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## Development and Consumer Acceptability of Beetroot-Enriched Hibiscus sabdariffa (Zobo) Drink

Obasanjo Muhammed Oguniola<sup>1\*</sup> & Oladejo Thomas Adepaju<sup>1</sup>

<sup>1</sup> Department of Human Nutrition and Dietetics, Faculty of Public Health, College of Medicine University of Ibadan, Nigeria.

\*Author for correspondence Email: [obasanjomuhammad95@gmail.com](mailto:obasanjomuhammad95@gmail.com)

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Drink,  
Antioxidants.*

Foods and drinks are essential for a healthy and active life. Sugar-sweetened carbonated drinks have grown in popularity and cost, though they have been linked with several cardiovascular diseases and dental problems, hence the need for non-sugar-sweetened drinks. This study was carried out to develop a Beetroot-enriched Hibiscus sabdariffa (Zobo) drink and evaluate its nutrient content and consumer's acceptability. Dry Hibiscus sabdariffa calyces and beetroot were purchased at Bodija Market, Ibadan, Nigeria. The beetroot (fresh) was blended, and the dry calyces were ground into powder. A 100 g of ground calyces was extracted with 1.5 L distilled water, boiled at 100 °C for 15 minutes, cooled and sieved (plain Zobo drink). The remaining ground calyces were mixed with ground beetroot in the ratio 90:10, 80:20, 70:30 and 60:40 w/w Calyx: Beetroot, respectively. Each portion was extracted with 1.5 L of distilled water and boiled at 100 °C for 15 minutes, sieved and allowed to settle. The five drinks were analysed for nutrient and phytochemical contents using standard methods of AOAC. Consumer acceptability of the drinks was done on a 9-point hedonic scale using 30 semi-trained panellists. Data was analysed using ANOVA and Duncan multiple range test at  $p < 0.05$ . Plain and enriched Zobo drinks contained 1.13-2.73 g protein, 0.21-0.40 g fibre, 6.307-8.52 g carbohydrate, 14.00-65.33 mg sodium, 47.70-91.00 mg calcium, 21.701-159.33 mg potassium, 61.33-97.70 mg magnesium, 110.33-160.00 mg phosphorus, 1.10-1.50 mg iron, 0.10 mg 212.80-25.70 µg β-carotene, and 32.10-40.10 mg vitamin E/100 ml of the drink. The addition of beetroot enhanced the total antioxidants, flavonoids, betaine, and polyphenol contents of the drinks. Hibiscus sabdariffa drink with 20% beetroot inclusion had the highest overall acceptability in most of the sensory parameters, while the plain Zobo drink was preferred in terms of odour. Beetroot-enriched Zobo drink is micronutrient-dense, well accepted, and compared well with plain Zobo drink. Beetroot-enriched Zobo drink contributes to the nutrient content of the drink; hence, its use in local refreshing drinks can improve the micronutrient intake of such drinks, thereby promoting the good health of consumers.

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**INTRODUCTION**

Ready-to-eat foods include fruits, fruit juices, nourishing drinks, snacks, and beverages (Orutugu *et al.*, 2015). Most of these conventional drinks contain phosphoric, malic, citric, and tartaric acids which corrode the surface of teeth and cause a variety of dental problems as well as osteoporosis (Hughes *et al.*, 2000). High-energy sweetened carbonated drinks can cause dental decay and overweight, which is a primary cause of obesity and diabetes (WHO, 2017). Adolescents in many middle-income nations have quickly progressed from being underweight to being overweight due to the rise in consumption of high-energy foods and drinks, leading to weight gain and poor long-term health outcomes (Saudi Medical Journal, 2017). The influence of sugar-sweetened beverage consumption on cardiovascular disease and death is attracting media and policymakers' attention, with various international societies proposing a reduction in the consumption of sugar-sweetened beverages (WHO & CDC, 2015). This shortcoming can be avoided by taking naturally blended fruit juice or beverages such as *Hibiscus sabdariffa* (Zobo) drink.

Plant-based foods and beverages have been shown to be free of saturated fats, sugars and salts, preventing the development of various chronic

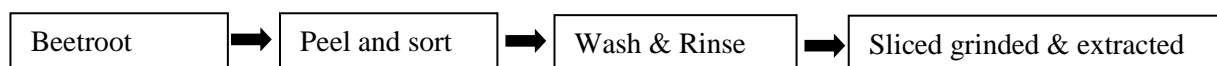
illnesses (Pires *et al.*, 2019)]. *Hibiscus sabdariffa* drink has been gaining popularity globally as a refreshing medicinal drink. The calyx of *Hibiscus sabdariffa* is used to make the popular non-alcoholic beverage known as "Zobo" drink in Nigeria. The plant is high in essential minerals including iron, copper, calcium, magnesium, and manganese, which are necessary for human development (Nkumah, 2015). The *Hibiscus sabdariffa* plant is widely cultivated for its strong fibre and is well known for its edibility and medicinal properties. The calyx is the most frequently used portion of the plant, while the leaves and seeds are often made into salads, curries, and potherbs.

Cardiovascular disease imposes a huge financial cost, being the leading cause of death worldwide. Consumption of conventional soft drinks has been linked with several cardiovascular diseases in man, hence the need for primary and secondary prevention through the consumption of natural local soft drinks such as Zobo drink (Salami *et al.*, 2020). High consumption of fruits and vegetables is associated with a reduced risk of some types of diseases (Wang *et al.*, 2011). Scientific evidence provides greater support for the role vegetables play in helping to protect against chronic diseases such as type-2 diabetes and cancers (Asif, 2011).

Beetroot, a root – vegetable, is known to be rich in antioxidant components because of the phenolic compounds called betalains responsible for the intense red colour of red beetroots (Winkler *et al.*, 2005; Azeredo, 2009; Wruss *et al.*, 2015). The pigment that gives beetroot juice its rich, red, and purple colouration is called "betaine". Some health benefits of beetroot include lowering blood pressure, preventing hypertension and stroke, improving stamina during exercise, treating anaemia, and improving blood circulation and oxygen-carrying capacity of erythrocytes, among others (Priya *et al.*, 2013).

Jams prepared from *Hibiscus sabdariffa* calyx extract with the addition of carrot, vanilla flavour and sugar showed improvement in the level of beta-carotene and suitability of the calyx extract as a good vehicle for vitamin A fortification (Adepoju *et al.*, 2010). However, the inclusion of beetroot in *Hibiscus sabdariffa* drink has been a neglected area worthy of investigation. The cost of beetroot is low compared to other iron-rich vegetables, and it can be stored easily. Due to the great potential of beetroot, it is imperative to investigate its nutritional importance in enriching the widely consumed homemade "Zobo drink" and evaluate the consumer acceptability of the products.

**Figure 1: Flow chart for Beetroot juice Extraction**



### Preparation of Zobo Drink

Plain Zobo drink was prepared by boiling 100 g of ground dried *Hibiscus sabdariffa* calyx in 1.5 L of distilled water at 100 °C for 15 mins, cooling and sieving with a muslin cloth. The filtrate was allowed to cool, kept in a plastic container, and labelled as sample A (Okereke *et al.*, 2016). The sample was refrigerated at 20 °C till when needed. This served as the control (plain Zobo drink).

### Preparation of Enriched Zobo Drink

The method of Adepoju *et al.* (2010) with some modifications, was used to prepare the enriched

### Study Objectives

The objectives of the study were to:

- Determine the proximate composition of *Beta vulgaris*-enriched *Hibiscus sabdariffa* drink compared to the commonly consumed plain *Hibiscus sabdariffa* drink.
- Determine the phytochemical composition of *Beta vulgaris*-enriched *Hibiscus sabdariffa* drink compared to the commonly consumed plain *Hibiscus sabdariffa* drink.
- Evaluate the acceptability of *Hibiscus sabdariffa* drink enriched with beetroot compared to the commonly consumed plain *Hibiscus sabdariffa* drink.

### MATERIALS AND METHODS

#### Sample Collection and Preparation

*Hibiscus sabdariffa* calyces and fresh beetroot were purchased from Bodija market in Ibadan, Oyo State, Nigeria. The calyx was ground into a fine powder while 100 g of fresh beetroot cut into small pieces was blended using a warring blender, and its juice was prepared as shown below.

Zobo drink. Ninety grammes (90 g) of ground *Hibiscus sabdariffa* calyx and 10 g of beetroot were extracted with 1.5 L of distilled water following the process in sample A and labelled sample B. Another 80 g of ground *Hibiscus sabdariffa* calyx and 20 g beetroot were extracted with 1.5 L of distilled water as in sample A above and labelled as sample C. Also, 70 g of ground *Hibiscus sabdariffa* calyx and 30 g beetroot were extracted with 1.5 L distilled water as in sample A and labelled as sample D. Finally, 60 g of ground *Hibiscus sabdariffa* calyx and 40 g beetroot were extracted with 1.5 L of distilled water as in sample

A and labelled sample E. Each sample was boiled for 15 mins at 100 °C, allowed to cool to room temperature, refrigerated at 20 °C and packaged in plastic containers till when needed for analysis.

## Chemical Analysis

### *Proximate Composition Determination*

The moisture content of the dry *Hibiscus sabdariffa* calyx and beetroot pulp was determined, while the nutrient and phytochemical contents determination of Samples A – E were done using standard methods of analyses of Association of Official Analytical Chemists (AOAC, 2005). The moisture content of the samples was determined by air oven (Plus 11 Sanyo Gallenkamp PLC UK) method at 105 °C for 4 hrs. The crude protein was determined using the micro-Kjeldahl method (Method No 978.04), crude lipid was determined by the Soxhlet extraction method (Method No 930.09) while the ash content was determined through incineration in a muffle furnace at 550 °C for 4 hrs (AOAC 2005 Method No 930.05). The carbohydrate content was obtained by difference.

### *Mineral Content Determination*

The potassium and sodium content of the samples was determined by digesting the ash of the samples with perchloric and nitric acid and taking the readings of the digests on Jenway digital flame photometer (PFP7 Model; Cecil; Japan)/spectronic20 (AOAC, 2005: (975.23). Calcium, magnesium, Iron, zinc, manganese, and copper were determined by the atomic absorption spectrophotometric method (Buck Scientific, Norwalk, Conn, USA), and their absorption was compared with the absorption of standard solutions of these minerals (AOAC, 2005: (975.23). The phosphorus content was determined using Vanado-molybdate colourimetric method (AOAC, 2005: (975.16).

### *Vitamin Content Determination*

**Beta-carotene Determination:** The beta-carotene content of the samples was determined through ultraviolet absorption measurements at 328 nm after extraction with chloroform. A

calibration curve of vitamin A acetate was made, and sample  $\beta$ -carotene concentration was read and estimated as microgramme of vitamin A acetate.

### **Thiamine (Vitamin B<sub>1</sub>) Determination:**

Thiamine content of the samples was determined by weighing 1 g of the sample into a 100 mL volumetric flask with the addition of 50 mL of H<sub>2</sub>SO<sub>4</sub> and boiled in a water bath with frequent shaking for 30 mins. Five millilitres of 2.5 M sodium acetate solution was added, and the flask was set in cold water to cool the contents below 50 °C. The flask was stoppered and kept at 45-50 °C for 2 hrs and thereafter made up to 100 mL. The mixture was filtered through No. 42 Whatman filter paper, discarding the first 10 mL, and then 10 mL was pipetted from the remaining filtrate into a 50 mL volumetric flask, followed by the addition of 5 mL acid potassium chloride solution with thorough shaking. Standard thiamine solutions were prepared and treated in the same way. The absorbance of the samples and the standard solutions was read on a fluorescent UV Spectrophotometer (CecilA20 Model) at 285 nm.

### **Riboflavin (Vitamin B<sub>2</sub>) Determination:**

Five millilitres of each sample was measured into a 250 mL volumetric flask, with 5 mL each of 1 M HCl and dichloroethene. The mixture was shaken, and 90 mL of de-ionised water was added. The whole mixture was thoroughly shaken and heated in a steam bath for 30 mins, cooled and made up to volume with de-ionised water. The resultant mixture was filtered, discarding the first 20 mL of the aliquot. Two millilitres of the filtrate obtained were pipetted into another 250 mL volumetric flask and made up to the mark with de-ionised water. Sample solutions were read on the fluorescent spectrophotometer at 460 nm. Standard solutions of riboflavin were prepared, and their readings were taken at 460 nm. The samples' riboflavin was obtained through calculation.

### **Niacin (Vitamin B<sub>3</sub>) Determination:**

Five millilitres of each of the samples was added to 100 mL of distilled water, and 5 mL of this solution was pipetted into a 100 mL volumetric flask and

made up to the mark with distilled water. Niacin standard solutions were prepared, and the absorbance of samples and standard solutions were measured at 385 nm on a spectrophotometer. The niacin concentration of the samples was estimated through calculation.

**Pantothenic Acid (Vitamin B<sub>5</sub>) Determination:**

Five millilitres of each of the samples was added to distilled water, and 5 mL aliquot of the sample was thoroughly mixed with 5 mL of 12% KBr and 10 mL of KMnO<sub>4</sub> solutions. The mixture was warmed in a boiling water bath for 10 mins, cooled in ice for 5 mins, and 20% freshly prepared H<sub>2</sub>SO<sub>3</sub> solution was added dropwise to obtain the colourless solution. To the colourless solution, 10 mL of 2, 4 - dinitrophenyl hydrazine (5 g/l) was added and mixed thoroughly. The mixture was heated in a steam bath for 15 mins and cooled to room temperature. The precipitate was dried at 100 °C for 30 mins in an air oven set at 100 °C and dissolved in a hot pyridine solution with thorough mixing. The suspension was filtered through Whatman No. 42 filter paper into a 50 mL volumetric flask and made up to mark with pyridine solution. To this solution was added 50 mL distilled water, followed by the addition of 5 mL 5 M NaOH solution. The absorbance of the samples and standard solutions of pantothenic acid was read on a spectronic21D spectrophotometer at 570 nm, and sample concentration was calculated in µg /100 g of sample.

**Pyridoxine (Vitamin B<sub>6</sub>) Determination:**

The vitamin B<sub>6</sub> content of the samples was determined by extracting 5 mL of the sample with 0.5 g of ammonium chloride, 45 mL chloroform and 5 mL absolute ethanol. The resultant mixture was thoroughly mixed in a separating funnel by shaking for 30 mins, followed by the addition of 5 mL of distilled water. The chloroform layer containing pyridoxine was filtered into a 100 mL volumetric flask and made up to the mark with chloroform. Standard solutions of 0-10 ppm of vitamin B<sub>6</sub> were prepared and treated in a similar way as samples, and their absorbance was measured on a Cecil 505E spectrophotometer at

415 nm. The amount of vitamin B<sub>6</sub> in the samples was then calculated.

**Folic Acid (Vitamin B<sub>9</sub>) Determination:**

Folic acid content of the samples was determined by measuring 5 mL of each sample into a 250 mL volumetric flask, followed by the addition of 100 mL of distilled water and shaken for 45 mins, and the mixture made up to the mark with distilled water. To 20 mL of the solution, 5 mL of 1% sodium dithionite solution was added. Standard solutions (0 – 10 µg / mL) of folic acid were prepared from folic acid stock. The absorbance of solutions of samples and standards were read at 445 nm on a spectronic21D spectrophotometer, and folic acid concentration was calculated.

**Ascorbic acid Determination:**

Ascorbic acid content of the samples was determined by titrating the solution of each sample with a solution of 2, 6 – dichlorophenol-indophenol dye to a faint pink endpoint.

**Tocopherol (Vitamin E) Determination:**

Five millilitres of each of the samples was measured into a 250 mL conical flask fitted with a reflux condenser wrapped in aluminium foil and refluxed with 10 mL of absolute ethanol and 20 mL of 1 M ethanolic sulphuric acid for 45 mins. The resultant solution was cooled for 5 mins, followed by the addition of 50 mL of distilled water and then transferred into a separating funnel covered with aluminium foil. The unsaponifiable matter in the mixture was extracted five times with 50 mL diethyl ether. The combined extract was washed free of acid and dry over anhydrous sodium sulphate, evaporated at a low temperature, and the residue obtained was immediately dissolved in 10 mL absolute ethanol. Aliquots of solutions of the samples and standards were transferred to a 20 mL volumetric flask, and 5 mL absolute ethanol was added, followed by the careful addition of 1 mL conc. HNO<sub>3</sub>. The contents were placed in a water bath set at 90 °C for 30 mins from the time the ethanol began to boil, followed by rapid cooling under running water. The absorbance of sample solutions was read at 470 nm.

### Phytochemical Analyses

The water, methanol, ethanol, ethyl acetate, and petroleum ether extracts of *Hibiscus sabdariffa* calyces were subjected to preliminary phytochemical screening to identify the chemical constituents. Determination of total antioxidants, titratable acidity, flavonoids, phenols, and betaine was done using the AOAC method with some modifications. Quantitative determination of the total antioxidants, flavonoids, phenols, and betaine of the samples was done using the gravimetric method.

### Sensory Evaluation of the Drinks

The sensory quality of the enriched *Zobo* drinks was carried out on colour, taste, odour, mouth feel, and overall acceptability using 30 semi-trained consenting panellists consisting of students from the University of Ibadan. The plain and enriched drinks were served chill in clean labelled cups, in the order of the level of enrichment: 0% (Plain *Zobo* drink), 10%, 20%, 30% and 40% beetroot inclusion levels, respectively. The quality indices were assessed on a 9-point Hedonic scale where 1 was = extremely dislike and 9 was = extremely like, as described by Mishra *et al.*, 2012.

### Ethical Consideration

Ethical approval (Number of UI/EC/21/0329) was obtained from the University of Ibadan/University

College Hospital (UI/UCH) ethics review board. Informed consent was also obtained from the panellists through their signatures.

### Data Analysis

Data were analysed using descriptive statistics on triplicate determinations and Duncan's multiple range test. One-way analysis of variance (ANOVA) was used to test for significant differences at  $p < 0.05$ .

### RESULTS

The proximate composition of plain and enriched *Hibiscus sabdariffa* drink is presented in *Table 1*. The plain *Zobo* drink (Sample A) was very high in moisture content and very low in other macronutrients. Sample A had the highest moisture content, followed by Sample B. There was a slight reduction in the moisture content of the enriched drinks as the level of the added beetroot increased ( $p < 0.05$ ). Generally, there was an increase in the macronutrient content of the drinks as more beetroot was added, with enriched *Hibiscus Sabdariffa* drink with 40% beetroot (Sample E) having the highest value in all the nutrients ( $p < 0.05$ ). However, the enriched *Hibiscus sabdariffa* drink with a 20% beetroot inclusion level (Sample C) had the highest carbohydrate content.

**Table 1: Proximate composition of plain and enriched *Hibiscus sabdariffa* and drink (g/100g)**

Sample	A	B	C	D	E	p-value
Moisture	91.73±0.03 <sup>a</sup>	90.04±0.03 <sup>b</sup>	89.13±0.03 <sup>c</sup>	88.64±0.03 <sup>d</sup>	86.93±0.02 <sup>e</sup>	<0.05
Crude Protein	1.13±0.05 <sup>a</sup>	1.43±0.06 <sup>b</sup>	1.92±0.04 <sup>c</sup>	2.23±0.04 <sup>d</sup>	2.73±0.06 <sup>e</sup>	<0.05
Fat	0.18±0.02 <sup>a</sup>	0.41±0.02 <sup>b</sup>	0.63±0.02 <sup>c</sup>	0.80±0.03 <sup>d</sup>	0.90±0.02 <sup>e</sup>	<0.05
Fibre	0.21±0.02 <sup>a</sup>	0.30±0.01 <sup>b</sup>	0.34±0.02 <sup>c</sup>	0.30±0.02 <sup>d</sup>	0.40±0.01 <sup>e</sup>	<0.05
Ash	0.52±0.10 <sup>a</sup>	0.70±0.03 <sup>b</sup>	0.83±0.02 <sup>c</sup>	0.74±0.02 <sup>d</sup>	0.90±0.02 <sup>e</sup>	<0.05
Carbohydrates	6.30±0.02 <sup>a</sup>	7.24±0.04 <sup>b</sup>	7.14±0.02 <sup>b</sup>	7.04±0.50 <sup>b</sup>	8.52±0.07 <sup>c</sup>	<0.05

*A = Roselle drink (Control), B = 10% Beetroot inclusion, C = 20% Beetroot inclusion, D = 30% Beetroot inclusion, E = 40% Beetroot inclusion drinks, respectively. Means that similar superscripts in the same row are not significantly different*

In *Table 2*, the plain *Zobo* drink was generally low in mineral content, except phosphorus. However, the addition of beetroot to the plain drink resulted in an increase in the values of the minerals

( $p < 0.05$ ). The concentration of sodium, potassium, calcium, phosphorus, and manganese was significantly highest in Sample E ( $p < 0.05$ ), with Sample C having the highest value of iron,

while Sample D had the highest value of magnesium and zinc ( $p < 0.05$ ).

**Table 2: Mineral composition of plain and enriched Hibiscus Sabdariffa and drink (mg/100g)**

Sample	A	B	C	D	E	p-value
Sodium	14.00±1.00 <sup>a</sup>	21.70±1.20 <sup>b</sup>	59.70±1.52 <sup>c</sup>	37.70±1.20 <sup>d</sup>	65.33±1.52 <sup>e</sup>	<0.001
Potassium	21.70±1.20 <sup>a</sup>	132.33±3.10 <sup>b</sup>	148.00±4.00 <sup>c</sup>	141.70±2.51 <sup>d</sup>	159.33±2.51 <sup>e</sup>	<0.001
Calcium	47.70±1.52 <sup>a</sup>	60.33±1.52 <sup>b</sup>	76.70±1.52 <sup>c</sup>	70.00±2.00 <sup>d</sup>	91.00±2.00 <sup>e</sup>	0.02
Magnesium	61.33±3.10	75.70±2.51	91.70±2.51	97.70±1.52	71.83±0.91	0.51
Phosphorus	110.33±1.52 <sup>a</sup>	117.33±1.52 <sup>b</sup>	150.33±1.52 <sup>c</sup>	138.00±1.00 <sup>d</sup>	160.00±2.00 <sup>e</sup>	<0.001
Iron	1.10±0.03 <sup>a</sup>	1.22±0.03 <sup>ab</sup>	1.50±1.52 <sup>c</sup>	1.40±0.04 <sup>bc</sup>	1.30±0.24 <sup>ab</sup>	0.02
Zinc	0.10±0.00 <sup>a</sup>	0.10±0.00 <sup>b</sup>	0.11±0.00 <sup>c</sup>	0.13±0.00 <sup>d</sup>	0.10±0.00 <sup>a</sup>	<0.001
Copper	0.20±0.00 <sup>a</sup>	0.21±0.00 <sup>b</sup>	0.30±0.00 <sup>c</sup>	0.31±0.00 <sup>d</sup>	0.32±0.00 <sup>e</sup>	<0.001
Manganese	1.20±0.00 <sup>a</sup>	1.23±0.00 <sup>b</sup>	1.40±0.00 <sup>d</sup>	1.50±0.00 <sup>c</sup>	1.60±0.00 <sup>e</sup>	<0.001

*A = Roselle drink (Control), B = 10% Beetroot inclusion, C = 20% Beetroot inclusion, D = 30% Beetroot inclusion, E = 40% Beetroot inclusion drinks, respectively. Means that similar superscripts in the same row are not significantly different.*

The plain *Zobo* drink (Sample A) was very high in  $\beta$ -carotene and vitamin E contents but low in water-soluble vitamins (Table 3). The addition of beetroot to the drinks significantly reduced the values of the  $\beta$ -carotene contents of the drinks ( $p < 0.05$ ) with no specific pattern in the level of

increase in water-soluble vitamins. However, the enrichment of the *Zobo* drink with beetroot significantly enhanced the values of vitamin E in the enriched samples ( $p < 0.05$ ). Sample E was highest in vitamins B<sub>1</sub>, B<sub>3</sub>, B<sub>6</sub>, B<sub>9</sub>, and C, while sample A was highest in  $\beta$ -carotene.

**Table 3: Vitamin composition of plain and enriched Hibiscus Sabdariffa drink (mg/100g)**

Sample	A	B	C	D	E	p-value
$\beta$ -Carotene ( $\mu\text{g}$ )	212.80±0.02 <sup>a</sup>	33.20±0.02 <sup>b</sup>	26.20±0.01 <sup>c</sup>	25.70±0.02 <sup>b</sup>	27.52±0.02 <sup>d</sup>	<0.001
Vitamin B <sub>1</sub>	0.20±0.02 <sup>a</sup>	0.20±0.02 <sup>b</sup>	0.25±0.02 <sup>b</sup>	0.20±0.01 <sup>b</sup>	0.30±0.01 <sup>c</sup>	<0.001
Vitamin B <sub>2</sub>	0.30±0.02 <sup>a</sup>	0.30±0.02 <sup>bc</sup>	0.30±0.02 <sup>ab</sup>	0.40±0.02 <sup>d</sup>	0.34±0.02 <sup>c</sup>	<0.001
Vitamin B <sub>3</sub>	2.80±0.02 <sup>a</sup>	2.90±0.02 <sup>b</sup>	2.82±0.02 <sup>a</sup>	2.90±0.02 <sup>b</sup>	3.01±0.04 <sup>c</sup>	<0.001
Vitamin B <sub>5</sub>	0.25±0.01 <sup>a</sup>	0.23±0.01 <sup>a</sup>	0.31±0.02 <sup>b</sup>	0.40±0.02 <sup>c</sup>	0.33±0.02 <sup>b</sup>	<0.001
Vitamin B <sub>6</sub>	0.90±0.01 <sup>a</sup>	0.80±0.01 <sup>b</sup>	0.10±0.02 <sup>c</sup>	0.91±0.02 <sup>d</sup>	0.93±0.02 <sup>d</sup>	<0.001
Vitamin B <sub>9</sub> ( $\mu\text{g}$ )	1.20±0.02 <sup>a</sup>	1.32±0.02 <sup>b</sup>	1.30±0.02 <sup>c</sup>	1.40±0.02 <sup>d</sup>	1.50±0.02 <sup>e</sup>	<0.001
Vitamin C	1.71±0.03 <sup>a</sup>	1.90±0.01 <sup>b</sup>	2.20±0.01 <sup>c</sup>	2.40±0.01 <sup>d</sup>	2.60±0.02 <sup>e</sup>	<0.001
Vitamin E	32.10±0.02 <sup>a</sup>	33.20±0.02 <sup>b</sup>	37.10±0.03 <sup>c</sup>	39.20±0.02 <sup>d</sup>	40.10±0.02 <sup>e</sup>	<0.001

The plain *Zobo* drink had acidic pH and contained an appreciable amount of titratable acidity, antioxidants, and phytochemicals (Table 4). The enriched drinks were more acidic, and the level of acidity increased with the level of inclusion of beetroot. Sample A was least in the phytochemical

composition and betaine content, while Sample E was significantly highest in phytochemical content. Sample B was lowest in acidity, total antioxidants, betaine, and polyphenols among the enriched samples.

**Table 4: Phytochemical composition of plain and enriched Hibiscus Sabdariffa and drink**

Sample	A	B	C	D	E	p-value
Titrateable acidity (g/100g)	1.95±0.02 <sup>a</sup>	2.10±0.01 <sup>b</sup>	2.30±0.02 <sup>c</sup>	2.20±0.03 <sup>d</sup>	2.40±0.02 <sup>e</sup>	<0.05
pH	2.70±0.00 <sup>e</sup>	2.54±0.01 <sup>d</sup>	2.40±0.00 <sup>c</sup>	2.42±0.01 <sup>b</sup>	2.32±0.01 <sup>a</sup>	<0.05
Total antioxidant (mg)	52.80±0.02 <sup>a</sup>	55.41±0.02 <sup>b</sup>	61.82±0.02 <sup>c</sup>	58.10±0.02 <sup>d</sup>	63.30±0.02 <sup>e</sup>	<0.05
Betaine (mol/l)	60.0±0.02 <sup>a</sup>	81.0±0.03 <sup>b</sup>	96.0±0.02 <sup>c</sup>	85.0±0.03 <sup>d</sup>	118.0±0.03 <sup>e</sup>	<0.05
Flavonoids (mg/100g)	3.00±0.00 <sup>a</sup>	4.00±0.00 <sup>b</sup>	6.00±0.00 <sup>c</sup>	8.00±0.00 <sup>d</sup>	9.00±0.00 <sup>e</sup>	<0.05
Polyphenol (mg/100g)	41.31±0.03 <sup>a</sup>	43.20±0.03 <sup>b</sup>	47.90±0.03 <sup>c</sup>	45.70±0.03 <sup>d</sup>	49.82±0.04 <sup>e</sup>	<0.05

In Table 5, the sensory scores for the colour and taste of the enriched drink samples decreased as the level of the beetroot added increased. Sample C (20% inclusion level) had the highest score for colour and taste, while the plain drink was the highest indoor and mouth-feel. In terms of general

acceptability, Sample C was the most acceptable drink, followed by Sample B (10% inclusion level) and Sample A. Sample D (30% inclusion level) was ranked the lowest among the drinks. There were no significant differences in all the sensory parameters assessed by the panellists.

**Table 5: Sensory attributes of Roselle drink enriched with beetroot**

Samples	Colour	Taste	Odour	Mouth-feel	Overall Acceptability
A	7.27±1.74	3.83±1.90	5.90±1.60	4.73±2.10	4.80±1.90
B	7.27±1.60	4.10±1.80	5.50±1.90	4.70±1.90	4.83±1.60
C	7.40±1.73	4.33±1.80	5.50±1.90	4.60±1.94	5.30±1.70
D	6.70±1.90	3.50±1.83	5.10±1.84	4.40±2.10	4.33±1.91
E	6.60±2.14	4.20±2.60	5.03±2.20	4.55±2.60	4.73±2.54
p-value	0.33	0.50	0.43	0.10	0.44

## DISCUSSION

The moisture content of the *Zobo* drink samples is very high, with little crude protein and carbohydrate contents and negligible fat and fibre contents. However, the addition of beetroot led to a significant reduction in moisture and a significant increase in the nutrient contents of the enriched drinks. The increased nutrient contents observed in this study are believed to be contributed by beetroot; its consumption is being promoted for its nutrient and bioactive substances (Clifford *et al.*, 2015). The observed low protein, fat, and fibre with relatively higher carbohydrate contents of the drinks are characteristic of refreshing drinks, which are usually low in these nutrients (Adepoju *et al.*, 2010). The low carbohydrate content of the drinks qualifies them as good and healthy refreshing drinks for everyone. Unlike carbonated drinks, both plain and enriched *Hibiscus Sabdariffa* drinks can contribute to the nutrient intake of consumers without adding unwanted energy intake to its consumers.

The *Hibiscus sabdariffa* drink was very low in micromineral, especially sodium. Its low sodium content qualifies it as a good refreshing drink for the hypertensive. The addition of beetroot to *Hibiscus sabdariffa* drink resulted in a significant increase in both macro and micronutrient contents of the drinks. The acidic property of the drinks can be an advantage in preventing microbial spoilage and increasing the shelf life of the drinks. The addition of beetroot to the *Hibiscus sabdariffa* drink increased the vitamin B<sub>9</sub>, C and E of the enriched samples. This observation is similar to the findings of Abiola *et al.* (2013), who reported that the ascorbic acid contents of *Hibiscus sabdariffa* drinks supplemented with garlic and ginger were higher than that of ordinary *Hibiscus sabdariffa* juice.

The most acceptable sample was the enriched *Hibiscus sabdariffa* with a 20% beetroot inclusion level (Sample C). The enriched drink can serve as a healthy and refreshing drink; and, if consumed often, will contribute immensely towards meeting the consumers' micronutrient requirements for the promotion of good health and longevity.



## CONCLUSION

Beetroot significantly improved the micronutrient and phytochemical content of the *Hibiscus sabdariffa* drink. The enriched *Zobo* drink had relatively higher levels of total antioxidants, fat-soluble vitamins and essential minerals, which qualify it as a good drink to be encouraged for a healthy lifestyle. Enriched drinks with 20% beetroot (Sample C) had the highest iron content, a micronutrient that is important for haemoglobin formation and prevention of anaemia; hence, it is the best choice among the drinks.

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