Full Paper

STUDIES ON EXTENSION OF SHELF-LIFE OF ROSELLE (*HIBISCUS SABDARIFFA*) EXTRACT

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ABSTRACT

The effect of pasteurization and addition of two preservatives (sodium metabisulphite and sodium benzoate) was studied on roselle juice (*zobo*) with a view to extending its shelf-life. Six samples of six treatments (A-conventional sample, B – pasteurized, C-conventional sample with 500mg/kg sodium benzoate, D conventional sample with 200mg/kg sodium metabisulphite, E – conventional sample with 500mg/kg sodium benzoate and pasteurization and F conventional sample with 200mg/kg metabisulphite with pasteurization) were prepared and stored for six weeks at ambient (28 ± 1 ^{0}C) and refrigeration temperatures (5 ± 1 ^{0} C). Physico-chemical (pH, titratable acidity and ^{o}Brix) and microbiological analyses of samples were carried out weekly using standard methods. Results showed pH, titratable acidity and ^{o}Brix values to range between 2.76 – 2.97, 0.075 - 0.207% and 9.0 - 9.6 respectively while coliform bacteria, yeast/moulds were nil over a period of six weeks. Sodium metabisulphite (200mg/kg) with or without pasteurization at 70 ^{o}C effectively preserved the product over the period of storage. These results have established that the shelf-life of roselle juice can be extended to between three and six weeks by pasteurization and addition of sodium metabisulphite ($Na_2S_2O_5$) or sodium benzoate at 200 or 500mg/kg respectively.

Keywords: *roselle juice, chemical preservatives, pasteurization, ambient, refrigeration*

1. INTRODUCTION

Demand for non conventional, health promoting beverages has been on the increase in recent times [1]. In most developing countries including Nigeria, economic realities and the desire to explore non-conventional beverages have forced many people to turn to roselle extract as a beverage. Beverages are foods consumed for their thirst-quenching properties, stimulating effects or simply because consumption is pleasurable. Food beverages are liquids or are consumed in the liquid state but the relative lack of actual food value differentiates them from others like milk and milk products [2]. The essential component of every beverage is the water that it contains. Other components such as stimulants, coloring and flavoring ingredients may perform some useful functions but they are not essential to the proper physiological function of the body [3]. Roselle drink is a beverage obtained by extracting the juice from the red, fleshy calyces of the leafy plant *Hibiscus sabdariffa* var *sabdariffa* with boiled water. The extract is usually sweetened with sugar and flavored with any of desirable flavors including that of pine apple, ginger or vanilla [4]. Roselle extract can be classified as a natural extract drink [5]. All over the world, the extract is known by many names including sorrel, sudan tea, and in Nigeria as *zobo*

The fleshy calyces containing anthocyanin pigments are largely used as tea and refreshing beverage but also as natural dye. The calyces contain high content of anthocyanic pigments, cyanidine (29%) and delphinidin (70.9%) [6]. *Zobo* drink is also consumed for its nutritional and medicinal properties. The drink is regarded as diuretic, cholerectic, febrifugal and hypotensive, antiseptic, aphrodisiac, astringent, laxative, sedative and stimulating intestinal peristalsis [7]. Roselle (*Hibiscus sabdariffa)* is widely grown in many countries in the Caribbean and Latin America, Africa and Asia. Nutritionally, it is a source of simple sugars, vitamin C which has been found to break mucus, detoxify the body and subsequently strengthen the immune system detoxify the body and subsequently strengthen the immune system. In addition, the calyces are rich in calcium, magnesium, zinc and iron [8].

The non-alcoholic beverage obtained from the calyces of *Hibiscus sabdariffa* is refreshing and highly accepted to consumers of different socio-economic levels in Africa, India, and Latin America. Roselle juice has been known to be prone to microbial spoilage within few days of production when stored at ambient temperature [10, 4 & 11]. This problem limits the use of the drink to only domestic use, preventing production on a large scale as industrial production requires a reasonable shelf life. In this study, application of hurdle technology in the form of chemical preservatives and pasteurization are explored for extension of shelf-life of roselle extract beyond few days. Microbial and physico-chemical changes associated with treated beverage samples under ambient and refrigeration storage were also investigated.

2. MATERIALS AND METHODS

2.1. Materials

Dried roselle calyces, pineapple andvanilla essences; and granulated sugar were obtained from a local market in Ile-Ife, Nigeria while Microbiological media (Lab M, United Kingdom) and other chemicals were of analytical grade.

2.2. Preparation of Roselle Juice

The dried roselle calyces were hand-picked to remove debris, stalks and other forms of dirt that might have been introduced in the

process of harvesting, and transportation. The cleaned calyces (10g/l) were then added to boiling water and allowed to boil for about 30 minutes to aid the extraction of the juice as well as destruction of the heat-sensitive microorganisms present. After boiling, the juice was filtered using muslin cloth. Granulated sugar (60g/l) was added to sweeten the clear juice. Vanilla (1%) and banana (1%) essences were then added to flavor roselle infusion [5, 10 & 12].

2.3. Preparation of Samples

After extraction, the juice was split into six portions labeled A-F. On cooling, one of the portions (Sample A) was dispensed into six tubes (25ml each) labeled $A_1 - A_6$. Tubes A_1- A_3 were stored at ambient temperature while $A_4 - A_6$ were stored at refrigeration temperature. Portion B was equally dispensed into six tubes labeled $B_1 - B_6$ and were thereafter pasteurized at 70 ^{0}C for 15 minutes. Samples $B_1 - B_3$ were kept at ambient temperature while $B_4 - B_6$ were kept at refrigeration temperature. Portions C and D were added 200 and 500 mg/l of sodium metabisulphite and sodium benzoate respectively, while still hot. They were subsequently dispensed into tubes and labeled as $C_1 - C_6$ and $D_1 - D_6$. The first three tubes in each case were stored at ambient temperature while the other tubes were stored at refrigeration temperature. Portions E and F were treated as Portions C and D but were pasteurized after receiving sodium metabisulphite and sodium benzoate respectively.

2.4. Analyses of samples

Physico-chemical and microbiological analyses of each of the samples were carried out weekly for six weeks. These include pH, titratable acidity and brix determinations as well as total aerobic, yeast, mould, and coliform counts. Physico-chemical analyses were also carried out after twelve weeks of storage.

2.5. Physico-Chemical Analysis

pH determination, titratable acidity (TTA) measured as malic acid , and ^{O}Brix were determined using standard methods (16).

2.6. Microbiological tests

Each sample was serially diluted until 10^{-6} dilution was obtained after which the microbial load of each dilution was estimated by plating 1.0ml of each using pour plate method. Nutrient agar and Potato Dextrose agar were used for enumeration of Total Aerobic Count, and Yeast/Mould Count respectively. Plates were incubated at 37 ^{0}C, and 28 ^{0}C,for 48 and 72 hrs respectively. Coliforms were enumerated with MacConkey agar using 37 ^{0}C incubation temperature and 48hrs of incubation. Following incubation, colonies were counted using a colony counter. Counts were then expressed as cfu/ml [13, 14 & 15]

2.7. Statistical Analysis

Data were obtained in triplicates and statistical analysis of all data was carried out with Origin Pro 70 (1999 - 2002) computer package. Statistically significant differences ($p \leq 0.05$) in all data were determined by analysis of variance while least significant difference was used to separate means.

3. Result And Discussion

3.1. Physico-Chemical Analysis

3.1.1 pH and Titratable acidity

The results of pH and titratable acidity of roselle juice with different treatments stored at room and refrigeration temperature are presented in Fig 1a and 1b; and Figs 2a and 2b respectively. The freshly prepared conventional juice with different treatments had a low pH range of 2.76 – 2.90. The pH of all the samples increased slightly over six weeks of storage. Extension of storage period to Week 12 produced a result that was not consistent with the trend from Weeks 1 to 6. This explains why the shelf-life is put at six weeks. The slight increase in pH could have been caused by increasing number of microbes and other chemical changes taking place in the extract. Most fruit juices are known to have a characteristic low pH [18].

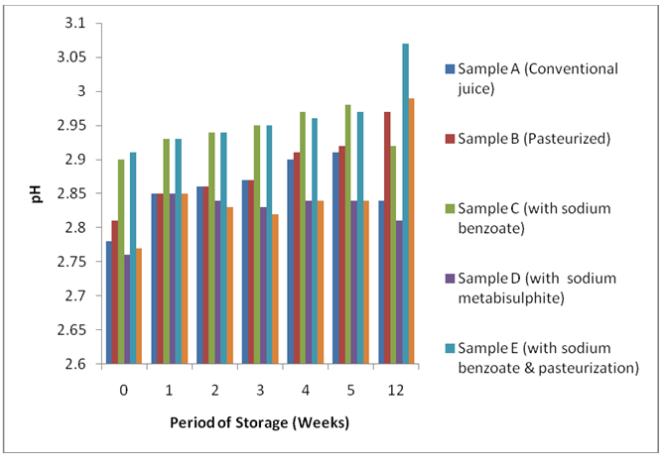

Fig 1a: pH of Roselle Juice Samples at Ambient Temperature

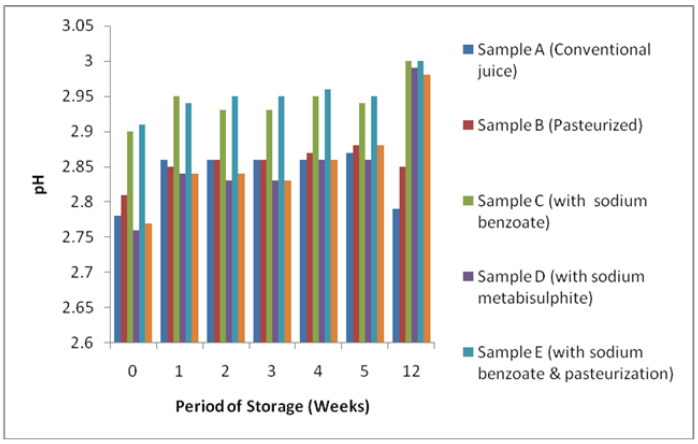

Fig 1b: pH of Roselle Juice Samples at Refrigeration Temperature

Result of the titratable acidity determined in terms of % malic acid are presented in Tables 5a and 5b. Since acidity and pH are inversely related, the acidities of all the samples were observed to decrease with time. The decrease was, however, not significantly different ($p > 0.05$) in each treatment over the period of storage. There was also no significant difference between the values of the refrigerated samples and those stored at room temperature and among treatments ($p > 0.05$).

3.1.2. ^{o}Brix

Results (Tables 4a and 4b) showed that the ^{o}Brix values obtained initially for all the samples was 9.6 and remained fairly constant during storage. The differences in the ^{o}Brix among the treatments and within the treatments were not significantly

different over the period of storage and even at the different storage temperatures at 5% level of significance.

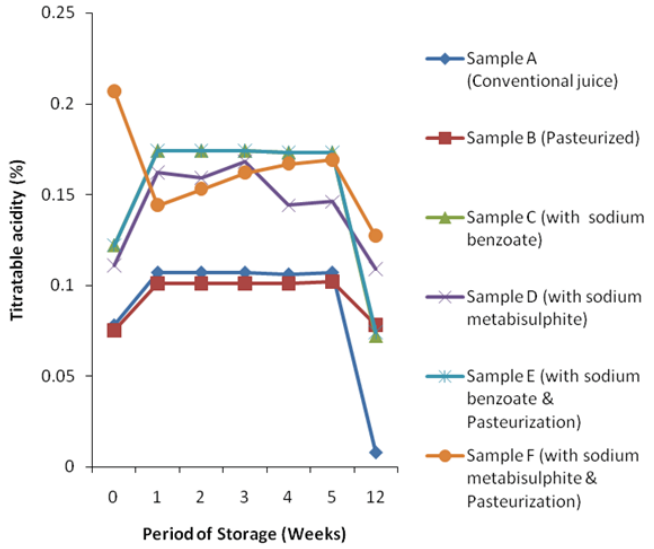

Fig 2a: Titratable acidity of Roselle Juice at Ambient Temperature

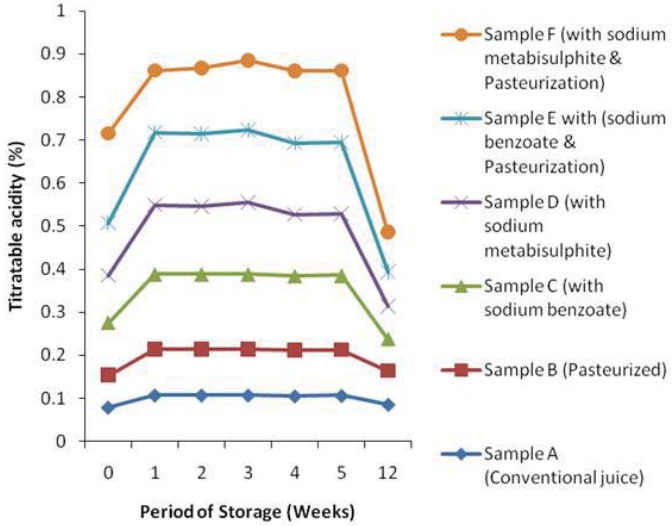

Fig 2b: Titratable acidity of Roselle Juice at Refrigeration Temperature

3.2. Microbial Analysis

The results obtained are shown in Tables 1 - 3. No microbial growth was observed on all plates containing freshly prepared samples as shown by total aerobic counts. This was probably due to the adequate heat treatment given to the samples and the hygienic conditions under which the samples were prepared. From Table 1a and 1b, samples A (conventional juice with no treatment) and B (conventional juice with pasteurization) had total aerobic counts of 1.7 x10^2 and 3.0 x 10^1cfu/ml respectively, after one week of storage at room temperature. Refrigerated samples showed no growth after a week of storage. However, after 2 weeks of storage, an increase of 276 and 100% in total aerobic count was recorded in samples A and B while C (conventional juice with sodium benzoate) showed a growth of 1.5 x 10^2 cfu/ml – an indication of the inability of the preservative to preserve the drink beyond 2 weeks. Growth was observed in sample E (conventional juice with sodium benzoate, and pasteurization) after 3 weeks of storage at both ambient and refrigeration temperatures. Samples D and F preserved with sodium metabisulphite, and sodium metabisulphite and pasteurization respectively, showed no growth at both temperatures of storage even after six weeks.

From Tables 2a and 2b, it can be seen that none of the investigated samples showed any coliform growth shortly after processing and up to two weeks of storage at both temperatures of storage. This is an indication that the product maintained its good sanitary standards for the first two weeks of storage. Samples A and B exhibited presence of coliform thereafter (3 and 4 weeks of storage respectively). Presence of coliforms in any food sample renders such food as unsafe for human consumption [13] From this point onward, the tested roselle samples are considered unsuitable for drinking. Yeasts and moulds follow the same trend except that they were not isolated until after 4 and 5 weeks (Tables 3a and 3b).

Application of sodium metabisulphite with or without pasteurization extended the shelf-life to six weeks. Although the actual mechanism of action of SO_2 is not known, several possibilities have been suggested; among these postulates are presence of undissociated sulphorous acid, strong reducing power of sulphites which can reduce oxygen tension to a point below that at which aerobic organisms can grow or by direct action on some enzyme system of the spoilage microbes [17]. Sodium benzoate extended the shelf-life of roselle drink by 2 weeks without pasteurization and 3 weeks with pasteurization. It has been established that sodium benzoate acts against microorganisms by inhibiting the cellular uptake of substrate molecules. The preservative was also shown to

Table 1a: Total Aerobic Count of Roselle Juice Samples Stored at Ambient Temperature (cfu/ml)

Period of Storage	Sample A	Sample B (Pasteurized)	Sample C (with sodium benzoate)	Sample D (with sodium metabisulphite)	Sample E (with sodium benzoate & Pasteurization)	Sample F (with sodium metabisulphite & Pasteurization)
Week 0	Nil	Nil	Nil	Nil	Nil	Nil
Week 1	1.7 ± 0.3 x 10^2	3.0 ± 0.2x 10^1	Nil	Nil	Nil	Nil
Week 2	2.6 ± 0.2 x 10^2	1.0 ± 0.1x 10^2	1.5 ± 0.4 x 10^2	Nil	Nil	Nil
Week 3	7.3 ± 0.4 x 10^2	2.0 ± 0.2 x10^2	2.1 ± 0.3 x 10^2	Nil	7.0 ± 0.3 x 10^1	Nil
Week 4	2.5 ± 0.4 x 10^4	3.0 ± 0.3 x 10^2	3.7 ± 0.3 x 10^2	Nil	1.4 ± 0.3 x 10^2	Nil
Week 5	2.9 ± 0.3 x 10^4	4.0 ± 0.3 x 10^2	4.4± 0.5 x 10^2	Nil	1.9 ± 0.3 x 10^2	Nil

Values are mean (± SD) of triplicates

Table 1b: Total Aerobic Count of Roselle Juice Samples Stored at Refrigeration Temperature (cfu/ml)

Period of Storage	Sample A	Sample B (Pasteurized)	Sample C (with sodium benzoate)	Sample D (with sodium metabisulphite)	Sample E (with Sodium benzoate & pasteurization)	Sample F (with sodium metabisulphite & pasteurization)
Week 0	Nil	Nil	Nil	Nil	Nil	Nil
Week 1	Nil	Nil	Nil	Nil	Nil	Nil
Week 2	1.2 ± 0.4 x 10^2	6.0 ± 0.2 x 10^1	7.0 ± 0.4x 10^1	Nil	Nil	Nil
Week 3	2.0 ± 0.1 x 10^2	1.3 ± 0.4 x 10^2	1.5 ± 0.3x 10^2	Nil	4.0 ± 0.1 x 10^1	Nil
Week 4	2.5 ± 0.4 x 10^2	1.8 ± 0.3 x 10^2	2.1 ± 0.4x 10^2	Nil	9.0 ± 0.2 x 10^1	Nil
Week 5	2.2 ± 0.3x 10^3	2.3 ± 0.3 x 10^2	2.7 ± 0.3x 10^2	Nil	1.6 ± 0.3 x 10^2	Nil

Values are mean (± SD) of triplicates

Table 2a: Coliform Count of Roselle Juice Samples Stored at Ambient Temperature (cfu/ml)

Period of Storage	Sample A	Sample B (Pasteurized)	Sample C with (sodium benzoate)	Sample D (with sodium metabisulphite)	Sample E (with sodium benzoate & pasteurization)	Sample F (with sodium metabisulphite & pasteurization)
Week 0	Nil	Nil	Nil	Nil	Nil	Nil
Week 1	Nil	Nil	Nil	Nil	Nil	Nil
Week 2	Nil	Nil	Nil	Nil	Nil	Nil
Week 3	6.0 ± 0.2 x 10^2	Nil	Nil	Nil	Nil	Nil
Week 4	3.0 ± 0.4 x 10^3	9.1 ± 0.2 x 10^2	Nil	Nil	Nil	Nil
Week 5	3.6 ± 0.4 x 10^3	1.0 ± 0.4 x 10^3	Nil	Nil	Nil	Nil

Values are mean (± SD) of triplicates

Table 2b: Coliform Count of Roselle Juice Samples Stored at Refrigeration Temperature (cfu/ml)

	Sample A	Sample B (Pasteurized)	Sample C (with sodium benzoate)	Sample D (with sodium metabisulphite	Sample E (with sodium benzoate & pasteurization)	Sample F (with sodium metabisulphite & pasteurization)
Week 0	Nil	Nil	Nil	Nil	Nil	Nil
Week 1	Nil	Nil	Nil	Nil	Nil	Nil
Week 2	Nil	Nil	Nil	Nil	Nil	Nil
Week 3	4.0 ± 0.2 x 10^2	Nil	Nil	Nil	Nil	Nil
Week 4	3.9 ± 0.1 x 10^2	1.2 ± 0.1 x 10^2	Nil	Nil	Nil	Nil
Week 5	1.6 ± 0.4 x 10^3	1.0 ± 0.4 x 10^3	Nil	Nil	Nil	Nil

Values are mean (± SD) of triplicates

Table 3a: Yeast/Mould Count of Roselle Juice Samples Stored at Ambient Temperature (cfu/ml)

Period of Storage	Sample A	Sample B (Pasteurized)	Sample C (with sodium benzoate)	Sample D (with sodium metabisulphite)	Sample E (with sodium benzoate & pasteurization)	Sample F (with sodium metabisulphite & pasteurization)
Week 0	Nil	Nil	Nil	Nil	Nil	Nil
Week 1	Nil	Nil	Nil	Nil	Nil	Nil
Week 2	5.6 ± 0.4 x 10^2	Nil	Nil	Nil	Nil	Nil
Week 3	8.0 ± 0.5 x 10^2	Nil	Nil	Nil	Nil	Nil
Week 4	1.0 ± 0.4 x 10^3	9.4 ± 0.4 x 10^2	Nil	Nil	Nil	Nil
Week 5	2.2 ± 0.4 x 10^3	1.0 ± 0.4 x 10^3	Nil	Nil	Nil	Nil

Values are mean (± SD) of triplicates

Table 3b: Yeast/Mould Count of Roselle Juice Samples Stored at Refrigeration Temperature (cfu/ml)

Period of Storage	Sample A	Sample B (Pasteurized)	Sample C (with sodium benzoate)	Sample D (with sodium metabisulphite)	Sample E (with sodium benzoate & pasteurization)	Sample F (with sodium metabisulphite & pasteurization)
Week 0	Nil	Nil	Nil	Nil	Nil	Nil
Week 1	Nil	Nil	Nil	Nil	Nil	Nil
Week 2	Nil	Nil	Nil	Nil	Nil	Nil
Week 3	Nil	Nil	Nil	Nil	Nil	Nil
Week 4	1.1± 0.1x 10^3	7.4 ± 0.4x 10^2	Nil	Nil	Nil	Nil
Week 5	3.9 ± 0.3X 10^2	1.2 ± 0.3x 10^2	Nil	Nil	Nil	Nil

Values are mean (± SD) of triplicates

Table 4a: ^{0}Brix of Roselle Juice Samples at Ambient Temperature

Period of storage	Sample A	Sample B (Pasteurized)	Sample C (with sodium benzoate)	Sample D (with sodium metabisulphite)	Sample E with (sodium benzoate & pasteurization)	Sample F (with sodium metabisulphite & pasteurization)
Week 0	9.6± 0.4	9.6± 0.4	9.6± 0.2	9.6± 0.4	9.6± 0.1	9.6± 0.4
Week 1	9.6± 0.3	9.6± 0.4	9.6± 0.3	9.6± 0.2	9.6± 0.4	9.6± 0.5
Week 2	9.4± 0.4	9.6± 0.2	9.6± 0.4	9.6± 0.3	9.6± 0.3	9.6± 0.4
Week 3	9.2± 0.2	9.4± 0.4	9.6± 0.3	9.4± 0.4	9.4± 0.3	9.6± 0.5
Week 4	9.4± 0.4	9.2± 0.4	9.4± 0.3	9.4± 0.4	9.4± 0.3	9.4± 0.1
Week 5	9.5± 0.4	9.4± 0.4	9.5± 0.3	9.4± 0.4	9.5± 0.3	9.4± 0.1
Week 12	6.4± 0.3	8.4± 0.3	8.6± 0.2	8.8± 0.2	8.8± 0.4	8.8± 0.3

Values are mean (± SD) of triplicates

Table 4b: ^{0}Brix of Roselle Juice Samples at Refrigeration Temperature

Period of storage	Sample A	Sample B (Pasteurized)	Sample C (with sodium benzoate)	Sample D (with sodium metabisulphite)	Sample E (with sodium benzoate & pasteurization)	Sample F (with sodium metabisulphite & pasteurization)
Week 0	9.6± 0.4	9.6± 0.4	9.6± 0.2	9.6± 0.4	9.6± 0.1	9.6± 0.4
Week 1	9.6± 0.2	9.6± 0.2	9.6± 0.3	9.6± 0.4	9.6± 0.2	9.6± 0.4
Week 2	9.6± 0.1	9.6± 0.4	9.6± 0.3	9.6± 0.4	9.6± 0.4	9.6± 0.4
Week 3	9.4± 0.1	9.4± 0.3	9.6± 0.4	9.6± 0.2	9.6± 0.3	9.6± 0.3
Week 4	9.0± 0.2	9.2± 0.1	9.4± 0.4	9.4± 0.3	9.4± 0.2	9.6± 0.4
Week 5	9.0± 0.1	9.2± 0.3	9.4± 0.2	9.5± 0.1	9.4± 0.3	9.4± 0.2
Week 12	6.6± 0.3	8.4± 0.4	8.8± 0.3	8.8± 0.2	9.0± 0.2	9.0± 0.2

Values are mean (± SD) of triplicates

be more effective against coliforms and yeast/moulds. Benzoic acid and benzoates are known to be particularly effective against yeasts and moulds [18 & 16]. Heat treatment by means of pasteurization alone preserved the drink for a week. Use of higher temperature and /or longer exposure time could have enhanced the efficiency of this form of heat treatment. However, the need to preserve the flavor, chemical constituents and organoleptic property makes this approach unappealing and hence the need to use hurdle technology which essentially involves minimal heat treatment with chemical preservatives. The study has shown that use of 200 mg/l sodium metabisulphite was significantly more effective with or without pasteurization than 500 mg/l sodium benzoate without pasteurization ($p < 0.05$). The action of the latter was accentuated with the use of pasteurization. It is known that preservative techniques often work in a synergy [9].

4. Conclusion

From this study, it can be concluded that the shelf life of conventional juice can be extended to six weeks with addition of 200 mg/kg sodium metabisulphite) with or without pasteurization at 70 ^{0}C for 15 minutes. Use of 500 mg/kg sodium benzoate when combined with pasteurization can maintain the wholesomeness of the drink for 4 weeks. This hurdle technology can assist in preservation of its organoleptic properties as well as maintaining a low microbial load. Hurdle technology can make industrial production of roselle juice with acceptable quality and enhanced shelf-life possible.

REFERENCES

[1] Anonymous, Beverage World, A cultured Trend. *www.beverageworld .com.*2009 Assessed on May 14, 2009

[2] A. I. Ihekoronye, & P.O. Ngoddy, *Integrated Food science and Technology for Tropics* (Macmillan Publishers Limited, London, 1985)

[3] N.N Potter, N.N.& J.H.Hotchkiss, *Food Science.* (5th ed. Chapmann and Hall Inc, New York, 1995)

[4] A. A. Aramide, S. H . Abiose, & H.A. Adeniran, Microbial Evaluation of Probiotic Beverage from Roselle Extract *African Journal of Food Science. 1(12)*, 2009, 385-392.

[5] A. A. Aramide, Studies on the Extension of Shelf-life of Roselle (*Hibiscus sabdariffa*) juice - *An unpublished B.Sc Thesis* Submitted to Department of Food Science & Technology, Obafemi Awolowo University, Ile-Ife, Nigeria, 2002

[6] P. Tsai, J. McIntosh, P. Pearse, B. Camden & B. Jordan, Anthocyanin and antioxidant capacity in roselle (*Hibiscus sabdariffa L)* Extract. *Food Res. Int. 35*: 351 -356

[7] L. M. Perry, *Medicinal Plants of East and South East Asia* (MIT Press, Cambridge, 1980).

[8] Y. Qi, K. L. Chin, F. Malekian, M. Berhane, & J. Gager, Biological Characteristics, Nutritional and Medicinal value of Roselle (*Hibiscus sabdariffa L), Urban Forestry Natural Resources and Environment Circular No: 604*, Agricultural research and Extension Centre, Los Angeles, 2005

[9] M. S. Rahman, *Handbook of Preservation* (CRC Press, Taylor & Francis, Boca Raton, 2007)

[10] R. A. Adejonwo, Microbial and Chemical Composition of Roselle (*Hibiscus sabdariffa*) juice - *An unpublished B.Sc Thesis* Submitted to Department of Food Science & Technology, Obafemi Awolowo University, Ile-Ife, Nigeria, 2001

[11] B. A. Sorunke, Further Studies on the Extension of Shelf-life of Roselle juice- *An unpublished B.Sc Thesis* Submitted to Department of Food Science & Technology, Obafemi Awolowo University, Ile-Ife, Nigeria, 2008

[12] R. McCaleb, *Roselle Production Manual* (*Hibiscus sabdariffa*) (Herb Research Foundation, U.S.A., 1996)

[13] W.F. Harrigan, *Laboratory Methods in Food Microbiology* (Academic Press, San Diego, CA, 1998)

[14] L. Mclandsborough, *Food Microbiology*, CRC Press, Boca Raton, FL, 2005)

[15] H.A. Adeniran, S.H. Abiose & T.M. Ukeyima, Microbiological Assessment of probioticated ginger-based beverages. *Nutrition and Food Science 40 (2)*, 2010, 209 -220.

[16] A.O.A.C., *Official Methods of Analysis*, 19th ed. (Association of Official Analytical Chemists, Washington D.C., 2004)

[17] J.M. Jay, *Modern Food Microbiology* (6th ed.Chapmann and Hall, London, 2000)

[18] H.A Krebs, D. Wiggins, M. Stubs, A. Sols, F. Bedoya, Studies on Mechanisms of Antifungal action of Benzoate. *Biochem J. 214:* 1983, 657-663