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Effects of Boiling Time on Mineral and Vitamin C Content of Three Varieties of *Hibiscus sabdriffa* Drink in Nigeria

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Abstracts: The effect of heat treatment on the mineral and vitamin C content of non-alcoholic drink obtained from three varieties of zobo calyx (*Hibiscus sabdariffa);* dark red, bright red, wine and subjected to different time of preparation conditions were investigated. 50g of each of the samples were boiled separately in one litre of water for 5, 10 and 15mins at 100±0.2°C to extract the juice. The ash, pH, vitamin C and individual mineral content were assayed for all varieties. The dark red variety has the highest ash content (16.37±0.84%) and least in bright red variety at 10mins (14.05±0.63). Bright red variety at 15 mins (137.48 mg L^{-1}) was found to have the highest calcium level while dark red at 5mins and wine at 5 mins recorded the least. Potassium was found to be high in dark red at 15mins (403.11 mg L^{-1}) and low in bright red at 5mins (324.32 mg L^{-1}). Sodium was high in wine variety at 10mins (9.98 mg L^{-1}) of boiling while the least was in bright red variety at 10mins (5.53 mg L^{-1}). Magnesium level was the highest in dark red variety at both 5 and 15mins (78.23 mg L^{-1}) and low in bright red variety at 5mins (54.67 mg L^{-1}). Dark red variety at 10mins has the highest level of vitamin C (36.24mg/100ml) while bright red variety at 5 mins has the least (25.66mg/100ml). Dark red variety at 15mins was rated highest for sensory evaluation, there was no significant difference with wine variety at 15 mins while bright red variety at 5mins was rated least. It was therefore concluded that heat treatment had no effect on the minerals but had an effect on vitamin C content. Furthermore, each of the varieties had its peculiar nutritional contents, therefore, the choice depends on the producer and consumer; however from the sensory evaluation the dark red variety at 10 and 15mins may be the best variety and time of preparation.

Key word: Dark red · Bright red · Sensory evaluation · Wine

INTRODUCTION

Zobo drink is a local, cheaper soft drink made from *Hibiscus sabdariffa* calyx which is an herbaceous medicinal plant grown in the tropics. It has a wide range of names worldwide depending on the locality which had earlier been documented by various authors [1]. In Nigeria, it is known as *Isapa pupa* in the South Western part of the country and *Zobo* in the Northern part [2]. Virtually all parts of the plant can be utilized for food, drink and medicine. Yadong *et al*, [3] reported that the calyx drink is rich in riboflavin, ascorbic acid (AandC), niacin, carotene, calcium and iron that are nutritionally important. The juice can be extracted by using either cold or hot water depending on choice. The drink serves as a cheaper alternative to the industrially produced carbonated soft drinks that is available in all parts of the country [4]. The demand for zobo drinks due to its low prices, nutritional and medicinal properties is on the high side [5, 6]. It is served indoors or at special occasion to people of various tribes and tradition, in fact the drink has gained a wide and general acceptance in Nigeria. There are two main types: *H. sabdariffa var. altissima and var. sadariffa. Altissima* is cultivated for its economic importance in jute-like fiber making while *sabdariffa* is exploited for its calyx and fiber [7]. In Nigeria, the processing method and choice of variety depends on individual's preference ranging from cold water extraction to hot water extraction and at different length of time (even for as long as thirty minutes) as long as the extract

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is out leading to variation in the quality. Many people process it without considering the effect of heat on the nutritional parameters imbedded especially the vitamins which are easily susceptible to destruction. Therefore this work was done to ascertain the effect of heat on the mineral and vitamin C Content of three varieties of *Hibiscus sabdariffa* drink found in Nigeria.

MATERIALS AND METHODS

Source of Sample: Matured dried dark red, bright red and wine petals of *H. sabdariffa* used for this study were obtained from the open market in Kano, Kano State, Nigeria.

Processing: The three varieties of zobo were picked to separate dirts from the calyx. 50g of each were weighed into a clean sterile five litre beaker and washed lightly to remove dusts. Each sample was poured into one litre water boiling at 100°C i.e. 1:50g(w:v).The extraction time was varied (i.e. 5, 10 and 15 min respectively) while the extraction temperature was kept constant (i.e. 100 ±2°C). At the end of each hot extraction, the extract was filtered using a clean muslin cloth after which the extract was cooled to ambient temperature (30° ±2°C) and stored in a pre-sterilized wide glass bottles and kept for further analysis.

Chemical Analyses: Ascorbic acid was determined by using titrimetric method as described by James [8]. The concentration of ascorbic acid in the sample was expressed as mg ascorbic acid /100 ml. The pH of beverage was measured using a digital pH meter (model EA513-055, ELE, England) standardized with buffer solutions of 4.0 and 7.0. The mineral was determined using the method described by AOAC [9].

Sensory Evaluation: A 15-member taste panel (members were familiar with the beverage) was requested to rate the sample using a nine- point hedonic scale (i.e 9 = like extremely; 5=neither like nor dislike; 1= dislike extremely). The scores from the rating were subsequently subjected to analysis of variance (ANOVA) and means separated using Duncan Multiple Range test [10, 11].

Statistical Analyses: All the analyses reported in this study were carried out in triplicates. In each case, a mean value and standard deviation were calculated. Analysis of variance (ANOVA) was also performed and separation of the mean values was carried out using Duncan Multiple Range Test at $p<0.05$ [12].

RESULTS AND DISCUSSION

The pH of the three varieties of zoborodo is shown in Table 1. The value ranges between 2.49±0.06 to 2.73±0.02 in all varieties and at 5, 10, 15 minutes of boiling which is indicating and confirming the high acidity usually noticed in zobo calyx and drinks. There was no significant difference among the three varieties. The presence of hibiscus, malic, oxalic, citric, succinic, tartaric and 3-indolyl acetic acids which naturally exist in acidic fruit is responsible for this high acid concentration [13, 14]. Low acid value of the three varieties zobo drink may affect people with stomach ulceration and may not be an ideal drink. High acidity will impact a preservative effect by inhibiting the growth of some microorganisms that are not tolerant to it thereby giving it a longer shelf life soft drink [13].

The ash and mineral contents are shown in Table 2. Generally, the dark red variety has the highest ash content of 16.37±0.84, 16.76±0.91 and 16.94.±1.09%, the bright red having 14.25±1.07, 14.05±0.63 and 14.12±0.29 while the wine variety has 14.40±1.79, 14.71±3.74 and 14.90±1.85 for 5,10 and 15 minutes respectively. The dark red variety at 15 mins has the highest while the bright variety at 10 mins has the lowest ash content. Ash content gives an indication of minerals present in a particular food sample and it is very important in many biochemical reactions which aid physiological functioning of major metabolic processes in the human body [15]. There was a significant difference ($p>0.05$) in the dark red variety as compared to the bright red and wine variety. There was no significant difference among the varieties at different time of boiling indicating that heat treatment had no significant effect on the ash content. It was observed that as the temperature increases, the bio-availability of the inorganic matter increased gradually and caused the slight increase.

Bright red variety at 15 mins (137.48 mg $L^{-}1$) was found to have the highest calcium level while dark red at 5mins and wine at 5 mins recorded the least indicating that at higher temperature the minerals are released as shown in Table 2. It was recorded to be the second highest level of mineral found in the beverage making this result to agree with the work of Okeniyi and Kolawole [16]. Calcium is vital for healthy teeth, bones, aids muscles growth and prevent muscles cramps. Consuming zoborodo will definitely contribute to the Required Daily Allowance (RDA) of calcium in infants and adults. Potassium content recorded the highest level of mineral in the drink and was found to be higher in dark red at 15mins (403.11 mg L^{-1}) and low in bright red at 5mins (324.32 mg L^{-1}). Potassium plays a large role in supporting the nervous system and

Table 1: The pH of dark red, bright red and wine variety of zobo drink prepared at different times Extraction time at 100 °C

Variety	5mins	10mins	15mins
Dark red	2.73±0.02[a]	2.56±0.04[ab]	2.61±0.04[a]
Bright red	2.52±0.07[ab]	2.53±0.07[a]	2.49±0.06[ab]
Wine	2.61±0.03[a]	2.62±0.08[a]	2.59±0.07[ab]

Mean values followed by the same superscripts in each column are not significantly different at P<0.05.

Table 2: The ash (%) and mineral content(mg L^{-1}) of dark red, bright red and wine variety of zobo drink

Sample	Ash (%)	Ca	K	Na (mg L^{-1})	Fe	Zn	Mg	Pb
Dark red								
5mins	16.37±0.84[a]	125.21[c]	396.25[a]	6.39[c]	0.67[b]	1.26[a]	78.23[a]	0.02[a]
10mins	16.76±0.91[a]	126.71[c]	402.34[a]	6.86[b]	0.67[b]	1.05[ab]	77.89[a]	0.02[a]
15mins	16.94.±1.09[a]	129.52[b]	403.11[a]	6.87[b]	0.83[ab]	1.20[a]	78.01[a]	0.03[a]
Bright red								
5mins	14.25±1.07[b]	133.83[ab]	324.32[c]	5.54[c]	0.58	1.00[ab]	54.67[c]	0.01[a]
10mins	14.05±0.63[b]	136.70[a]	326.68[c]	5.53[c]	0.67[b]	0.98[b]	55.56[c]	0.02[a]
15mins	14.62±0.29[b]	137.48[a]	332.24[c]	5.56[c]	0.68[b]	1.12[ab]	57.11[c]	0.02[a]
Wine								
5mins	14.40±1.79[b]	127.66[c]	386.36[b]	9.43[a]	0.98[a]	1.13[ab]	74.32[b]	0.02[a]
10mins	14.71±3.74[b]	125.93[c]	387.49[b]	9.98[a]	0.96[a]	1.00[ab]	75.55[b]	0.02[a]
15mins	14.90±1.85[b]	126.06[c]	391.04[a]	9.01[a]	1.00[a]	0.99[b]	75.89[b]	0.02[a]

Values are means of triplicate determinations ±SEM; Means bearing different superscripts on the same column are statistically different (p<0.05)

natural heart rhythm. It stabilizes blood pressure and helps in electrochemical transmissions and has been shown to prevent strokes and works with sodium to maintain a proper water balance in the body [17]. Zobo drink being highly rich in potassium may be used therapeutically in the area of medicine and to meet the RDA in infants and adults. Generally, the bright red variety is lower in potassium content as compared to dark red and wine varieties. Sodium level is quite low in all the samples as seen in Table 2, but the highest was observed in wine variety at 10mins of boiling while the least was in bright red variety at 10mins. The trend follows as; wine to dark red to bright red. In most body cellular function especially in Na^+/K^+ pump. This causes influx of K^+ into intracellular fluids and efflux of Na^+ accompanied with Cl^- and water resulting in diuresis. This could account for the acclaimed diuretic effect and property of zobo drink as claimed by Osuntogu and Aboaba, [18] and Delgado-Vargas and Paredes-López [19]. Iron content in all the samples was low, though it is regarded as trace mineral. There was no significant difference in all the samples and at different time of boiling. All were in the range of 1.00 mg L^{-1} in wine at 15mins to 0.58 mg L^{-1} bright red at 5 mins. It was observed that at higher temperature of 15 mins, the Iron content increased indicating that heat did not deactivate or destroy it. Iron is vital for the production of haemoglobin, formation of red blood cells and the oxygenation of red blood cells. It also improves circulation, digestion, elimination and respiration. Zinc is also one of the trace elements that is required in small quantity in the body. The recommended daily intake is between 8-15 mg L^{-1} and doses larger than 25 mg may cause anaemia and copper deficiency [20]. Zinc promotes a healthy immune system, taste, smell, joint and connective tissue, cell division, repair and growth and helps in the proper functioning of insulin [17]. The range for the three varieties was very low between 1.26-0.98 mg L^{-1} and there was no significant difference (p>0.05) among them. Zobo drink can still be used to supply part of the daily requirement. Magnesium is rated the third highest among the minerals in zobo drink. The level is the highest in dark red variety at both 5 and 15 mins and is low in bright red variety at 5mins. This result agrees with the work of Okeniyi and Kolawole [16], who discovered that the drink is very rich in Magnesium. It is reported that magnesium is a co-factor in a number of enzyme system and is involved in neuro-chemical transmission and muscular excitability along with calcium and vitamin C.

Lead level in the drink was very low (0.03-0.01 mg L^{-1}) and quite negligible. The body is exposed to high level of lead on daily basis due to fumes from generators and exhaust pipes of cars. Lead is very toxic when accumulated in the body system and can even cause lead poisoning or death. The low level makes the drink safe for consumption.

Table 3: The vitamin C content(mg/100g) of the three varieties of zobo drink prepared at different times

Sample	5mins	10mins	15mins
Dark red	34.32±2.13[ab]	36.24±1.76[a]	33.71±1.09[b]
Bright red	25.66± 0.62[c]	26.12±1.62[c]	21.33±0.99[d]
Wine	27.21 ± 1.21[c]	29.07± 2.70[b]	24.39±2.01[d]

Mean values followed by the same superscripts in each column are not significantly different at P<0.05.

Table 4: Physical characteristics of the three varieties of zobo drink prepared at different time

Sample	Appearance/colour	Taste
Dark red		
5mins	red	slightly sour
10mins	dark red	sour
15mins	brilliant red	very sour
Bright red		
5mins	moderately pink	slightly sour
10mins pink	slightly	sour
15mins	red	slightly sour
Wine		
5mins	dirty red	slightly sour
10mins	red	slightly sour
15mins	wine/deep purple	sour

Table 5: Sensory evaluation of dark red, bright red and wine variety of zobo drink after 13% sugar inclusion

Sample	Appearance	Taste	Aroma	General Acceptance
Dark red				
5mins	5.9[b]	7.3[a]	6.2[b]	6.3[b]
10mins	6.6[a]	5.7[b]	6.9[a]	6.8[a]
15mins	7.8[a]	5.6[b]	6.7[a]	7.1[a]
Bright red				
5mins	2.1[d]	5.4[b]	6.0[b]	4.2[d]
10mins	4.1[c]	5.1[b]	6.2[b]	5.3[c]
15mins	5.5[b]	5.0[b]	6.9[a]	6.0[b]
Wine				
5mins	4.3[c]	5.9[b]	5.9[b]	5.5[c]
10mins	5.8[b]	5.0[b]	6.3[b]	6.0[b]
15mins	7.1[a]	5.4[b]	6.7[a]	6.2[b]

Means not followed by the same letter in the same column differ at 5% level of significance

The ascorbic content of the samples are shown in Table 3. Dark red variety at 10mins has the highest level of vitamin C (36.24mg/100ml) while bright red variety at 5 mins has the least (25.66mg/100ml). Generally, the dark red sample seems to record higher vitamin C level followed by wine variety and bright red. It was also observed that they all follow the same trend of action at 10mins in all varieties; the level was higher than at 5mins and 15mins. It can be deduced that at 5mins the aggregation of vitamin C has just begun (being water soluble vitamin) while at 10mins it has reached the peak and any further subjection to heat tends to destroy it. At 15mins in all varieties, they are gradually subjected to denaturation, further heating may tend to bring the level to zero as said by Nagy and Smoot [21]. The presence of ascorbic acid in the beverage essentially confirms the nutritional benefit of the drink to the consumers and agrees with Mat Isa [22] and Tee *et al*. [23] who indicated that *H.sabdariffa* calyx and drink contain higher vitamin C than guava, orange and black currant.

From Table 4, it was observed that the physical appearance of the beverage especially colour largely depends on the pigment anthocyanin which is responsible for the characteristics red colour of most *H. sabdariffa* as reported by Hong and Wrostlad, [24] and Tsai and Huang, [25].

An increase in boiling time seems to favour its extraction making the colour intensity deeper from red at 5mins in dark red to brownish red at 15mins. Also, from moderately pink at 5 mins in bright red variety to red and from dirty red colour in wine variety at 5 mins to wine at 15 mins. The stability or degradation of the brilliant red colour of the beverage is dependent on such factors as pH, light, temperature and oxygen [26].

Therefore, the variation in the colour intensity of zobo drink is most probably related to the calyx variety, water ratios and the extent of boiling time. The sourness in all variety may be related to the high vitamin C content which has a pleasant acidic/sour property without the addition of sweetener [27].

The sensory evaluation rating of zobo drink after adding 13% sugar and 100mls of strawberry flavour is presented in Table 5. Dark red variety at 15mins was rated highest by the panel member in terms of appearance while there was no significant difference with wine variety at 15 mins while bright red variety at 5mins was rated least. The variety of H.*sabdariffa* to a large extent determines the colour and appearance of the drink. Commercially, colour and taste are known to play a principal role in the acceptability of zobo beverage by the consumers [28]. All the varieties at 5 mins were rated higher than at 10 and 15mins and this could be that the lightness of the drink at that time favours sugar addition, thereby making it sweeter than when sour. Although, dark red variety is rated higher than other varieties, all other varieties compete favorably. Dark red variety at 15mins has the highest general acceptability among all.

CONCLUSION

Present research has shown that high temperature reduces the ascorbic acid content and had no significant effect on mineral content on the three zobo varieties. It was therefore concluded that the three varieties had their peculiar nutritional benefits, therefore, the choice depends on the producer and consumer. However, from the sensory evaluation, the dark red variety at 10mins and 15mins has a higher vitamin C content and general acceptability respectively, it can thus be said that dark red variety at 10 and 15mins may be the best variety and time of preparation, respectively.

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