

Chemoprotective Potential of Selected Preservatives against Spoilage Microorganisms Associated with Stored Zobo and Their Resultant Effects on the Juice's pH and Ascorbic Acid

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Authors' contributions

The work is a fruition of multi-disciplinary collaboration among all authors. Author LOA designed the study and wrote the protocol. Author SJJ managed the literature searches. Author OPO wrote the first draft of the manuscript. Author TOF performed the statistical analysis and interpreted the results. All authors took part in the analyses, read and approved the final manuscript.

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ABSTRACT

Samples of extracts from *Hibiscus sabdariffa* calyxes were stored with different preservatives (sodium benzoate, sodium metabisulphite, boric acid and citric acid) for eight weeks and studied fortnightly for change in certain parameters. A sample (control) was also stored without preservative for the same period. The research was carried out to study the possible effect(s) of these chemical preservatives on the pH, ascorbic acid and microbial growth of the juice. Up to 4

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weeks of storage, all the samples were not statistically different at 95% confidence level except sample preserved with sodium benzoate with respect to vitamin C. In the same manner, with exception of samples without preservative and the one stored with sodium metabisulphite, other samples were not significantly different at $P < 0.05$ with respect to pH. Generally, the findings revealed an increase in acidity (decrease in pH), loss of vitamin C and increase in microbial population as the duration of storage was elongated in all the samples but much more explicit in the sample without preservative. Likewise, the rate of change of pH and percentage loss of vitamin C with respect to storage time in the sample without preservative increased tremendously compared to other samples. The results bare that the use of preservatives prohibited rapid loss of nutrients and inhibited the microorganisms that cause spoilage on storage of this juice of hidden health benefits. The use of preservatives to prolong the shelf life of this juice is therefore advocated so as to make it more available round the year.

Keywords: *Hibiscus sabdariffa*; preservatives; storage time; ascorbic acid; acidity; microorganisms.

1. INTRODUCTION

Roselle (*Hibiscus sabdariffa*) is a tropical plant of considerable economic potentials. Its calyxes have been used as food colourants, emulsifier for carbonated drinks, jam manufacture, juice and wine production, beverage, tea, jelly, syrup, gelatin, puddings, ice cream, butter, pies, sauces, food preserves and soup thickening [1,2,3,4].

It also has many medicinal applications to cure kidney stone, pyrexia, liver damage, hypertension and leukemia [5]. *H. sabdariffa* had proved to have a wide range of therapeutic properties as reported by many researchers elsewhere, among which are: antioxidant [2], antibacterial [6,7], antihypertensive [8,9], hepatoprotective [10]; antihyperlipidemic [11]; and anticancer [12], to mention but a few. The calyx of roselle is traditionally used as antiseptic, aphrodisiac, astringent, cholagogue, demulcent, digestive, diuretic, emollient, purgative, refrigerant, sedative, stomachic and tonic [13]. The calyxes are potentially a good source of anthocyanins, ascorbic acid, dietary fibre, vitamins, minerals and bioactive compounds such as organic acids, phytosterols and polyphenols, some of which have antioxidant properties [13,14,15].

Vitamin C, also known as ascorbic acid, which is a water soluble vitamin, is synthesized from sugars which are produced during the process of photosynthesis in plants [16]. Humans depend on exogenous sources of vitamin C which include fruits, vegetables, food supplements and pharmaceutical preparations because human body cannot synthesize it. It has been confirmed that vitamin C aids healing of wounds and

fractures [17], prevent blood clotting, enhances the body resistance to diseases but sensitive to some factors like heat, air and storage [18].

Roselle juice popularly called *Zobo* in Nigeria, which is a conventionally made from water extraction of *H. sabdariffa* calyxes, is now popular with daily consumption in the country and in many other tropics. However, the dried calyxes used in the production of this drink, like any other raw material or food is susceptible to deterioration by food borne microbes which can lead to reduction in quality of the drink in terms of colour, taste and nutrition. A large number of lactic acid bacteria, coliforms, moulds and yeasts cause spoilage as they can use the carbohydrate content for fermentation processes, producing undesirable changes. The products of fermentation affect organoleptic properties [19]. Most of the fungal contaminants can cause spoilage and they are known to produce mycotoxins which are detrimental to human health. Aflatoxin, a group of toxic metabolites produced by certain *Aspergillus* species present in fermented roselle drink [19], had been found to be carcinogenic, tetragenetic and mutagenic to several species of experimental animals [20,21].

Nutrients stability in roselle juice depends on a combination of various factors such as structure of anthocyanins, pH, temperature, oxygen, light and water activity. Enzymatic degradation and interactions with food/drink components such as ascorbic acid, sugars, metal ions, sulphurdioxide and condiments are no less important [22]. The presence of natural organic acids and antioxidants prevent rapid degradation and browning of fruit juices. In this way, they prolong the shelf life of the juice. Addition of synthetic preservatives improves the shelf life of such fruit juices [4]. Production of high quality fruit juices

on commercial scale has been affected by a number of factors, which include excessive browning, hazes or precipitates formation and flavour change on storage [4]. Chemical additives such as sulphites have been applied in browning [23], thereby prolonging the shelf life of fruit juice and wine. Preservatives such as sodium metabisulphite (0.5g/L) are known to inhibit the growth of undesirable microorganisms [24].

Fruit drinks from apple, pineapple and orange have been long in the market while *zobo* drink prepared from *H. sabdariffa* is new, but it is a proven competitor among packaged fruit juices. Despite the consumer acceptability of drink from *H. sabdariffa* calyxes and its health benefits [14,19,25], it is prone to microbial deterioration on storage. In the light of the above submission, this research studied the inhibitory potential of some chemical preservatives against microorganisms that are responsible for its spoilage and the resultant effect on the pH and ascorbic acid contents of *H. sabdariffa* juice extract, thereby aiming at determining their potencies in prolonging the shelf life of this drink of concealed health benefits.

2. EXPERIMENTAL DETAILS

2.1 Sample Treatment and Preparation

The calyces of *H. sabdariffa* (purple type popularly cultivated in the Northern part of Nigeria) used in the present study was purchased from Eke market, Afikpo, Nigeria. The samples were sorted to remove dirt and stones. They were then sun-dried for three days after which a part was rinsed with distilled water and blanched in boiled water for about 30 minutes to extract the juice from the calyces. Filtration was carried out and the filtrate was divided into five parts. Four different preservatives were added to each portion separately while no preservative was added to a portion, which serves as the control. The quantities of preservatives added were followed according to IPAN [26] prescriptions. The samples were bottled separately, covered and pasteurized and labeled as follows: sample with sodium benzoate as preservative (SSB), sample stored with sodium metabisulphite (SSM), sample preserved with boric acid (SBA), sample stored with citric acid (SCA), and sample without preservative (SWP). They were cooled and stored under room temperature through out the period of study.

Vitamin C contents, pH values and colony counts of the samples were determined on the day of production and at two weeks interval for the period of eight weeks, according to standard methods [27,28].

2.2 Data Analysis

Data were analyzed statistically by one way analysis of variance (ANOVA) technique. Means were compared by the Duncan' multiple range test and significance was established at 95% level ($P = .05$) using SPSS 2008 version 15.0 package.

3. RESULTS AND DISCUSSION

3.1 Results

With exception of SWP and SSM, other samples were not significantly different at $P = 0.05$. Generally, prolonging the storage time gave rise to increase in acidities in all the samples.

Up to four (4) weeks of storage, all the samples were not statistically differ at 5% confidence level except SSB. As the storage time was prolonged, there was general decrease in vitamin C contents in all the samples.

3.2 Discussion

On production day, the pH of the sample without preservative was 3.62. However, there were either increases or decreases in the pH values in other samples, which may be due to the influence of the chemical nature of preservatives used. The value obtained for the fresh juice was consistent with what was obtained by Fasoyiro et al. [5] on the same plant calyx extract in which the pH was found to be 3.10. It was less acidic compared to what was recorded (pH = 2.73) by Abuo-Arab et al. [1]. It was acidic compared to other locally made beverages like kunu (pH = 4.9) [29]. pH is an index of acidity of a substance. At low pH (high acidity), the growth of microbes is inhibited.

The microflora of finished products depends on the processing and storage conditions. Food microorganisms thrive in medium rich in fermentable substances such as sugars, which can easily be degraded with production of acids [19]. This might be responsible for the drop in pH values as the storage time was extended. The pH of the drinks decreased with time reflecting

an increase in acidity (Table 1). The result indicated a significant ($P < 0.05$) decrease in the pH of the samples stored with preservatives compared to the control. There were slight differences in the pH values of all the samples with respect to storage time, except sample without preservative (Fig. 1).

The high acidity of the juice is attributed to the presence of naturally occurring organic acids such as malic, citric and oxalic acids in the extract of *Hibiscus sabdariffa* [30]. Therefore, ulcer patients and people with other related problems are advised to desist from large consumption of the extract. However, with proper fortification with other less acidic fruit juices [31], it will be a good drink for all and sundry. There was a slight change in the acidity (pH) of the sample without preservative just like those with preservatives for the first two weeks of storage. This suggests that the juice contains enough

natural acids and antioxidants which prevented it from fast degradation. This is an indication that the drink is stable for at least two weeks without addition of preservatives.

Vitamin C (Ascorbic acid) is an organic substance required in minute amount for efficient metabolism of the body. Vitamin C is involved in many aspects of human metabolism. Some of which are the role it plays in the utilization and metabolism of amino acids, lipids and collagen formation [32,33]. It also guards against scurvy and boosts human immune system. It is an antioxidant which is very important in human nutrition. Its deficiency in human is known to cause scurvy, a disease of bones and teeth [17]. Addition of different preservatives maintained the vitamin C contents of the samples being comparable to the control (without preservative) on the day of production (Table 2).

Table 1. Results of pH values of the juice samples

| Sample | Prod. day | 2 weeks | 4 weeks | 6 weeks | 8 weeks |
|--------|------------------------|------------------------|------------------------|--------------------------|------------------------|
| SWP | 3.62±0.01 ^a | 3.58±0.01 ^b | 3.45±0.12 ^c | 3.36±0.16 ^d | 3.25±0.21 ^e |
| SSB | 3.65±0.05 ^a | 3.64±0.00 ^a | 3.61±0.01 ^b | 3.60±0.06 ^b | 3.58±0.09 ^c |
| SSM | 3.65±0.10 ^a | 3.63±0.02 ^b | 3.57±0.02 ^c | 3.53±0.00 ^d | 3.50±0.12 ^e |
| SBA | 3.55±0.14 ^a | 3.54±0.01 ^a | 3.50±0.06 ^b | 3.48±0.05 ^{b,c} | 3.46±0.17 ^c |
| SCA | 3.53±0.03 ^a | 3.52±0.07 ^a | 3.49±0.11 ^b | 3.47±0.01 ^{b,c} | 3.46±0.03 ^c |

Values followed by the same superscript alphabets in the same row are not significantly different but those followed by different alphabets are significantly different using Duncan's multiple range test at $P = .05$

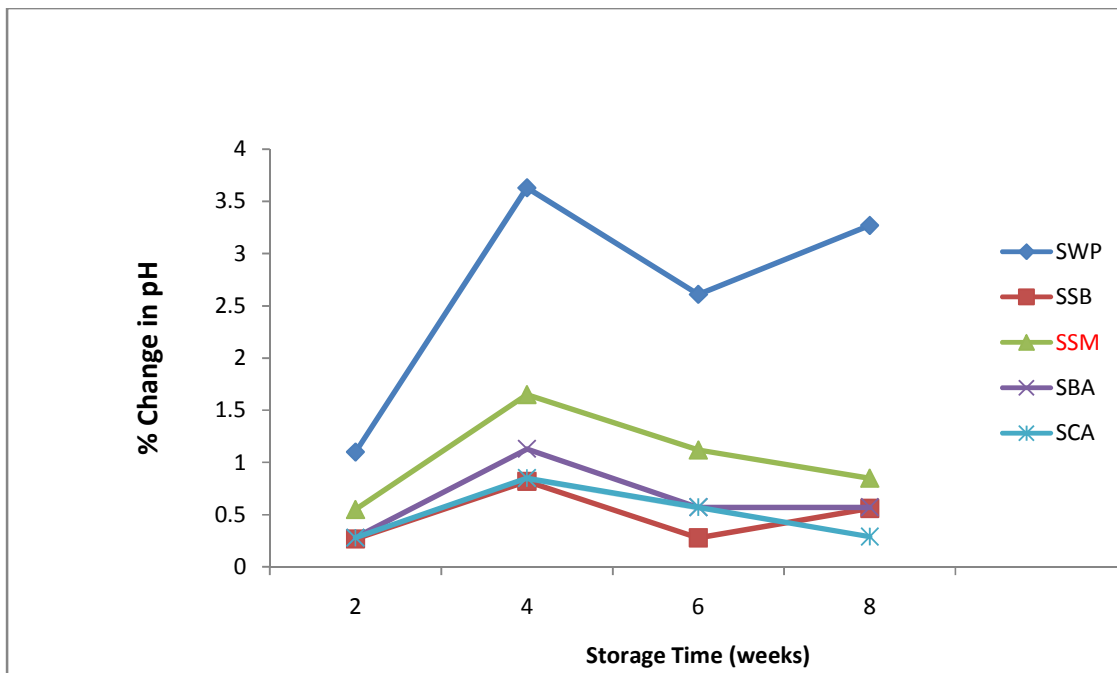


Fig. 1. Change in pH of the juice samples with respect to time

Generally, the result of ascorbic acid revealed that there was a general significant ($P < 0.05$) decrease as the storage time was elongated. Although, the values for samples with preservatives were generally lower than that of the control (SWP), the difference were not statistically significant ($P > 0.05$). The vitamin C content of the juice before addition of preservatives and storage gave 34.11mg/100g. This value was high enough and impressive as it was within the values found in many other fruits [34]. It was also in agreement with 31.33mg/100g reported by Fasoyiro et al. [5] but lower than 140.13mg/100g recorded for the same calyx extract by Abuo-Arab et al. [1]. The lower value of vitamin C of roselle juice as reported in the present study could be attributed to nutrients losses during drying of the calyces, processing, cultural used and climatic factors. Vitamin C is water soluble [5] and therefore could also be lost during boiling, heating and cooking.

Either with or without preservatives, fresh juice samples gave the highest ascorbic acid contents when compared with values obtained for other weeks of storage (Table 2). It was observed that there was a linear decrease in the vitamin C contents as the duration of storage was extended in all the samples, but it was much more pronounced in the sample without preservative, that is, deterioration (% loss) was found to be at the highest in SWP (Fig. 2). This was evidenced in its microbial population (Table 3).

No microorganism was detected in the fresh drinks on the day of production (Table 3). This may be as a result of aseptic procedures that were observed during processing since the drinks were pasteurized, which might have killed the errant microbes inherent in ingredients and processing apparatus. Microbial population

increased as the storage time was prolonged in most samples with exception of SSM but spoilage was very pronounced in the absence of preservative (Table 3). Microbiological stability is of great concern to soft drink producers. Most beverages contain between 90-99% water and therefore have a relatively high risk of spoilage if not properly treated. Spoilage occurred between four and six weeks after production (Table 3). Apart from microbial growth, other signs of spoilage include sedimentation, cloud formation, presence of offensive odour or an increase in carbon dioxide due to fermentation [26]. It has been established that the use of high temperature (pasteurization) destroyed most mesophilic organisms while addition of preservatives control the proliferation of those remaining during storage [19]. This could be the reason why the unpreserved juice had most microbial counts.

There was a drop in the pH and vitamin C contents of the drinks as the time of storage elongated. This is an indication that storage negatively affected all the samples either with or without preservatives, though the increase was more obvious in sample stored without preservative. This result agreed with the earlier reports [19,31]. The difference in results of pH and vitamin C in our report and those of other authors on the same calyx extracts could be due to factors that affect ascorbic acid levels and pH values in fruits, which include processing technique, climate, temperature and amount of nitrogen fertilizers used in growing the plants [34].

Sodium metabisulphite and citric acid retarded the growth of microorganisms up to six weeks. Others that showed less inhibitory actions against microbial growth may not be potentially active in *zobo* (which is slightly acidic) but may be active in basic substances.

Table 2. Results of vitamin C content (mg/100g) of the juice samples

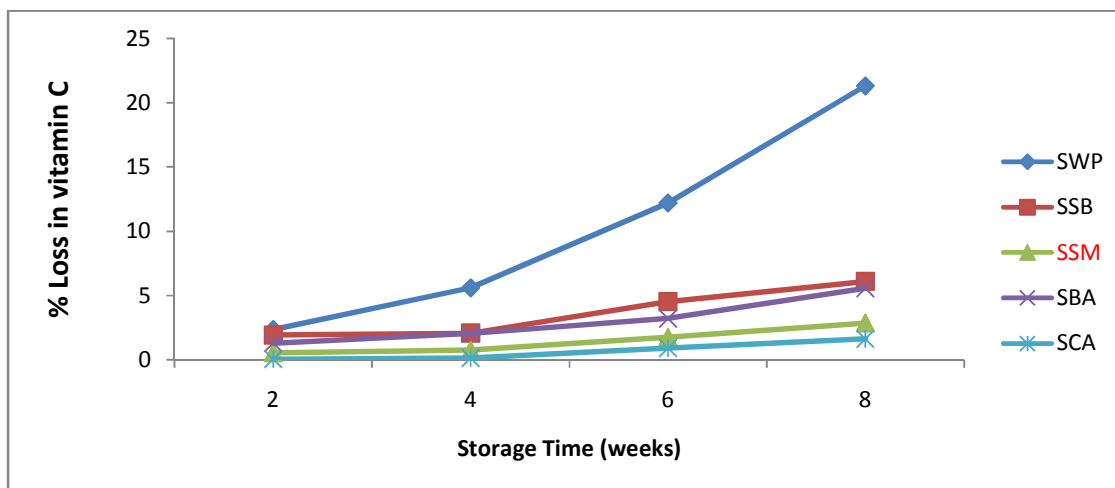
| Sample | Prod. day | 2 weeks | 4 weeks | 6 weeks | 8 weeks |
|--------|-------------------------|---------------------------|---------------------------|---------------------------|-------------------------|
| SWP | 34.11±0.25 ^a | 33.31±0.41 ^a | 32.20±0.32 ^{a,b} | 29.95±0.40 ^b | 26.84±2.70 ^c |
| SSB | 34.67±0.07 ^a | 34.00±0.04 ^b | 33.95±0.58 ^b | 33.10±0.09 ^c | 32.56±0.28 ^c |
| SSM | 34.52±0.09 ^a | 34.33±0.36 ^{a,b} | 34.26±0.21 ^{a,b} | 33.91±0.49 ^{b,c} | 33.53±0.03 ^c |
| SBA | 34.55±0.05 ^a | 34.10±0.04 ^{a,b} | 33.84±0.53 ^{a,b} | 33.44±0.57 ^b | 32.63±0.63 ^c |
| SCA | 34.42±0.03 ^a | 34.40±0.40 ^a | 34.37±0.07 ^{a,b} | 34.10±0.15 ^{a,b} | 33.86±0.31 ^b |

Values followed by the same superscript alphabets in the same row are not significantly different but those followed by different alphabets are significantly different using Duncan's multiple range test at $P = .05$

Table 3. Results of colony forming units (CFU) of the juice samples

| Sample | Production day | 2 weeks | 4 weeks | 6 weeks | 8 weeks |
|--------|----------------|---------|---------|---------|---------|
| SWP | ND | 10 | 25 | 32 | 36 |
| SSB | ND | ND | 8 | 19 | 24 |
| SSM | ND | ND | ND | ND | ND |
| SBA | ND | ND | 5 | 12 | 21 |
| SCA | ND | ND | ND | ND | 7 |

ND = Not Detected

**Fig. 2. Loss of vitamin C in the samples with respect to time**

4. CONCLUSION

Zobo drink is cheaper and provides more nutrients than many other sugar laden drinks prominent in the market. It is therefore recommended that industrial production with effective preservatives like sodium metabisulphite among others be encouraged in order to ensure all year round availability of the drink so as to meet up with the longing for fluid intake in tropical countries.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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