STUDIES ON THE USE OF CHEMICAL PRESERVATIVES IN THE PRESERVATION OF ROSELLE DRINK AND PINEAPPLE FLAVOURED ROSELLE DRINK CONCENTRATES

Gbadegesin Adewumi Ronke[1*] and Odunlade Tunji Victor[1]
[1] Department of Food Science and Technology, Obafemi Awolowo University, Ile-Ife, Nigeria
Email: ronkeadewumi@gmail.com

Abstract
Roselle drink concentrate and pineapple flavoured roselle drink concentrates were produced through the reduction of water used for extraction. This was with the additional intent of evaluating the effects of chemical preservatives (0.1% sodium benzoate and 0.01% ethylenediaminetetraacetic acid (EDTA)) on the elongation of the shelf life of roselle drink concentrate and pineapple flavoured roselle drink concentrates. These effects were investigated by determining the pH, titratable acidity (% malic acid), total soluble solids, colour intensity, vitamin C content and the sensory evaluation of the samples weekly during storage at ambient temperature for 4 weeks. Microbial analyses were also carried out on the samples before and after 4 weeks of storage. The results showed that the range of values for pH, titratable acidity, total soluble solids, colour intensity and vitamin C for all the samples were between 1.81 to 2.12, 1.26 to 1.50% malic acid, 18.60 to 23.50 ºBrix, 2.160 to 2.224 and 32.09 to 37.24 mg/100 g, respectively. There was no detectable microbial growth in any of the samples before and after storage. The sensory properties of the samples were kept throughout the storage period. The study recommends the combinatorial usage of sodium benzoate (0.1%) and EDTA (0.01%) to extend the shelf life of roselle drink and pineapple flavoured roselle drink concentrates.

Keywords: *Hibiscus sabdariffa*, *Ananas comosus*, vitamin C, pH, total soluble solids

Submitted: 25.05.2016 Reviewed: 15.07.2016 Accepted: 02.09.2016

1. INTRODUCTION

Roselle, also known as Sorrel or Jamaica flower, has been expended by people for preparing soft drinks and in traditional medicine (Juliani *et al*., 2009). The word *zobo* is derived from *zoborodo*, a northern Nigerian (Hausa) name for roselle plant (Mukhtar, 2007). Roselle drink is an aqueous extract of *Hibiscus sabdariffa* calyces and is a non-alcoholic beverage made from the reddish purple dried calyces. It has been proven to reduce blood pressure and cholesterol levels (Olajide, 2012). The drink is a good source of ascorbic acid and minerals such as calcium, iron and phosphorous (Mohamed *et al*., 2007). It has been shown to possess medicinal properties including antiseptic, diuretic, antioxidant, anti-diabetic, anti-hypertensive and anti-inflammatory properties (Osueke and Ehirim, 2004).
Economically, it is cheaper when compared with other available soft drinks. In spite of the nutritional, medical and economic benefits of this dink, its greatest limitation is that it deteriorates rapidly. It has a very short life of 24 h if not refrigerated (Omemu *et al*., 2006). It contains microorganisms which can cause food spoilage (Omemu *et al*., 2006). Roselle drink is often contaminated with enteropathogenic microorganisms with as much as 2.49 x 10^4 cfu/ml, which could be harmful to persons who consume large quantities of the drink (Bukar *et al*., 2009). Major points of contamination of the drink includes; the packaging material, as most retailers package the drink in already used plastic bottles and polyethene bags, which are not properly disinfected prior to packaging (Nwafor and Ikenebomeh, 2009a). The dried calyces are also a major point of contamination as they harbor spoilage organisms such as *Penicillium* and *Aspergillus spp*. (Amusa *et al*., 2005) and the retailers, who seldomly prepare the drink under aseptic conditions and often do not do enough boiling to reduce the microbial load in the preparation of the beverage.
Pineapple is known for its attractive flavour. Sucrose is a sweetener and can also act as a

preservative when used in sufficient concentration (Taubes, 2011).
Sodium benzoate is an antimicrobial compounds permitted in foods by the FDA (Jay, 1992). It is a GRAS preservative and it is permitted to a maximum level of 0.1% by weight (Branen *et al*., 2002). Ethylenediaminetetraacetic acid (EDTA) is also a GRAS food additive. It is permitted to a level of 33 ppm in soft drinks to promote flavour and colour retention (FDA, 2014). The maximum level permitted refers to foods ready for consumption. Therefore, for concentrates, the levels apply to the drinks when diluted according to manufacturer's recommendations (FDA, 2014).

2. MATERIAL AND METHODS

Materials

Dried roselle calyces, ripe pineapples and sucrose were purchased from New market in Ile-Ife, Osun State, Nigeria. Sodium benzoate and EDTA were purchased from Sigma Chemicals (St. Louis, MO). All chemicals used for analyses were of analytical grade and were obtained from Fisher Scientific (Oakville, ON, Canada) and Sigma Chemicals (St. Louis, MO).

Methods

Production of pineapple juice

Fresh, mature and ripe pineapples (*Ananas comosus*) were washed with water, peeled and sliced (2 cm × 2 cm) manually with kitchen knife. The fruit slices were blended using SAISHO Magic Blender S-742 at maximum speed for 30 seconds and then sieved with a clean muslin cloth (Masamba and Mndalira, 2013).

Production of pineapple flavoured roselle drink concentrate

The pineapple flavoured roselle drink concentrate was produced according to a modified method of Braide *et al.* (2012). Dried calyces of *Hibiscus sabdariffa* were freed from dirts and extraneous materials by manual sorting and winnowing. Calyces (400 g) were weighed and washed slightly with water. The washed calyces were boiled in 4000 ml water for 30 min to aid the extraction as well as destruction of heat-sensitive microorganisms present. It was then filtered into a pre-sterilized bowl using a clean muslin cloth to obtain roselle drink concentrates.

Pineapple juice was added at different proportions (0, 15, 20 and 25% [v/v]) to serve as flavour. Sucrose (20% [w/v]) was added to each pineapple flavoured roselle drink concentrate and then stirred to hasten dissolution. Each sample was boiled for 5 min to prevent post production contamination. Preservatives (0.1% sodium benzoate and 0.01% EDTA) were added and packaged immediately. The samples were pasteurized at 70 ºC for 15 min and then stored at ambient temperature (27 ± 2 ºC) for four weeks.

Determination of pH

The pH was measured using a digital pH meter (Hanna checker Model HI1270, USA). The instrument was calibrated using two standard buffer solutions, pH 7.0 and pH 4.0. The pH electrode was inserted into a beaker filled with 10 ml of sample (Meei *et al*., 2012).

Determination of titratable acidity

Titratable acidity was determined by titrimetric method using 0.1 N sodium hydroxide. A sample of 10 ml was pipetted into a beaker and titrated with 0.1 N NaOH to a pH endpoint of 8.2. Results were expressed as malic acid equivalent (%). Titratable acidity was calculated using the equation below (Meei *et al*., 2012).

$$\text{Titratable acidity (\% malic acid)} = \left(\frac{\text{volume of titration} \times 0.1\ \text{N NaOH} \times 0.067 \times 100}{\text{volume of juice}} \right)$$

Determination of total soluble solids

This was carried out using a hand refractometer. The refractometer prism surface was cleaned with distilled water and tissue paper, followed by placing a drop of the sample on the prism of the refractometer.

The reading was taken by looking through the eyepiece of the refractometer and the soluble sugar was expressed in °Brix at 25 °C (AOAC, 2000).

Determination of colour intensity

The colour of the samples was determined using a UV-VIS spectrophotometer (model SP9, Pye Unicam, UK) set at wavelength of 420 nm. The absorbance was taken as an index of colour intensity of the beverage (Idolo *et al*., 2012).

Determination of vitamin C (ascorbic acid)

The amount of vitamin C contained in each sample was determined by titrimetric method (using 2, 6-dichlorophenol indophenol dye) described by Hassan and Hassan (2008).

Sensory evaluation

Drinks were prepared from the roselle drink concentrate and pineapple flavoured roselle drink concentrate samples by diluting each sample with 500% water. The samples were presented to 10 semi-trained panelists who are used to roselle drink. The panelists evaluated the drinks by sipping on a drink, then rinsing their mouth with water after testing each drink. The drinks were ranked by colour, flavour, taste and overall acceptability using a 9-point hedonic scale where 9 represents like extremely and 1 for dislike extremely.

Microbial Analyses (total bacterial and coliform counts)

Each sample was serially diluted until 10^{-5} dilution was obtained after which the microbial load of each dilution was estimated by plating 1 ml of each using pour plate method on nutrient agar and eosin methylene blue agar for total bacterial and coliform counts respectively. The plates were incubated at 35-37 °C for 48 h. (Adeniran *et al*., 2010).

Statistical Analysis

Statistical analysis of all data was carried out using the Statistical Package for the Social Sciences software version 16.0 (SPSS, Chicago, USA). Statistically significant differences ($p< 0.05$) in all data obtained were determined using one-way Analysis of Variance (ANOVA) procedure and the means were separated using Duncan multiple range tests. Values were expressed as means ± standard deviation of triplicate measurements.

3. RESULTS AND DISCUSSION

pH and titratable acidity

The results of pH and titratable acidity (% malic acid) during storage are shown in Figures 1 and 2, respectively. These indicated a general significant decrease ($p < 0.05$) in pH and corresponding increase in titratable acidity (TTA) of the samples.

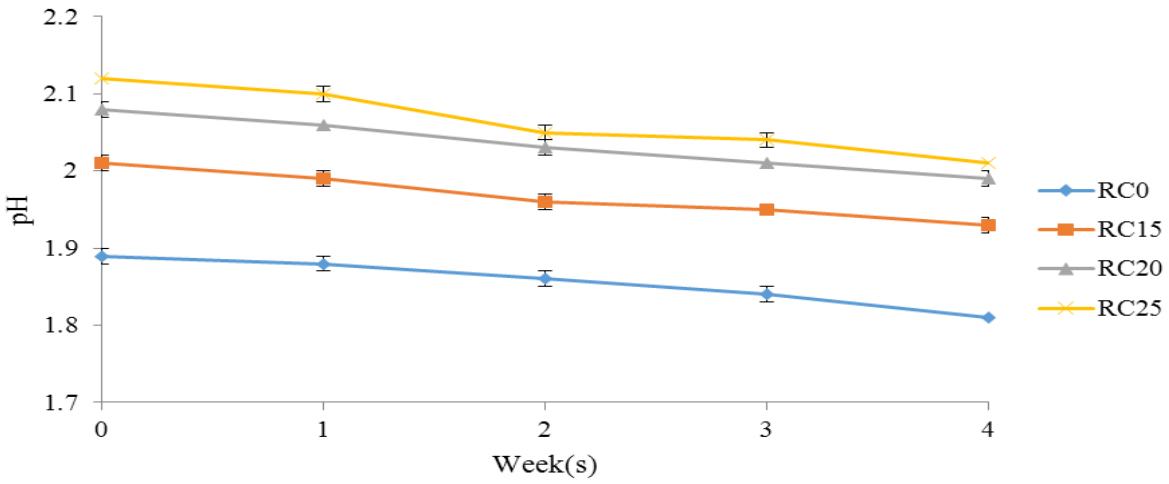

Fig. 1 pH of roselle drink concentrate and pineapple flavoured roselle drink concentrates during storage
RC0: 100% roselle drink concentrate, **RC15:** 85% roselle drink concentrate and 15% pineapple juice, **RC20:** 80% roselle drink concentrate and 20% pineapple juice, **RC25:** 75% roselle drink concentrate and 25% pineapple

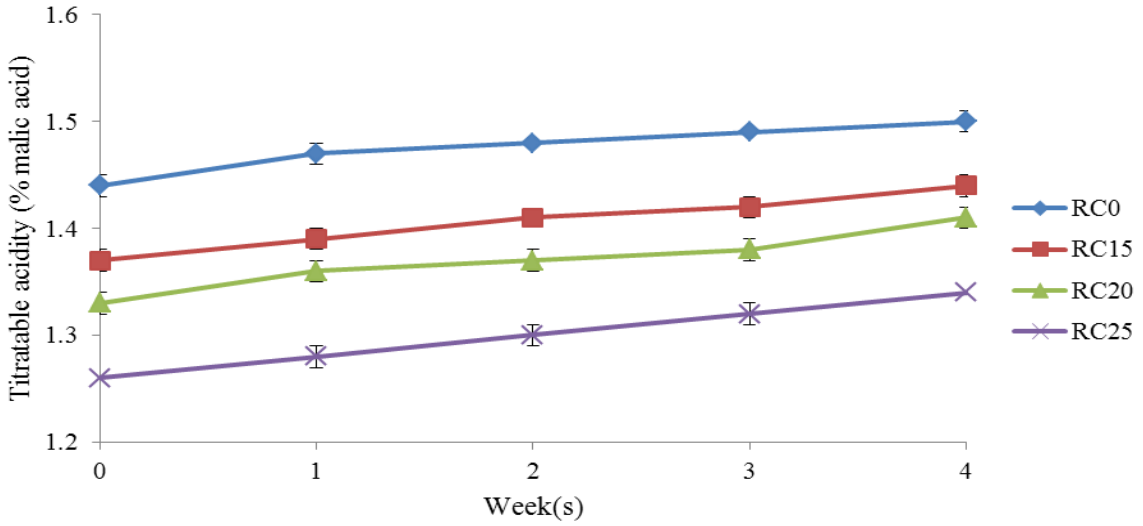

Fig. 2 Titratable acidity of roselle drink concentrate and pineapple flavoured roselle drink concentrates during storage

RC0: 100% roselle drink concentrate, **RC15:** 85% roselle drink concentrate and 15% pineapple juice, **RC20:** 80% roselle drink concentrate and 20% pineapple juice, **RC25:** 75% roselle drink concentrate and 25% pineapple juice

The pH of the samples varied between 1.81 and 2.12 while the TTA varied between 1.26 and 1.50% malic acid throughout the period of storage. This shows the efficacy of the preservatives in preventing chemical degradation. This observation agrees with the postulation that TTA and pH are inversely related (Nwafor and Ikenebomeh, 2009a, b; Egbere *et al.*, 2007).

Decrease in pH and increase in acidity imply decrease in microbial population (Stainer *et al.*, 1987; Prescott *et al.*, 1999; Doughari *et al.*, 2007). Similar result of decrease in pH and corresponding increase in titratable acidity was reported by Nwokocha *et al.* (2012) during the storage of roselle drink prepared with ethanolic extracts of alligator pepper and ethanol at ambient temperature. Dauda and Adegoke (2014) also reported similar trend of result during the storage of soy-milk based juice at ambient temperature.

Total soluble solids

The result of the total soluble solids (TSS) of the samples during storage is shown in Figure 3. TSS of the samples were in the range of 18.60 and 23.50 ºBrix over the storage period. The results were stable for all the samples for 2 weeks. However, there was slight but significant ($p < 0.05$) decrease in all the samples by weeks 3 and 4. The percent reduction in the total soluble solids of the samples ranged between 0.97 to 1.37%. The TSS values showed a controlled reduction in the samples.

The stability and slight decrease of TSS in the samples could be attributed to the effect of the preservatives added. Larger decrease could have been due to the utilization of sugars by fermenting organisms leading to sugar degradation (Nwokocha *et al.*, 2012).

This showed that the preservatives were effective in preventing microbial decomposition of sugar.

Similar results were reported by Abiose and Adeniran (2010) and Nwokocha *et al.* (2012) during the storage of roselle drink. Comparable result was also reported by Dauda and Adegoke (2014) during the storage of soy-milk based juice at ambient temperature.

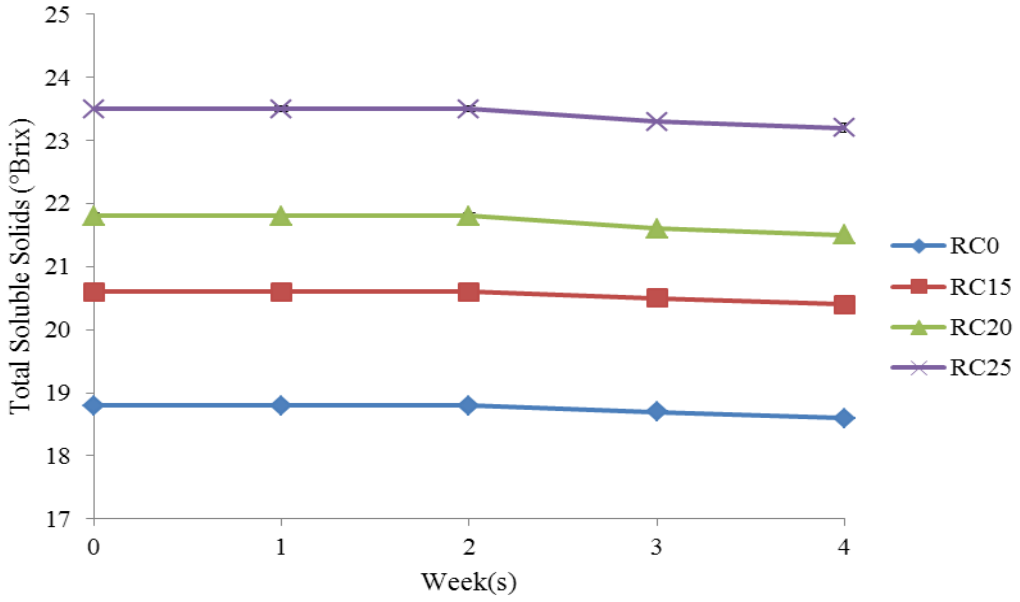

Fig. 3 Total soluble solids of roselle drink concentrate and pineapple flavoured roselle drink concentrates during storage

RC0: 100% roselle drink concentrate, **RC15:** 85% roselle drink concentrate and 15% pineapple juice, **RC20:** 80% roselle drink concentrate and 20% pineapple juice, **RC25:** 75% roselle drink concentrate and 25% pineapple juice

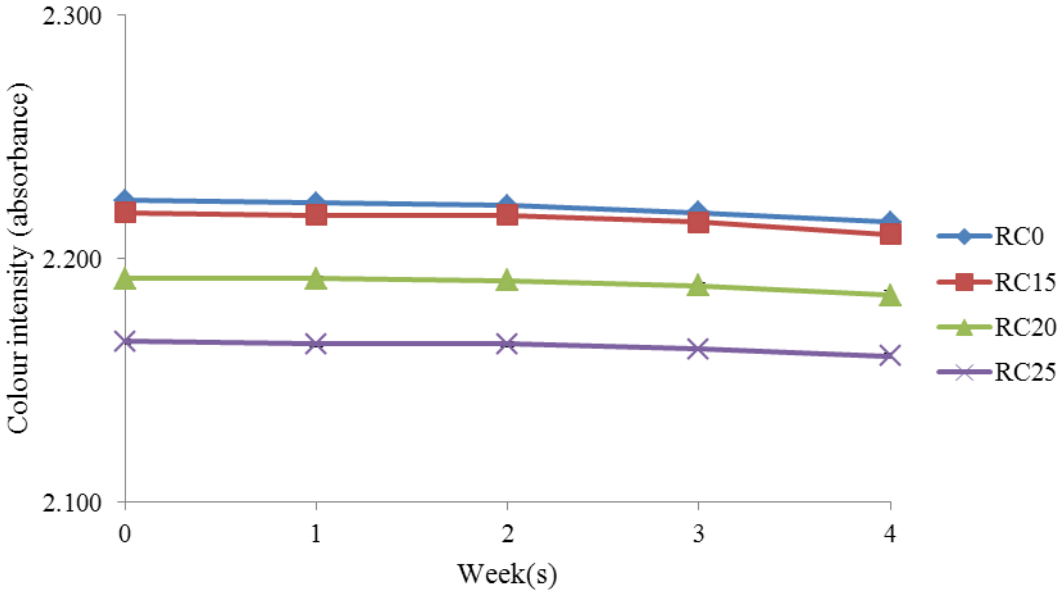

Fig. 4 Colour intensity of roselle drink concentrate and pineapple flavoured roselle drink concentrates during storage

RC0: 100% roselle drink concentrate, **RC15:** 85% roselle drink concentrate and 15% pineapple juice, **RC20:** 80% roselle drink concentrate and 20% pineapple juice, **RC25:** 75% roselle drink concentrate and 25% pineapple juice

Colour intensity

Colour was objectively measured using pigment concentration based on Wrolstad *et al.* (2005) approach. The measurement of absorbance at 420 nm determines the stability of the anthocyanin of the roselle drink concentrates. The result of the colour intensity during storage is shown in Figure 4.

The values ranged between 2.160 and 2.224 for all the samples. There was stability in the colour intensity of all the samples for the first two weeks of storage. However, there was slight decrease which was significantly different ($p < 0.05$) for all the samples by the third and fourth week. The stability of the absorbance of the samples for the first two weeks might be due to the presence of EDTA which has been shown to promote colour retention (FDA, 2014). It might also be due to the presence of higher amount of sucrose (20%, w/v). Sucrose preserves anthocyanins than freezing during storage and also inhibits browning and formation of polymeric pigments (Huang, 1956). Nikkhah *et al*. (2007) reported that the use of 20% sugar (sucrose) has protective effect on anthocyanins in berries. Also, reduction of water activity with sucrose can also prevent anthocyanin destruction (De-Ancos *et al*., 1999).

Vitamin C

Vitamin C is an index of nutrient quality of fruits and vegetable and it is much more sensitive to various modes of degradation in food processing and subsequent storage (Ozkan *et al*., 2004; Shaw, 1992). It is also used as a standard for monitoring the quality of juice in storage. The result of the vitamin C content of the samples during storage is presented in Figure 5. The values of all the samples varied between 32.09 and 37.24 mg/100 g throughout the period of storage. There was significant decrease ($p < 0.05$) in the vitamin C content of all the samples as the storage period increased. Similar pattern of results was reported by Egbere *et al.* (2007) and Nwokocha *et al.* (2012) during the storage of roselle drink. Vitamin C loss of between 9.94 and 11.33% in the samples after 4 weeks of storage at ambient temperature indicated that the preservatives helped in minimizing degradation and assist in the retention of over half of the Vitamin C contained initially. The percentage loss increased with increase in concentration of pineapple juice to roselle extract.

The percentage losses were 9.94, 10.24, 10.96 and 11.33% for samples RC0, RC15, RC20 and RC25 respectively. Vitamin C loss is known to increase with exposure to heat, light and oxygen. The decrease observed could also be attributed to oxidation, which occurs in fruit juices during storage and is highly dependent on the presence of oxygen in the head space or dissolve in the samples (Costa *et al.*, 2003).

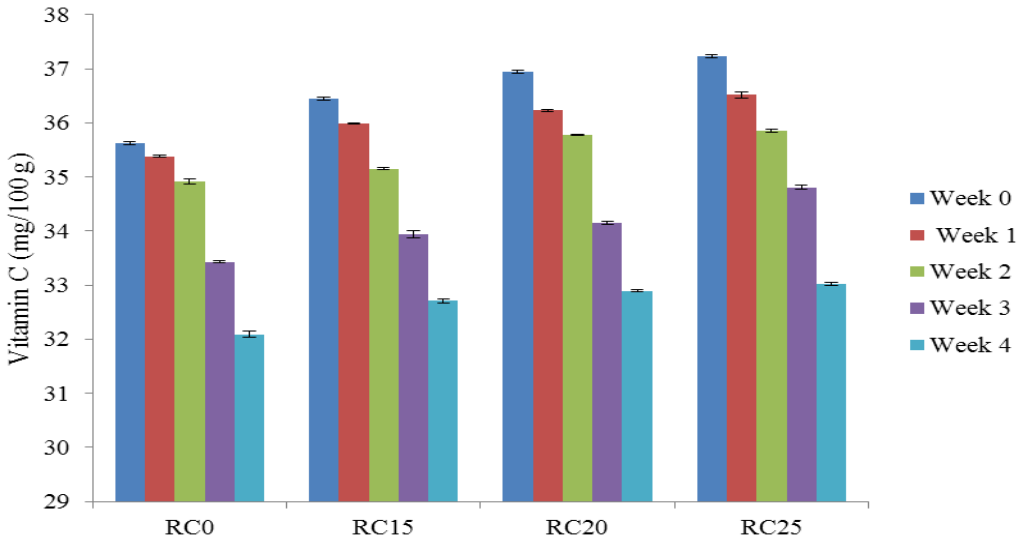

Fig. 5 Vitamin C content of roselle drink concentrate and pineapple flavoured roselle drink concentrate during storage

RC0: 100% roselle drink concentrate, **RC15:** 85% roselle drink concentrate and 15% pineapple juice, **RC20:** 80% roselle drink concentrate and 20% pineapple juice, **RC25:** 75% roselle drink concentrate and 25% pineapple juice

Table 1 Sensory evaluation of reconstituted samples from roselle drink concentrates

Sample	Colour	Flavour	Taste	Overall acceptability
RC0	7.5^{a}	6.0^{c}	6.0^{c}	6.2^{c}
RC15	7.6^{a}	6.8^{b}	6.8^{b}	7.2^{b}
RC20	7.6^{a}	7.4^{ab}	7.8^{a}	7.6^{ab}
RC25	7.6^{a}	8.0^{a}	8.4^{a}	8.4^{a}

The mean values along the same column with different superscripts are significantly different ($p < 0.05$)
RC0: 100% roselle drink concentrate, **RC15:** 85% roselle drink concentrate and 15% pineapple juice, **RC20:** 80% roselle drink concentrate and 20% pineapple juice, **RC25:** 75% roselle drink concentrate and 25% pineapple juice

Table 2 Sensory evaluation of reconstituted samples from roselle drink concentrate and pineapple flavoured roselle drink concentrates during storage

Sample	Colour	Flavour	Taste	Overall acceptability
		Week 1		
C0	7.5^{a}	6.0^{c}	6.0^{c}	6.1^{c}
RC15	7.5^{a}	6.7^{b}	6.7^{b}	7.1^{b}
RC20	7.5^{a}	7.4^{ab}	7.7^{a}	7.6^{ab}
RC25	7.6^{a}	8.0^{a}	8.3^{a}	8.2^{a}
		Week 2		
RC0	7.5^{a}	6.0^{c}	5.9^{d}	5.9^{c}
RC15	7.5^{a}	6.5^{b}	6.4^{c}	7.0^{b}
RC20	7.5^{a}	7.3^{a}	7.5^{b}	7.4^{b}
RC25	7.4^{a}	7.8^{a}	8.2^{a}	8.0^{a}
		Week 3		
RC0	7.5^{a}	5.9^{d}	5.7^{c}	5.8^{c}
RC15	7.4^{a}	6.3^{c}	6.4^{b}	6.8^{b}
RC20	7.4^{a}	7.1^{b}	7.4^{a}	7.3^{ab}
RC25	7.4^{a}	7.7^{a}	7.9^{a}	7.8^{a}
		Week 4		
RC0	7.4^{a}	5.7^{bc}	5.6^{c}	5.6^{c}
RC15	7.4^{a}	6.1^{b}	6.1^{b}	6.5^{ab}
RC20	7.4^{a}	6.9^{a}	7.2^{a}	7.0^{a}
RC25	7.4^{a}	7.4^{a}	7.5^{a}	7.4^{a}

The mean values along the same column with different superscripts are significantly different ($p < 0.05$)
RC0: 100% roselle drink concentrate, **RC15:** 85% roselle drink concentrate and 15% pineapple juice, **RC20:** 80% roselle drink concentrate and 20% pineapple juice, **RC25:** 75% roselle drink concentrate and 25% pineapple juice

Sensory evaluation

The results of the sensory evaluation of the roselle drink concentrates after reconstitution (1 part of concentrate: 5 parts of water) shortly after production are shown in Table 1. There was no significant difference in the colour of the drinks prepared from the concentrates. Sample RP25 (sample with 25% pineapple juice) had the highest mean hedonic scores in flavour, taste and overall acceptability. The results of the sensory evaluation during storage of the samples are presented in Table 2. Throughout the period of storage, there was no significant difference ($p > 0.05$) in the colour of drinks prepared from the stored samples. There was also no major significant difference in the flavour, taste and overall acceptability of each of the samples for 3 weeks but slight differences were observed after week 4. From the results of the sensory evaluation during storage, the pineapple flavoured roselle drinks prepared from the concentrates were still preferred to other samples even after 4 weeks of storage.

Table 3 Microbial counts of roselle drink concentrate and pineapple flavoured roselle drink concentrate before and after storage

	RC0	RC15	RC20	RC25
Coliform count (cfu/ml)				
Week 0	Nil	Nil	Nil	Nil
Week 4	Nil	Nil	Nil	Nil
Total bacterial count (cfu/ml)				
Week 0	Nil	Nil	Nil	Nil
Week 4	Nil	Nil	Nil	Nil

RC0: 100% roselle drink concentrate, **RC15:** 85% roselle drink concentrate and 15% pineapple juice, **RC20:** 80% roselle drink concentrate and 20% pineapple juice, **RC25:** 75% roselle drink concentrate and 25% pineapple juice

Microbial counts

Microbial analyses (total bacterial and coliform counts) were done before and after storage of the roselle drink concentrates for 4 weeks at ambient temperature to ensure the safety of the samples. The results of the total bacterial and coliform counts of the samples are shown in Table 3. There was no coliform and bacterial growth in the samples shortly after production. This may be attributed to the adequate heat treatment given to the samples, the hygienic conditions under which they were prepared and the boiling carried out after the addition of pineapple juice and sucrose.

After 4 weeks of storage, there was no coliform growth in all the samples. This is an indication that the products maintained good sanitary standards throughout the 4 weeks of storage. This may also be an indication that the processing method was adequate and that the preservatives used prevented the growth of coliform. The result of the coliform count obtained was comparable to the report of Abiose and Adeniran (2010) during the storage of roselle drink at ambient temperature. Presence of coliforms in any food sample renders such food unsafe for human consumption (Harrigan, 1998). Also, there was no bacterial growth in all the samples after 4 weeks of storage. This may be attributed to the adequate heat treatment given to the samples, the hygienic conditions under which they are prepared, addition of preservatives and the consequent pasteurization at 70 °C for 15 min. The FAO critical microbial count for ready to eat foods and drinks is 10^4 cfu/ml (FAO, 1979). From the results, it can be deduced that the roselle drink concentrate and pineapple flavoured roselle drink concentrates stored at ambient temperature for 4 weeks are safe for consumption.

4. CONCLUSIONS

From the study, it can be concluded that boiling after the addition of pineapple juice and sucrose, the use of chemical preservatives (0.1% sodium benzoate and 0.01% EDTA) and pasteurization (70 °C) could be used to increase the shelf life of roselle drink concentrate and pineapple flavoured roselle drink concentrate at ambient temperature for four weeks and could also be used to improve the convenience of production of the drink in homes and offices by simply diluting with water.

5. REFERENCES

[1] Abiose, S. H. and Adeniran, H. A. Studies on the extension of shelf-life of roselle (Hibiscus sabdariffa) extract. Ife Journal of Technology, 2010, 19 (1), 34-39.

[2] Adeniran, H. A., Abiose, S. H. and Ukeyima, T. M. Micribiological assesment of probioticated ginger-based beverages. Nutrition Based Food Science, 2010, 40 (2), 209-220.

[3] Amusa, N. A., Ashaye, O. A., Aiyegbayo, A. A., Oladapo, M. O., Oni, M. O. and Afolabi, O. O. Microbiological and nutritional quality of hawked Sorrel drinks (Soborodo, the Nigerian locally brewed soft drink) widely consumed and notable drinks in Nigeria. Journal of Food, Agriculture and Environment, 2005, 3 (3- 4), 47-50.

[4] AOAC. Official methods of analysis of AOAC International. 17th Edition. Association Official Analytical Chemists, Arlinton, United State of America. 2000.

[5] Braide, W., Oranusi, S. and Peter-Ikechukwu. A. I. Perspectives in the hurdle techniques in the preservation of a non-alcoholic beverage, zobo. African Journal of Food Science and Technology, 2012, 3 (2), 46-52.
[6] Branen, A. L., Davidson, P. M., Salminen, S. and Thorngate, J. H. Food Additives, Second Edition, Revised and Expanded. Marcel Dekker, Inc. New York, Basel, 2002, 584.
[7] Bukar, A., Uba, A. and Oyeyi, T. I. Occurrence of some enteropathogenic bacteria in some minimally and fully processed ready-to-eat foods in Kano metropolis, Nigeria. African Journal of Food Science, 2009, 4 (2): 32-36.
[8] Costa, M. C. O., Maia, G. A., Figueiredo, R. W., Souza Filho, M. M., Brasil, I. M. Storage stability of cashew apple juice preserved by hot fill and aseptic processes. African Journal of Food Science, 2003, 23, 106-109.
[9] Dauda, A. O. and Adegoke, G. O. Preservation of some physico-chemical properties of soymilk-based juice with Aframomum danielli spice powder. American Journal of Food Science and Technology, 2014, 2 (4), 116-121.
[10] De-Ancos, B., Gonzalez, U. E. and Cano, M. P. Differentiation of raspberry varieties according to anthocyanic composition. Food Research and Technology, 1999, 208, 33-38.
[11] Doughari, J. H., Alabi, G. and Elmahmood, A. M. Effect of some Chemical preservatives on the Shelf-life of 'zobo' drink. African Journal of Microbiology, 2007, 2, 037-041.
[12] Egbere O. J, Anuonye J. C., Chollom P. F. and Okpara P. V. 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, 2007, 5 (3), 225-228.
[13] Food and Agricultural Organization (FAO). Manuals of food quality control. Food and Nutrition Paper, United Nations, Rome, 1979, 14 (4), A1 – F10.
[14] Food and Drug Administration (FDA). Ethylenediaminetetraacetic acid (EDTA). Department of Health and Human Services. United States, 2014.
[15] Hassan, A. S. and Hassan, H. S. Quantitative estimation of vitamin c in some local fruits. Science World Journal, 2008, 3 (2), 113-115.
[16] Harrigan, W. F. Laboratory methods in Food Microboilogy. Academic Press, San Diego, CA. 1998.
[17] Huang, H. T. The kinetics of the decolourization of anthocyanins by fungal anthocyanese. Journal of the American Chemical Society, 1956, 78, 2390-2393.
[18] Idolo, I., Taiwo, O. O. and Johnson O. A. Production and quality attributes of vegetable wine from Hibiscus sabdariffa Linn. African Journal of Food Science, 2012, 6 (7), 212-215.
[19] Jay, J. M. Modern Food Microbiology, 5th ed., Chapman and Hall, New York, 1992.
[20] Juliani, H. R., Welch, C. R., Wu, Q., Diouf, B., Malainy, D., and Simon, J. E. Chemistry and quality of hibiscus (Hibiscus sabdariffa) for developing the natural-product industry in Senegal. Journal of Food Science, 2009, 74 (2), 113-121.
[21] Masamba K. G. and Mndalira K. Vitamin C stability in pineapple, guava and baobab juices under different storage conditions using different levels of sodium benzoate and metabisulphite. African Journal of Biotechnology, 2013, 12 (2), 186-191.
[22] Meei, C. Q., Nyuk, L. C. and Yus, A. Y. Optimisation and comparative study on extraction methods of soursop juice. Journal of Food, Agriculture and Environment, 2012, 10 (3-4): 245-251.
[23] Mohamed, R., Fernandez, J., Pineda, M. and Aguilar, M. Roselle (Hibiscus sabdariffa) seed oil is a rich source of gamma-tocopherol. Journal of Food Science, 2007, 72 (3): 207-211.
[24] Mukhtar, A. M. The effect of feeding roselle (Hibiscus sabdariffa) seed on broiler chick's performance. Journal of Animal Veterinary Science, 2007, 2, 21-23.
[25] Nikkhah, E., Khayamy, M., Heidari, R. and Jamee, R. Effect of sugar treatment on the stability of anthocyanin pigments in berries. Journal of Biological Sciences, 2007, 7: 1412-1417.
[26] Nwafor, O. E. and Ikenebomeh, M. J. Effect of sodium benzoate on the growth and enzyme activity of Aspergillus niger and Penicillium citrunum on zobo drink during storage at 30±2 °C. African Journal of Health Technology, 2009a, 8 (12), 2843-2847.
[27] Nwafor, O. E. and Ikenebomeh, M. J. Effect of different packaging materials on microbiological physicochemical and organoleptic quality of zobo drink stored at room temperature. African Journal of Biotechnology, 2009b, 8 (12), 2843-2447.
[28] Nwokocha, J. V., Okoronkwo, N. E., Eze, S. O. and Nwokocha, N. J. Comparism of alligator pepper and ginger extracts on zobo liquor during storage at ambient temperature. Academic Research International, 2012, 2 (3), 2223-9944.
[29] Olajide, K. All Natural 'Zobo' Hibiscus drink to exhibit summer fancy food show in Washington D.C., V.K. fields and Co. Public Relations, 2012.
[30] Omemu, A. M., Edema, M. O., Atayese, A. O., and Obadina, A. O. A survey of the micoflora of Hibiscus sabdariffa (roselle) and the resulting 'zobo' juice. African Journal of Biotechnology, 2006, 5, 245-259.
[31] Osueke, J. C. and Ehirim, F. N. Chemical, nutritional and sensory analysis of zobo drink (Var Sabdariffa) and selected soft drinks. Journal of Agriculture and Food Science, 2004, 2, 33-37.
[32] Ozkan, M., Kirca, A. and Cameroglu, B. Effect of hydrogen peroxide on the stability of ascorbic acid during storagein various fruit juices. Food Chemistry, 2004, 88, 591-597.

[33] Prescott, L. M., Harley, J. P. and Kleen, D. A. Food Microbiology, 5th Edition, McGraw Hill, New York, 1999, 352-627.
[34] Shaw, P. E. Shelf life and aging of citrus juices, drinks and related soft drinks. In: Redd, J. B. and Hendrix, D. L. (eds.) Quality control manual for citrus processing plants. Florida, Aubandale, Agricscience, 1992, 173-179.
[35] Stainer, R. Y., Ingram, J., Wheellis, M. L. and Painter, M. General Microbiology, 5th Edition. London University Press, 1987, 137-141.
[36] Taubes, G. Is Sugar Toxic? The New York Times, 2011.
[37] Wrolstad, R., Durst, R. W. and Lee, J. Tracking color and pigment changes in anthocyanin products. Trends in Food Science and Technology, 2005, 16, 423-428.