (Bola and Aboaba, 2004). Zobo is commonly found hawked around, packaged in transparent polythene sachets or plastic containers in most northern and some southern parts of Nigeria.

According to Bola and Aboaba (2004) the *H. sabdariffa* plant commonly known as Roselle is native to India and Malaysia where it is commonly cultivated and is now found in many tropical and subtropical countries of both hemispheres. It is a dicotyledonous plant belonging to the sub-class Archichlamydea, Order malvale and family, malvaceae (Onoja, 1996; Bola and Aboaba, 2004). In Nigeria, it is grown, commonly in the middle belt regions of Nigeria like plateau, Nasarawa and Benue States and south western states like Ondo and Osun. Phytochemical analysis of extracts of roselle showed that it contains anthraquinones, glycosides alkaloids, tannins, polyphenols and saponins (Onoja, 1996; Bola and Aboaba, 2004). The medicinal value of the plant has been claimed to include anti-hypertensive, antiseptic astringent diuretic and purgative activities remedy for cancer, abscesses, cough, debility, dysuria, laxative, scurvy and fever (Onoja, 1996).

Corresponding Author: O.J. Egbere, Department of Microbiology, University of Jos PMB 2084, Jos Plateau State, Nigeria

Among the many challenges of traditionally processed food products in Nigeria are that processing methods are crude, manual and of unsanitary conditions hence products are of unpredictable quality (Okafor, 1983). Many of the indigenously prepared foods have limited shelf-lives of about 2-3 days been susceptible to chance contaminating yeast, molds and bacteria which end up deteriorating the quality of the food. The overall objective of this research was therefore, to standardized Zobo processing and preservation. The specific objective of this present study was to assess the nutritional, microbiological and storage qualities of laboratory prepared Zobo drink following combinations of heat and chemical treatment.

MATERIALS AND METHODS

The fresh dried calyces of *Hibiscus sabdariffa* var *Sabdariffa* (dark red in colour) granulated sugar and straw berry flavour were purchased from Jos main market (Nigeria). Processing, packaging and microbiological evaluation of the samples used for experimentation were under strict and standard aseptic conditions as described by Cheesbrough.

Laboratory processing of Zobo drink: One hundred grams of the dried calyces were sorted out and rinsed under tap water and ground in a sterile laboratory blender to obtain a paste. Two litres of warm sterile water (at 60°C) was added to the paste and left for 1 h to enable extraction. The mixture was then filtered through a Muslim and then flavoured with sugar (200 g) and strawberry to taste.

Sample preparation for analyses: The bulk prepared drink was divided into three, designated as control (for non-treated samples), PO (for pasteurized samples only) and BOP (for samples treated with 0.1% sodium benzoate and pasteurization). Sodium benzoate treatment was according to the US Code of Regulations on chemical additives as reported by Frazier and Westhoff (1988).

Packaging: Fifty milliliters of the beverage drinks were aseptically dispensed into sterilized polythene sachets and then sealed back using a thermo-electric sealing machine to prevent contamination.

Pasteurization: The various samples for pasteurization were labeled accordingly and then immersed in sterile water contained in a water bath set at 70°C and left for 20 min. Thereafter the samples were immersed in sterile cold water to cool.

Analysis

Proximate analysis: The proximate composition (moisture content, fat, crude fibre and ash, contents) were determined following the methods described by the Association of Official Analytical Chemists (AOAC, 2000).

The pH values of the samples were determined using the laboratory pH meter (Jen Way 30/5/corning model 10) that has been previously standardized using buffer solutions of pH 4 and 7, respectively. The Total Titratable Acidity (TTA) calculated as lactic acid was determined as described by Pearson.

Vitamin C determination: The amount of vitamin contained in each sample was determined by the titrimetric method (using 2, 6-dichlorophenol indophenol dye) as described by Pearson.

Microbiological analyses: An aliquot of 1mL of the various serially diluted samples was placed each on Nutrient agar (for total bacterial load estimation) Potato Dextrose Agar (PDA) (for fungal isolation) according to Bradshaw (1979).

The nutrient agar plates were incubated at 37°C while the PDA plates were incubated at room temperature 28±2°C for 3-5 days. Pure cultures of the isolates morphological cultural and biochemical characteristics were established.

Storage studies: The different samples-control, Pasteurized (PO) and samples treated with sodium Benzoate Plus Pasteurization (BOP) were all kept at room temperature (28±2°C). They were then monitored periodically for changes in their microbial and physico-chemical characteristics.

RESULTS AND DISCUSSION

Nutritional quality of Zobo: The results (Table1) showed that Zobo drink has very low protein (0.046-0.463%) and fat (0.15-0.24%) content but high levels of dry matter (11.12-11.54%) content. The low protein value though a disadvantage to populations with protein malnutrition, it is on the other hand an ideal diet for a select people with liver problems (hepatic cirrhosis, hepatitis or hepatoma).

Table 1: Proximate composition (%) of control and experimental Zobo samples

Sample	Moisture	Protein	Fat	Ash	Fiber	Carbohydrate	Dry mater
Control	88.88	0.046	0.15	0.61	0.12	10.64	11.12
BOP_0	88.60	0.31	0.13	0.54	0.16	10.40	11.40
BOP_{40}	88.46	0.43	0.24	0.66	0.20	10.01	11.54

Key: BOPo-Sample on day zero. BOP_{40}-Sample on day 40

Table 2: Vitamin C content of control and experimental samples of Zobo

Sample	Vitamin C (mg 100 mL)	Decrease in vitamin C (%)
$Control_0$	1,105.88	N.a
$Control_4$	1,044.84	5.52
PO_0	515.23	53.41
PO_{14}	438.31	60.37
BOP_0	437.25	60.46
BOP_{40}	327.96	70.34

Key: The subscript denotions, 0, 4, 14 and 40 were for the respective days the sample were analysed for Vitamin C content

Table 3: Microbial isolates identified from the sample drinks

	Bacterial isolates		Fungal isolates	
Sample	First day of storage	End of storage (Spoilt sample)	First day of storage	End of storage (Spoilt sample)
Control	*Escherichia. Coli* sp., *Staphylococcus aureus* *Lactobacillus* sp., *Bacillus* sp.	*Lactobacillus* *Bacillus* sp.	*Saccharomyces cerevisiae*	*S. cerevisiae* *Aspergillusniger* *Mucor* sp.
PO	*Bacillus* sp., *Lactobacillus sp* *Lactobacillus* sp.	*Bacillus* sp., *Lactobacillus* sp. *Lactobacillus* sp.	No fungal isolate	*S. cerevisiae* *A. niger* *Mucor* sp.
BOP		*Bacillus* sp.	No fungal growth	*S. cerevisiae*

Key: PO: Pasteurized Only, BOP: Treated with Sodium Benzoate and Pasteurized

Table 4: Total plate count of control and treated samples of Zobo drink

Sample	Initial plate count (cfu mL^{-1})	D-value of microbial treatment	Final plate count (cfu mL^{-1})	%Decrease in plate count on storage	Shelf life* (days)
Control	1.2×0^5	Na	1.1×10^4	90. 83	3
PO	5.0×10^3	1.38	9.8×10^2	99.18	14
BOP	1.6×10^3	2.079	2.8×10^2	99.78	40+n

Key: PO: Pasteurized Only BOP: Pasteurized and treated with 0.1% sodium benzoate, Na: Not applicable, *Shelf-life: Determined by noting objectionable sensory features such as presence of gassy bubbles, cloudy appearance, alcoholic odour and over-soured taste., n: Number of days beyond the 40th day on storage of the cfu: Colony forming units

who need little or no proteins in their menu. Since, Zobo has been used as folk remedy of cancer its low protein content then makes the drink a possible ideal diet and therapy for liver cases. The low fat content of Zobo equally makes it a possible ideal drink for the obsess or those watching their weights. The high water content of the drink (88.60%) underpins its role in thirst-quenching characteristics for which it is known (Bola and Aboala, 2004). The vitamin C content (1105.88 mg 100 mL) is of great health significance (Table 2). However, pasteurization and sodium benzoate application resulted in significant reduction of vitamin C content (by 50-60%). Despite the losses in the vitamin, the residual amounts (437.24-515.23 mg 100 mL) were still appreciable quantities that still met the Recommended Daily Intake (RDI) value of 45 mg for a 70 kg adult human, the excess of which could be excreted from the body by urination (Fisher and Bender, 1985). Vitamin C loss due to storage was relatively

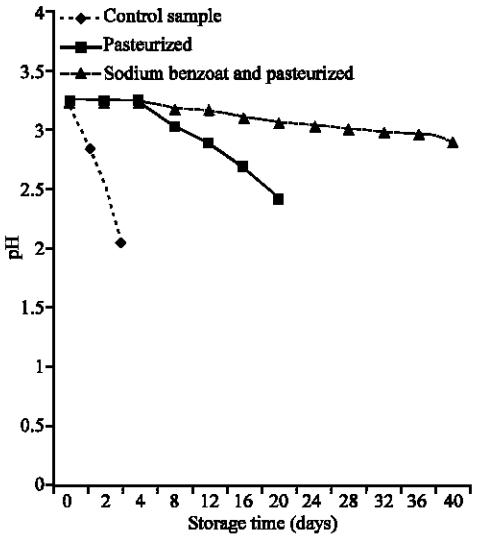

Fig. 1: Changes in pH profile of Zobo drinks with storage

minimal (5-10%) when compared to the losses due to the preservation techniques applied. Vitamin loss is known to increase with exposure to heat (as in pasteurization), light and oxygen (Cameroon, 1982). Although, the general fear on the consumption of Zobo has been its high acidic nature, it should however, be noted that the acidity is organic in nature and therefore less corrosive to the gastro intestinal tracts. However, person with peptic and gastric ulcers should be discouraged from high and frequent consumption of the beverage.

Physio-chemical quality: The pH value of the freshly prepared Zobo drink being 3.40 (Fig. 1) imply that it belongs to a class of foods referred to high acid foods (Frazier and Westhoff, 1988). An advantage of an acid food is that it does not support survival of many pathogenic organisms but favours the proliferation of acidic organisms. This explains the domination of *bacillus*, *Lactobacillus* sp. and *S. cerevisiae* in occurrence in the beverage (Table 3). The graphical representations of the pH and TTA of the beverage with respect to storage time indicated a general decrease in pH and corresponding increase in acidity. The pattern is obviously due to the acid producing activities of spoilage bacteria, *Lactobacillus* and *Bacillus* sp. (Table 2). The increase in acidity account largely for the decrease in microbial populations as indicated in Table 4.

Microbial quality: The microbial load of the control sample was at the border limit of (10^5 cfu mL^{-1}) expected for-ready to consume food products as specified by legislative organs (Frazier and Westhoff, 1988).

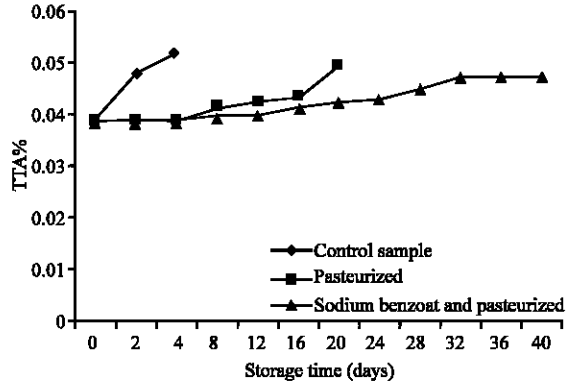

Fig. 2: Changes in Total Titratable Acidity (TTA) of sample drinks with storage time

However, pasteurization of 70°C for 25 min successfully reduced the microbial load, by a D-value of 1.38 (more than 90% reduction in the microbial load). Combination of pasteurization and treatment with sodium benzoate had a much more effect in lowering the microbial populations and thus resulting to a shelf life of beyond 40 days) for the product sample (BOP). The various decreases in the microbial loads of product on storage could be due to corresponding increase in the acidity in the product thereby eliminating organisms that could not tolerate very high-acidity.

The results on microbial isolates (Table 4) indicated that pasteurization was effective in eliminating pathogenic organisms *E. coli* and *Staphyloccocus aureus.* Combination of pasteurization and sodium benzoate was more effective in reducing the organism excepting *Lactobacitlas* sp. which could have been heat resistant strains. However, on storage *Bacillus* and *Lactobacillus* which have been known for acid tolerance and acid production emerged in all the spoilt (BOP) samples. The presence of *Saccharomces cerevisiae* in all the spoilt samples infers that the organism was responsible for the alcoholic odour/spoilage in Zobo drinks stored for up to 3-4 days at ambient temperature.

Storage stability: Studies on Zobo drink (Table 1-3) has shown that pasteurization and application of sodium benzoate preserved the product the most (beyond 40 days) followed by pasteurization (for 14 days). Control or untreated samples could store for a maximum of three days bsfore spoilage ensued with characteristic sourness, alcoholic odour, cloudy appearance and gassy bubbles on shaking the beverage. However, the reddish colour of Zobo drink remained unchanged even after deterioration of the product's quality (Fig. 2).

CONCLUSION

Combination and pasteurization and sodium benzoate application 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. The drink is rich in vitamin C, low in protein and fat content and so could be useful as dietary supplement for patients with scurvy, hepatic diseases and obesity, respectively. There is a strong prospect that industrial production of the Zobo drink could be rewarding to a growing economy like that of Nigeria since the acceptability of beverage cuts across religious and ethnic divides. If the production of the beverage is developed commercially, it can replace red wine as a drink of choice, increase revenue generation and increase job opportunities.

REFERENCES

Adams, M.R. and M.O. Moss 1995. Food Microbiology. The Royal Society of Chemistry, Cambridge CB 4, pp: 398.

Adepegba, A.O., 1999. The Chemical Analysis of Hibiscus Sabdariffa and Parkia biglobosa Proc. 23rd Ann. NIFST. Conf., pp: 189-1990.

A.O.A.C., 2000. Official Methods of Analysis, Association of Analytical Chemists, Washington, D.C.

Bradshaw, L.J., 1979. Laboratory Microbiology, W.B. Saunders Pub. Company, Philadelphia, pp: 13.

Bola, O. and O.O. Aboaba, 2004. Microbiological and Physico-Chemical Evaluation of some Non-alcoholic Beverages. Pak. J. Nut., 3: 188-192.

Cameroon, A., 1982. The Science of Food and Cookry (2nd Edn.), Whitstable Litho Ltd Kent Great Britian, pp: 123.

Esan, E.O. and R.O. Okafor, 1995. Basic Statistical Methods. University of Lagos Press, Lagos, pp: 171-175.

Fisher, P. and A. Bender, 1995. The Values of Foods. (3rd Edn.), Oxford University Press, pp: 44-49.

Frazier, W.C. and D.C. Westhoff, 1988. Food Microbiology McGraw Hill Inc. New York, pp: 189- 210.

Okafor, N., 1983. Processing of Indigenous Fermented Foods: A chance for innovations. Nig. Food J., 1: 32-37.

Onoja, R., 1996. Chemical Constituents of Yakwua Hibiscus Sabdariffa. University Press Jos, pp: 4-5.