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Assessment of Nutritive and Preservative Impact of Enriching Zobo (*Hibiscus sabdariffa* Calyx) Drink with Moringa Extract

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Authors' contributions

This research work was done in collaboration with all the authors. Author SOB designed the work and wrote the protocol. Authors EDO and ME conducted the experiment and wrote the first draft of the manuscript. Author ME did the literature search. All authors read and approved the final manuscript.

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ABSTRACT

This study was aimed to determine the nutritive and preservative potential of enriching Zobo drink with *Moringa* leaf extract. Hot water extraction of Zobo drink produced with ginger and garlic gloves was separated into four different samples M_0, M_{10}, M_{20} and $M_{30.}$ To samples M_{10}, M_{20} and M_{30} 40 g, 80 g and 120 g of *Moringa* leaf powder respectively, were added. Samples analyzed in triplicate for their sensory characteristics, vitamins, minerals and microbial content. Sensory evaluation showed no significant difference in flavour, colour, tartness and sweetness amongst the samples. Overall acceptability was significantly (p<0.05) higher in sample M_{30} (6.90±2.36) probably due to high levels of tartness. Vitamin C was significantly (p<0.05) higher in sample M_{30} (4.50±0.10), compared to M_0 (4.00 ±0.01), M_{10} (4.00 ±0.02) and M_{20} (4.20 ±0.01) samples. Vitamin A content (mg/100 mL) of M_{30} (0.13±0.300) was also significantly (p<0.05) higher than values obtained for M_0 (0.1 0 ±0.20), M_{10} (0.12 ±0.30) and M_{20} (0.12 ±0.10) samples. pH differed significantly (p<0.05) amongst

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the different samples, i.e. M_0 (2.70 ±0.00), M_{10} (2.80±0.00), M_{20} (3.00±0.00) and M_{30} (3.20±0.00). Microbial load result revealed the presence of coliform, total viable count, total aerobic, *Salmonella typhi, Aspagillus niger* and *Strep.* Spp. which was absent in sample M_0 but were present in significantly ($p<0.05$) higher levels in sample M_{30} (867.00±0.20, 982.00±0.10, 982.00±0.10, 28.00±0.10, 7.00±0.10 and 93.00±0.10 respectively). Significantly ($p<0.05$) higher levels of iron (1.30 ±0.12 mg/100 mL) in M_{30} compared with M_{10} (0.34±0.02), M_{20} (0.83±0.02) and M_{30} (1.30 ±0.12) and magnesium was highest in M_{20} (0.05 ±0.03 mg/100 mL) compared with M_0 (0.00 ±0.00), M_{10} (0.00±0.00) and M_{30} (0.02±0.31) samples, In conclusion, this study has revealed that *Moringa* improve the nutritive and overall sensory characteristics of Zobo drink but result in a limited shelf life.

Keywords: H. sabdariffa; calyx drink; Moringa.

1. INTRODUCTION

Hibiscus sabdariffa (Linn) [1], is an annual dicotyledonous herbaceous shrub popularly known as 'Gongura' in Hindi or 'PulichaKeerai' in Tamil [1]. This plant is well known in Asia and Africa and is commonly used to make jellies, jams and beverages [1]. The flower is cultivated in the Northern part of Nigeria. The calyx of *Hibiscus sabdariffa* flowers is used for the production of valued food and medicinal products in different parts of the world including Nigeria [2].The flowers of *H. sabdariffa* are rich in vitamins and other antioxidants [3].

Zobo drink is a traditional non-alcoholic beverage made from the reddish purple and acid-succulent calyxes of the flower *Hibiscus sabdariffa* by mainly hot water extraction [4]. The proximate composition of Zobo drink is 90% water, 0.7% protein, 8% carbohydrate, 1.4%, fiber and 1.1% fat. Iron, niacin, riboflavin, thiamine, Beta-carotene, phosphorous and calcium are also present in various proportions [5]. The purported medicinal value of the Zobo drink include antihypertensive, antiseptic, astringent, diuretic, purgative activities, remedy for cancer, abscesses, cough, debility, scurvy and fever [6,7].

The shelf life of Zobo drink is less than two days [5]. This instability of Zobo drinks stored at ambient temperature shortly after production is a major hindrance to its large scale production and marketability which is largely due to the fact that Zobo drinks can ferment naturally due to microbial activity, if kept unpreserved [8].

Spices are known and used for their aroma and to some extent for their preservative qualities [9,4]. Garlic, cinnamon and cloves have shown to inhibit bacterial and mold growth [10]. The use of our cheap, readily available and largely underutilized local spices as an additive to Zobo drink will have a dual effect of improving the taste as well as prolonging the shelf-life. *Moringa*, ginger and garlic have antimicrobial activity [11], hence their use as preservatives.

It has been reported that the nutritional value of *Moringa* using proximate method, showed that the dried leaves had crude protein levels of 30.3% and 19 amino acids and constituents such as alkaloids, carotenoids, tannins, anthrax-quinones, anthocyanins and proanthocyanidns were identified [12,13].

Nutritional composition of the plant plays a role in nutritional, medicinal and therapeutic values [12,14]. *Moringa oleifera* is considered as one of the world's most useful trees, as almost all parts of the plant have some medicinal uses, i.e. treatment of ascites, rheumatism, cardiac and blood disorders [15]. Leaves of *Moringa* are known to have various biological activities, including hypo-lipidaemic, anti-atherosclerotic, and antioxidant [16]. The seed kernels of *Moringa* showed promising effect in the treatment of bronchial asthma [17,18].

Chemical preservatives have an adverse effect on humans, they are expensive and usually not affordable by some producers of the drink, who also may not afford refrigerator. There is therefore the need for an alternative source of preservative that is natural and human-friendly, affordable and readily available. Hence, the need to investigate the impact of spicing Zobo drinks with a natural preservative such as *Moringa*.

2. MATERIALS AND METHODS

2.1 Collection of Samples

2.1.1 *H. sabdariffa* calyx

Matured dried petals of *H. sabdariffa* used for this study were obtained from a local market

(Watt market) in Calabar, Cross River State, Nigeria. Debris was removed by hand picking. Moringa leaves were obtained from a farm yard in Nko, Cross-river state of Nigeria. They were removed from the stems and carefully sorted, the bad ones were removed. The sorted leaves were rinsed several times with clean tap water to remove dust particles and debris. The leaves were shaded to dry for 7 days at room temperature (±37°C). The dried *Moringa* leaves were blended into powder using a hand blender and stored in a container corked tightly to prevent air penetration.

2.1.2 Ginger

Dry blended ginger was bought from Spar in Calabar, Cross River State, produced and packaged by Gel Spice Co. Inc. Bayonne (USA).

2.1.3 Garlic

Dry blended garlic was bought from Spar in Calabar, Cross River State, produced and packaged by Gel Spice Co. Inc. Bayonne, USA.

2.1.4 Sugar

Granulated dietary sugar was obtained from the local market.

2.2 Preparation of Zobo Drink

One thousand grams (1000 g) of Zobo leaves ere weighed and washed thoroughly and boiled for 30 minutes in a pot containing 20 liters of water. Thereafter, it was filtered using a muslin cloth and the filtrate (juice) was poured into four containers with a capacity of 4 liters each and labeled A, B, C and D.

To samples A, B, C and D, 8 g of garlic and 20 g of ginger were added. In addition, to sample B, C and D 40 g, 80 g and 120 g of *Moringa* leaf powder respectively, were added. Each sample was stirred with a spatula (to aid even distribution) and allowed to stand for 45 minute. The 4 samples were filtered, bottled and labeled as M_0, M_{10}, M_{20} and M_{30}. The samples were kept at room temperature for 48 hours. All analysis, sensory evaluation and laboratory test were performed after 48 hours.

2.3 Sensory Characteristics

Sensory evaluation was performed 48 hours after the Zobo drink was prepared. The flavour, colour, tartness acceptance, sweetness and overall acceptability of the Zobo drink sample were evaluated. A panel of twenty regular Zobo drink consumers was used. They were aged between 18-30 years. A questionnaire with 9-point Hedonic scale was used: 1 – disliked extremely; 5 – neither liked nor disliked and 9 – liked extremely. The samples were served with a bottle of drinking water for palate cleansing between tasting [19].

2.4 Wet Digestion of Extract

Ten millilitres (10 mL) of the samples were measured and evaporated. Twenty milliliters (20 mL) of nitric acid and 10 mL of perfluoric acid were added to the mixture and kept in a fume hood overnight at ambient temperature to predigest the sample. As the digest began, the temperature was gradually increased to about 120°C. The digestion was completed in about 70-80 minutes indicated by the appearance of white fumes. The mixture was left to cool and the content of the tubes were transferred to a 100 mL volumetric flask. The volume of the contents was diluted to 100 mL with distilled water. The wet digested solution was transferred to plastic bottles and labeled for use in mineral determination [20,21].

2.5 Mineral Element Analysis

Sodium and potassium contents of the samples were determined using a flame emission spectrophotometer [20].

Calcium, magnesium, zinc, copper, manganese were determined form the digest using a PerkinElmer Model 2280 atomic absorption spectrophotometer with hollow-cathode lamps [20].

Phosphorus was analyzed using the Vanado Molybedate method [22]. The concentration of each mineral was calculated from known standards.

Calculation:

Na, K, Ca, Mg, Zn, Cu, Mn and Fe were calculated from the formula:

mg/L = (Absorbance of sample x dilution factor x gradient factor) / Weight of sample

Phosphorus was calculated using the formula:

mg/L = A x Fv x D / Wt

Where

A = conc. of sample
Fv = final volume of sample extract or digest weight of sample
D = dilution factor
Wt = weight of sample

2.6 Microbiological Analysis

2.6.1 Total bacteria count

Aliquots (0.1 mL) of the dilutions were spread onto duplicate sterile plates of Nutrient Agar (NA), MacConkey Agar (MCA) and Eosin methylene blue Agar (EMB) for total aerobic count of *Escherichia. coli* and *Staphylococcus aureus*. The samples were serially diluted and 1 mL of an appropriate dilution was used to inoculate each of the plates in duplicate. The culture plates were inoculated at 37°C for 24-48h and calories counted. The mean of duplicate results were recorded as the colony count [23].

2.6.2 Coliform count

The serially diluted samples were also inoculated into lactose and MacConkey agar both for coliform count. Varying volumes of the sample are added to lactose broth tubes containing inverted Durham tubes to indicate production of gas and these were incubated for 48h at 37°C. The positive samples were plated into differential and selective media and characterization was done thereafter [23].

2.6.3 Isolation and identification of colonies

Discrete colonies of the organism were selected and sub cultured from the mixed cultures of the plates to respective NA plates and incubated at 37°C for 48 hours. Each pure isolate was processed following standard procedure [24]. Preliminary identification of isolates was from Gram's reaction and morphological characteristics. Further characterization was carried out with various biochemical tests [24]. These test include spore stain, motility, sulphide production, catalase, coagulase, lactose formulation using triple sugar iron (TSI) agar, carbohydrate test, citrate utilization and indole production. All media were prepared according to manufacturer's specification and sterilized at 121°C for 15 minutes.

2.7 Vitamin Composition

2.7.1 Determination of vitamin A

Two grams (2 g) of sample was weighed into a flat bottom reflux condenser where after ten milliliters (10 mL) of distilled water was added, followed by careful shaking to form a paste. This was followed by the addition of 25 mL of an alcoholic potassium hydroxide (KOH) solution. The mixture was heated in a water bath at 35°C for 1 hour with frequent shakin, 35 mL of distilled water was added. The hydrolysate obtained was transferred into a separating funnel and the solution was extracted three (3) times with 250mL quantities of chloroform. Two grams (2 g) of anhydrous sodium tetraoxosulphate (Na_2SO_4) was thereafter added to the extract to remove any traces of water. The mixture was filtered into a 100 mL volumetric flask and brought to volume with chloroform (0 to 50 µg/mL) standard solution. Averages of the reference value were taken and the absorbance read for vitamin A. (B-carotene is µg/100 mL). Absorbance of each sample and standards waere read on a spectrophotometer at a wavelength of 328 nm [25,26].

Calculation:

Vitamin A (µg/100 g) = (Absorbance of sample x Dilution factor/Weight of sample) x (100/1)

2.7.2 Determination of vitamin C

Equal weights of each samples and 3% metaphosphoric acid were individually, mechanically blended and each portion mixed to obtain a homogenous mix. Five grams (5 g) of the mixture was transferred using a pipette into a 100 mL volumetric flask and made up to mark with 3% metaphophoric acid. Each mixture was filtered, discarding the first portion of the filtrate and 10 mL of the aliquot was pipetted into a 50 mL volumetric flask and titrated with the standard dye solution of 2, 6-dichlorophenol indophenol to a faint pink colour which persisted for 15 seconds [27].

$$\text{Vitamin C 100g} = ([W_1 + W_2] \times [100 (V \times F)] / [W_1 \times W_3])$$

Where

W1 = weight of sample (g)
W2 = weight of extracting acid (g)
W3 = weight of mixture removed for analysis
V1 = volume to which mixture sample is diluted (mL)
V2 = volume of filtration taken for titration (mL)
V = volume of dye solution used for titration
F = ascorbic acid equivalent of dye (mg/mL)

2.8 Statistical Analysis

All data were expressed as mean ± standard deviation. The data were analyzed using one-way ANOVA followed with post hoc test (Duncan test) assuming equal variances using the IBM SPSS statistic software version 25 (SPSS: Statistical Package for Social Sciences, USA). Differences at $p<0.05$ were considered significant.

3. RESULTS AND DISCUSSION

3.1 Mineral Composition

The mineral composition of the different samples of Zobo drink is summarized in Table 1. Iron content of M_0, M_{10}, M_{20} and M_{30} were 0.89±0.10, 0.34±0.02, 0.83±0.02 and 1.30±0.12. It was significantly ($p<0.05$) lower in M_{10} and M_{30} samples compared to M_0 and M_{20}. Calcium was significantly ($p<0.05$) higher in M_0 (0.17±0.01) compared to M_{10} (0.01 ±0.01^b), M_{20}, (0.08 ±0.01) and M_{30} (0.00±0.00). The magnesium contents of samples M_0, M_{10}, M_{20} and M_{30} were 0.00 ±0.00, 0.00 ±0.00, 0.05 ±0.03 and 0.02 ±0.31, it was absent in samples M_0 and M_{10}. Manganese content was significantly ($p<0.05$) different among the different samples. It was highest in M_{30} (0.05 ±0.03) and least in M_{10} (0.15 ±0.20). Sodium was significantly ($p<0.05$) lower in M_{20} (0.35 ±0.01) compared to other samples M_0 (0.50 ±0.11), M_{10} (0.40±0.03) and M_{30} (0.41±0.02). Potassium was significantly ($p<0.05$) higher in M_{10} (0.20±0.01) compared to other samples M_{30} (0.10±0.01), M_{30} (0.20±0.00) and M_{30} (0.10 ±0.03). Zinc content of M_{10} (0.40±0.03) was the highest and significantly ($p<0.05$) higher compared to other samples M_{30} (0.85 ±0.10), M_{30} (0.85±0.03) and M_{30} (0.69±0.13). M_{30} had the least content of zinc. Copper content of the different samples were significantly different from each other. The zinc content of M_0, M_{10}, M_{20} and M_{30} were 1.60±0.02, 2.17±0.21, 2.57±0.10 and 0.68 ±0.10. it was highest in M_{20} and least in M_{30}. Phosphorus was significantly ($p<0.05$) higher in M_{10} 44.60±0.04 compared to other group samples M_0 (17.40 ±0.00), M_{20} (22.20±0.10) and M_{30} (7.50 ±0.03).

3.2 Sensory Evaluation

The results for the sensory evaluation of the different samples are listed in Table 2. The result of flavour of the control sample M_0 (6.20 ±2.17) was not significantly ($p<0.05$) different from M_{10} (5.85 ±1.90), M_{20} (5.85 ±2.23) and M_{30} (6.05 ±2.58).

Table 1. Mineral composition of the different juice samples

Mineral	M_0 (mg/100 mL)	M_{10} (mg/100 mL)	M_{20} (mg/100 mL)	M_{30} (mg/100 mL)
Fe	0.89 ±0.10^a	0.34 ±0.02^b	0.83 ±0.02^a	1.30 ±0.12^c
Ca	0.17 ±0.01^a	0.01 ±0.01^b	0.08 ±0.01^c	0.00 ±0.00^d
Mg	0.00 ±0.00^a	0.00 ±0.00^a	0.05 ±0.03^b	0.02 ±0.31^c
Mn	0.33 ±0.21^a	0.15 ±0.20^b	0.55 ±0.01^c	0.44 ±0.01^d
Na	0.5 ±0.11^a	0.4 ±0.03^a	0.35 ±0.01^b	0.41 ±0.02^a
K	0.10 ±0.01^a	0.20 ±0.01^b	0.10 ±0.00^a	0.10 ±0.03^a
Zn	0.85 ±0.10^a	1.59 ±0.10^b	0.85±0.03^a	0.69 ±0.13^c
Cu	1.60 ±0.02^a	2.17 ±0.21^b	2.57 ±0.10^c	0.68 ±0.10^d
P	17.40 ±0.00^a	44.60 ±0.04^b	22.20 ±0.10^c	7.50 ±0.03^d

Values are expressed as mean ±SD.
Mean values in the same row with different letters in superscript are significantly different ($p<0.05$)

Table 2. Sensory evaluation

Sample	Flavour	Colour	Tartness acceptance	Sweetness	Overall acceptability
M_0	6.20±2.17^b	7.50±1.36^d	5.95±1.64^b	6.25±2.16^a	5.45±2.74^a
M_{10}	5.85±1.90^b	7.40±1.23^d	6.50±1.50^a	6.40±1.50^a	6.20±2.12^a
M_{20}	5.85±2.23^b	7.30±1.45^d	7.00±1.92^c	6.85±1.27^c	6.75±1.86^a
M_{30}	6.05±2.58^b	7.35±1.65^d	7.00±1.65^c	6.50±2.28^b	6.90±2.36^f

Values are expressed as mean ±SD
Mean values in the same column with different letters in superscript are significantly different ($p<0.05$)

The colour attribute for sample M_0 (7.50 ±1.36) which served as the reference (control) was not significantly different from M_{10} (7.40 ±1.23), M_{20} (7.30 ±1.45) and M_{30} (7.35 ±1.60).

Sweetness increased from M_0 to M_{20}. The level of sweetness in the samples showed that sample M_{20} (6.85 ±1.27) was the highest and was significantly ($p<0.05$) different from the reference (6.25 ±2.16), M_{10} (6.40 ±1.50) and M_{30} (6.50 ±2.28). Sample M_{30} was significantly ($p<0.05$) higher than M_{10}.

Overall acceptability showed that there was increased acceptability as *Moringa* was added to the drink. This could be attributed to increased level of tartness in sample M_{30}.

3.3 Vitamin Composition (mg/100 mL)

In in Table 3, vitamin C values for the control sample (M_0) (4.00 ±0.01) was significantly ($p<0.05$) reduced when compared to sample M_{20} (4.20 ±0.01) and M_{30} (4.50 ±0.10). Sample M_{10} (4.00 ±0.02) was not significantly different from sample M_0 (4.00 ±0.01). Sample M_{30} was significantly ($p<0.05$) higher than M_{10} and M_{20}.

Vitamin A content of sample M_{30} (0.13 ±0.30) was significantly ($p<0.05$) higher than M_0 (0.10 ±0.20), M_{10} (0.12 ±0.30) and M_{20} (0.12 ±0.10). Sample M_{10} and M_{20} was significantly ($p<0.05$) higher than sample M_0. Sample M_{10} and sample M_{20} showed no significant ($p<0.05$) difference (Table 3).

The vitamin A content of samples M_0 was significantly low, while samples M_{30} was significantly higher than M_0, M_{10} and M_{20}.

3.4 pH and Microbial Examination

The results showed that the pH values increased steadily from M_0 to M_{30} (Table 4). The pH value of sample M_{30} (3.20 ±0.00) was significantly ($p<0.05$) higher than sample M_{20} (3.00 ±0.00), M_{10} (2.80 ±0.00) and M_0 (2.70 ±0.00). pH of sample M_{20} was higher ($p<0.05$) than sample M_{10}.

In Table 4, the presence of coliform was highest ($p<0.05$) in sample M_{30} (867.00 ±0.20) when compared to sample M_{20} (713.00 ±0.10) and M_{10} (54.00 ±0.10). Sample M_0 showed no presence of coliform. Total viable count was highest ($p<0.05$) in sample M_{30} (982.00 ±0.10) when compared to sample M_{20} (817.00 ±0.10) and M_{10} (70.00 ±0.10).

Total aerobic count was highest ($p<0.05$) in sample M_{30} (982.00 ±0.10) when compared to sample M_{20} (817.00 ±0.10) and M_{10} (70.00 ±0.10). Total fungi levels in sample M_{10} (6.00 ±0.10) was significantly ($p<0.05$) lower, compared to M_{20} (16.00 $\pm$ 0.10) and M_{30} (16.00$\pm$0.10). It was absent in M_0.

Salmonella typhi bacteria was present in sample M_{10} (12.00 ±0.10) which is significantly ($p<0.05$) lower when compared to samples M_{20} (21.00 ±0.10) and M_{30} (28.00 ±0.10). Sample M_{30} had the highest ($p<0.05$) level when compared to the other samples. The growth of *Aspergilus niger* was highest ($p<0.05$) in sample M_{30} (5.00 ±0.10) and sample M_{10} (2.00 ±0.20). No growth was observed in sample M_0.

The level of *streptococcus* spp was highest ($p<0.05$) in sample M_{30} (93.00 ±0.10) when compared to sample M_{20} (75.00 ±0.10) and sample M_{10} (5.00 ±0.10). Sample M_{20} was significantly ($p<0.05$) higher than sample M_{10}.

4. DISCUSSION

Hibiscus sabdariffa is rich in mineral elements which are mainly inorganic [1]. Their bioavailability and metabolic functions are related to the forms in which they are found.

4.1 Mineral Elements

The different samples differed significantly ($p<0.05$) in their mineral composition (Table 1).

Table 3. Vitamin composition

Sample	Vitamin C (mg/100 ml)	Vitamin A (mg/100 ml)
M_0	4.00 ±0.01^a	0.10 ±0.20^a
M_{10}	4.00 ±0.02^a	0.12 ±0.30^b
M_{20}	4.20 ±0.01^b	0.12 ±0.10^b
M_{30}	4.50 ±0.10^c	0.13 ±0.30^c

Values are expressed as mean ±SD. Mean values in the same column with different symbols in superscript are significantly different (p<0.05)

Table 4. Microbiological analysis

	M_0	M_{10}	M_{20}	M_{30}
pH	2.70 ±0.00^a	2.80 ±0.00^b	3.00 ±0.00^c	3.20 ±0.00^d
Coliform	-	54.00 ±0.10^a	713.00 ±0.10^b	867.00 ±0.20^c
Total viable count-		70.00 ±0.10^a	817.00 ±0.10^b	982.00 ±0.10^c
Total aerobic-		70.00 ±0.10^a	817.00 ±0.10^b	982.00 ±0.10^c
Total anaerobic-		-	-	-
Total fungi-		6.00 ±0.10^a	16.00 ±0.10^b	16.00 ±0.10^b
Rhizopus stolonifera? -		-	-	-
Cholera vibrio-		-	-	-
Salmonella typhi-		12.00 ±0.10^a	21.00 ±0.10^b	28.00 ±0.10^c
Aspergillus niger-		2.00 ±0.20^a	5.00 ±0.10^b	7.00 ±0.10^c
Streptococcus spp-		5.00 ±0.10^a	75.00 ±0.10^b	93.00 ±0.10^c
Lactobacillus spp.-		-	-	-
Bacillus? spp.-		-	-	-
Botulinum spp-		-	-	-

Values are expressed as mean ±SD; Mean values in the same row with different symbols in superscript are significantly different (p<0.05)

Addition of *Moringa* extract to the Zobo drink significantly improved the mineral composition especially for iron and manganese.

The iron content of the different samples was all lower than the recommended dietary allowance for either male (1.37 mg) or female (2.93 mg) [28]. Iron is an important trace element which plays numerous biochemical roles in the body, including oxygen binding with haemoglobin and in enzymes catalytic reactions [29]. M_{30} had the highest iron content amongst the samples, therefore fortifying Zobo drinks with *Moringa* could improve dietary iron intake.

Calcium contents of *Moringa* supplemented samples were significantly lower, compared to the unfortified samples (control). This shows that *Moringa* did not improve the calcium content of Zobo drink. However, this finding is contrary to what was reported [1], which showed higher calcium content than values obtain in this study.

All the four samples in this study had lower magnesium contents compared to values in previous report which indicated higher magnesium content in Zobo drink. However, this study, the addition of *Moringa* to Zobo drinks improved the magnesium content as higher concentrations of manganese were recorded in Zobo supplemented samples M_{20} and M_{30}.

Relatively lower sodium content were found in Zobo drinks of this study, compared to reports by [4], showing sodium content of up to 50.67 mg/L. Potassium content of our Zobo drinks were also lower than values reported by [4]. The addition of *Moringa* did not improve the potassium levels of the Zobo drinks.

Zinc content of 0.39 to 0.90 mg/100 mL in Zobo drink reported by [30] are similar to the values obtained in this t study. *Moringa* leave extract improved the zinc contents of Zobo drinks. Copper was highest in M_{10} and M_{20} samples, followed by M_0.

Phosphorus is an important mediator of DNA and RNA biological activities [30]. Phosphorus was the most abundant element in the Zobo drinks, with M_{10} having the highest phosphorus content of 44.60 ±0.04 mg/100 mL.

4.2 Vitamin Composition

Vitamin C is a water soluble vitamin which acts as an anti-oxidant. Zobo contains vitamin C which raises the immune system of the body and this study confirmed it with the presence of moderate concentrations of vitamin C which is higher in samples M_{30} (4.50 ±0.10) when compared to pure Zobo drink M_0 (4.00 ±0.01). This work demonstrates that the vitamin A and C contents of Zobo drinks supplemented with *Moringa oleifera* extract was higher than that of Zobo juice (control). This means that the vitamin A and C status of Zobo drinks can thus be improved.

4.3 Sensory Evaluation

The result for sensory evaluation in Table 3 showed that there was no significant difference among the different samples analysed (M_0, M_{10},

M_{20} and M_{30}) for flavor, colour, and tartness. Addition of *Moringa* improved the sweetness of Zobo drink. Overall acceptability showed that there was increased acceptability as *Moringa* was added to the drink. This could be attributed to increased level of tartness in sample M_{30}.

4.4 pH and Microbial Analysis

The pH of the Zobo samples blended with *Moringa oleifera* were higher than that of the Zobo juice (control) which contain no extract. The pH value 3.20 ±0.00 obtained from Zobo drinks blended with *Moringa oleifera* extract (M_{30}) was lower when compared to the pH value of 7.60 ±0.00 of Zobo drink reported by [2]. This implies that Zobo drinks are slightly acidic and should not be consumed without a snack or on an empty stomach. A recent study revealed that the pH of fruit flavoured Zobo drinks had a pH value ranged between 2.19 and 3.62 [4].

The result for microbiological analysis in Table 3 showed that coliform, total viable count, total aerobic, *Salmonella typhi*, *Aspagillus niger* and *Strep. spp* was absent in samples M_0 and a high significant level in samples M_{30}. The absence of coliform, total viable count, total aerobic, *Salmonella typhi*, *Aspagillus niger* and *Streptococcus* spp in samples M_0 (0.00 ±0.00). The occurrence of high levels of microorganisms in samples M_{30} indicates that *Moringa oleifera* enhances the growth of micro-organism.

5. CONCLUSION

In conclusion, the use of *Moringa oleifera* extract for the enrichment of Zobo drink impacted on its overall sensory acceptability. Vitamins A and C, iron, manganese, magnesium and phosphorus contents of the enriched Zobo drinks improved. However, *Moringa* enhanced growth of microorganisms in Zobo drinks, hence the need to identify alternative natural preservative methods to increase the shelf life of Zobo drinks if the goal of commercialization is to be achieved.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Kuriyan R, Kumaryl R, Kurpad A. An evaluation of the hypolipidemic effect of an extract of *Hibiscus sabdariffa* leaves in hyperlipidemic Indians: A double blind, placebo controlled trial. Compl Alt Med. 2010;10:27-34.
2. Adesokan IA, Abiola OP, Adigun MO, Anifowose OA. Analysis of quality attributes of *Hibiscus sabdariffa* (*zobo*) drinks blended with aqueous extract of ginger and garlic. Afr J Food Sci. 2013;7(7):174-177.
3. Kochlar SI. Tropical crops: Textbook of economic botany. Macmillan Publishers Ltd., London. 1991;25-62.
4. Olayemi F, Adedayo, R, Muhummad R, Bamishaiye E. The nutritional quality of three varieties of Zobo (*Hibiscus sabadariffa*) subjected to the same preparation condition. Plant Food Human Nutr. 2011;6:10-15.
5. Fasoyiro SB, Ashaye OA, Adeola A, Samuel FO. Chemical and storability of fruit-flavoured (*Hibiscus sabdariffa*) drinks. World J Agri Sci. 2005;1(2):161-164.
6. Anjorin TS, Ikokoh P, Okolo S. Mineral composition of *Moringa oleifera* leaves, pods and seeds from two regions in Abuja, Nigeria. Int J Agri Biol. 2010;12(3):431-434.
7. Ogbo A. Nutritional values of some tropical foods with allergy/ toxicants. Computer Edge Publishers, Enugu. 2002;30-35.
8. Ukwuru MU, Uzodinma CC. Preservative effect of spices and their flavor acceptability in zobo drink. Nig Food J. 2010;28(2):265-274.
9. Hintz T, Karl K, Matthews I, Rong D. The use of plant antimicrobial compounds for food preservation. Biol Med Res Int. 2015;20(15):12.
10. Paster N, Menashorov M, Ravid U, Juven B. Antifungal activity of oregano and thyme essential oils applied as fumigant fungi attacking stored grain. J Food Protect. 1995;58:81-85.

11. Gull I, Sohail M, Aslam MS, Athar MA. Phytochemical, toxicological and antimicrobial evaluation of *Lawsonia inermis* extracts against clinical isolates of pathogenic bacteria. Annal Clin Micro Antimicro. 2016;12(36):1-2.
12. Moyo B, Masika PJ, Hugo A, Muchenje V. Nutritional characterization of Moringa (*Moringa oleifera* Lam.) leaves. *Afr J Biotech*. 2013;10(60),12925-12933.
13. Padayachee B, Baijnath H. An overview of the medicinal importance of Moringaceae. J Med Plant Res. 2012;6(48):5831-5839.
14. Anwar F, Latif S, Ashraf M, Gilani AH. *Moringa oleifera*: A food plant with multiple medicinal uses. Phytother Res. 2007;21(1):17-25.
15. Jongrungruangchok S, Bunrathep S, Songsak T. Nutrients and minerals content of eleven different samples of *Moringa oleifera* cultivated in Thailand. J Health Res. 2010;24(3):123-127.
16. Matshediso PG, Cukrowska E, Chimuka L. Development of pressurised hot water extraction (PHWE) for essential compounds from *Moringa oleifera* leaf extracts. Food Chem. 2015;172:423-427.
17. Farooq F, Rai M, Tiwari A, Khan AA, Farooq S. Medicinal properties of *Moringa oleifera*: An overview of promising healer. J Med Plant Res. 2012;6:4368-4374.
18. Mugal KT, Haq IU. *Moringa oleifera*: A natural gift-A review. J Pharm Sci Res. 2010;51:6558-6563.
19. Stone H, Sidel JL. Sensory evaluation practices, 3rd Edition. Elsevier Academic Press, London. 2004;87-90.
20. Horwitz W. Official Methods of Analysis of AOAC International. 17th Ed. AOAC International, Gaithers-burg, MD. 2000;1(2).
21. Greenfield H, Southgate DAT. Food composition data: Production, management and use. First Edition, Elsevier Science Publishers. 1992;83:97.
22. Olaniyi OO, Bankefa EO, Folasade IO, Familoni TV. Nutrient enrichment of manganase-treated Cassava peels and Corn cob. Res J Microbiol. 2015;10:533-541.
23. Grabow WO, Hilner CA, Coubrough P. Evaluation of standard and modified M-FC, MacConkey, and Teepol media for membrane filtration counting of fecal coliforms in water. Appl Envir Microbiol. 1981;42(2):192-199.
24. Cowan ST, Steel KJ. Manual for the Identification of Medical Bacterial. 4th Edn, Cambridge University Press, London. 1985;45-60.
25. Capel ID, Dorrell HM. Abnormal antioxidant defence in some tissues of congenitally obese mice. Biochem J. 1984;219:41-49.
26. Adelekan AO, Arisa NU, Alamu AE, Adebayo YO, Popoola GJT. Production and Acceptability of Fruits Enhanced Zobo Drink. Food Sci Tech Letter. 2014;5(1): 046-051.
27. Adeboye OC. Phyto-constituent and anti-oxidant of the pulp of snake tomato *(Trichosanthes cucumerina).* Afri J Trad Compl Alt Med. 2008;5(2):173-179.
28. WHO. WHO estimates of the global burden of foodborne diseases. Foodborne Diseases Burden Epidemiology Reference Group 2007–2015, World Health Organization, Geneva, Switzerland; 2015.
29. Geissler CA, Power HJ. Human Nutrition. 11th edition. Elsevier, Churchill Livingstone. 2005;236-243.
30. Okeniyi SO, Kolawole JA. Quantitative mineral ion content of a Nigerian local refreshing drink "Zobo" (Water Extract of *Hibiscus sabdariffa* Calyx). Res J Pharm. 2007;1:23-26.

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