



Nutritional Quality and Antioxidant Properties of Roselle-Tiger Nut Beverage

Mtam, N.P. ^{a*} and Iombor, T.T. ^b

^a Department of Home Science and Management, Joseph Sarwuan Tarka University, Makurdi, Nigeria.

^b Department of Nutrition and Dietetics, Joseph Sarwuan Tarka University, Makurdi, Nigeria.

Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Background: A beverage is a liquid prepared from cereals, grains and leaves/calyxes for human consumption. Beverages are classified into alcoholic and non-alcoholic beverages.

Objective: The study evaluated the nutritional quality and antioxidant properties of Roselle-tiger nut beverage.

Methods: Roselle-tiger nut beverage was produced from the control samples roselle and tiger nut (% v/v) using the ratios 100:0 Roselle beverage, 0:100 tiger nut beverage and 80:20 Roselle-tiger nut beverage. Subsequently, Proximate, mineral, phytochemical compositions, antioxidants properties and sensory properties were determined using established methods.

Results: There was an elevation in protein (16.29 ± 2.39^a) carbohydrate (11.08 ± 9.59^a), crude protein (16.29 ± 2.39^a) and ash content (3.09 ± 0.56^a) but a decrease in moisture and crude fat content. Also, an increase in Sodium (0.09 ± 0.01^b), Zinc (0.21 ± 0.00^b) and Copper (0.09 ± 0.00^b) content with a decrease in Potassium, Phosphorus, Manganese, Magnesium, Calcium and Iron levels. For phytochemical compositions, an increase in flavonoids (1.73 ± 0.43^a), saponins (1.25 ± 0.75^a) and

*Corresponding author: Email: patnguhemenmtam@gmail.com;

alkaloids (1.36 ± 0.13^a) contents with a decrease in tannins, oxalates and phytate contents was observed. There was an elevation in free radical scavenging activity (0.36 ± 0.00^{ab}) and ferric reducing power (0.32 ± 0.00^c) but a decrease in hydroxyl scavenging activity and metal chelating ability. Sample MNP836 of Roselle-tiger nut beverage was the most preferred by panelists for appearance (8.10), taste (8.24), flavor (8.38), mouth feel (8.38) and overall acceptability (8.48).

Conclusion: Substitution of roselle beverage with 20% tiger nut beverage improved the nutritional quality of roselle-tiger nut beverage.

Keywords: Antioxidants; roselle; tiger nut; beverage and nutritional; non-alcoholic beverages; free radicals.

1. INTRODUCTION

A beverage is a liquid prepared from cereals, grains and leaves/calyxes for human consumption. Beverages are classified into alcoholic and non-alcoholic beverages. Each of this is further classified into industrial and homemade beverages [1]. Global beverage consumption stood at about 145 billion liters in 2017 for alcoholic beverages, and 812 billion liters for non-alcoholic beverages [2]. Beverages are rich sources of carbohydrates, micronutrients (vitamins and minerals) and anti-oxidants (carotenoids and flavonoids). High levels of alcoholic beverage intake have been reported to be associated with cancer, cardiovascular diseases, high blood pressure, diabetes mellitus, obesity and degenerative diseases of man [3], while high levels of non-alcoholic beverage intake are associated with weight gain.

Hibiscus sabdariffa Linn is a tropical plant that belongs to the super order *Malvaceae*. The plant is cultivated and consumed as a vegetable and beverage, whereas other *Hibiscus* varieties are cultivated because of their rich fiber content. *Hibiscus sabdariffa* is commonly known as Roselle or Sorrel, *Zobo* (Hausa), *Ashwe* (Tiv) in Nigeria [4]. "Various types of highly valued food and medicinal products have been produced from *Hibiscus sabdariffa*. Roselle beverage made from the calyxes of *Hibiscus sabdariffa* is reported to be highly nutritive with many medicinal potentials (lowers blood pressure, anti-diabetic, weight reduction, anti-hyperlipidemia, hepatoprotective, anti-cancer, as well as an antioxidant).

Phytochemicals are non-nutritive plant chemicals that possess disease protective and preventive properties. They are non-essential nutrients implying that they are not required to sustain life of humans. However, these chemicals produced abundantly by this plant, have been shown to have various chemo therapeutic and chemo preventive effects against many diseases of

man" [5]. "These phytochemicals have the ability to protect our cells from oxidative damage and limit the risk of developing certain types of diseases as a result of their antioxidant activities" [6]. Flavonoids, oxalate, tannin, phytate, polyphenols, alkaloids and saponins are phytochemicals present in Roselle.

Vitamins A, C and E are nutritive antioxidants which confer additional therapeutic functions to the calyxes of *Hibiscus sabdariffa* by scavenging free radicals generated in the body. They do this by binding to the free radicals in order to make them less toxic to the cell. Vitamin C provides the first line of defense against oxidative stress. Antioxidants can donate electrons and thus, can inactivate free radicals and convert them to less harmful compounds like water" [7].

Tiger nut (*Cyperus esculentus*) is a tuber crop that belongs to the family *Cyperaceae*, which is cultivated throughout the world and is widely found in the northern parts of Nigeria. In Nigeria, it is commonly known as "Aya" in Hausa, "Ofio" in Yoruba, "Ishoho" in Tiv and "Akihausa" in Igbo, where three varieties (black, brown and yellow) are cultivated [8]. It has been reported to contain appreciable quantities of myristic, oleic and linoleic fatty acids [9]. Tiger nut have been reported as helping in the prevention of heart attacks and thrombosis by enhancing blood circulation. In addition, it is believed to assist in reducing the risk of colon cancer attributed to its high vitamin C, K, E and metabolic antioxidant power. Tiger nut is rich in energy content (starch, fat, sugars and protein), mineral (phosphorus, potassium) and vitamins E and C [10].

2. METHODOLOGY

Roselle (*Hibiscus sabdariffa*) calyxes and tiger nut (*Cyperus esculentus*) were purchased from Wadata Market, Makurdi, Benue State. The flow chart below showed how Roselle-Tiger nut beverage was prepared.

2.1 Proximate Composition Determination

Proximate composition refers to the basic components of a food item, including moisture, ash, lipid, protein, and carbohydrates. It is an important aspect of nutrition analysis and can be used for product development, quality control and regulatory purposes [11,12].

2.2 Moisture Content Determination

The oven drying method was used as described by AOAC [13]. The samples (2g)

were accurately weighed into moisture dishes. The moisture dishes were washed, dried in the oven at 85°C for 30 minutes and placed inside the desiccators to cool, its weight was noted (W_1). Each of the dishes were weighed together with the sample (W_2) and then placed inside the oven and was heated for 20 minutes at 105°C and was also weighed (W_3). This was repeated until the weight became constant. The percentage of moisture content (MC) was calculated from the weight loss using the formula below:

$$\% \text{Moisture Content} = \frac{W_2 - W_3}{W_2 - W_1} \times 100 = \frac{\text{Weight loss after drying}}{\text{Weight of sample before drying}} \times 100 \quad (1)$$

Where;

W_1 = Initial weight of empty pan

W_2 = Weight of the pan + Sample before drying

W_3 = Final weight of pan + Sample after drying

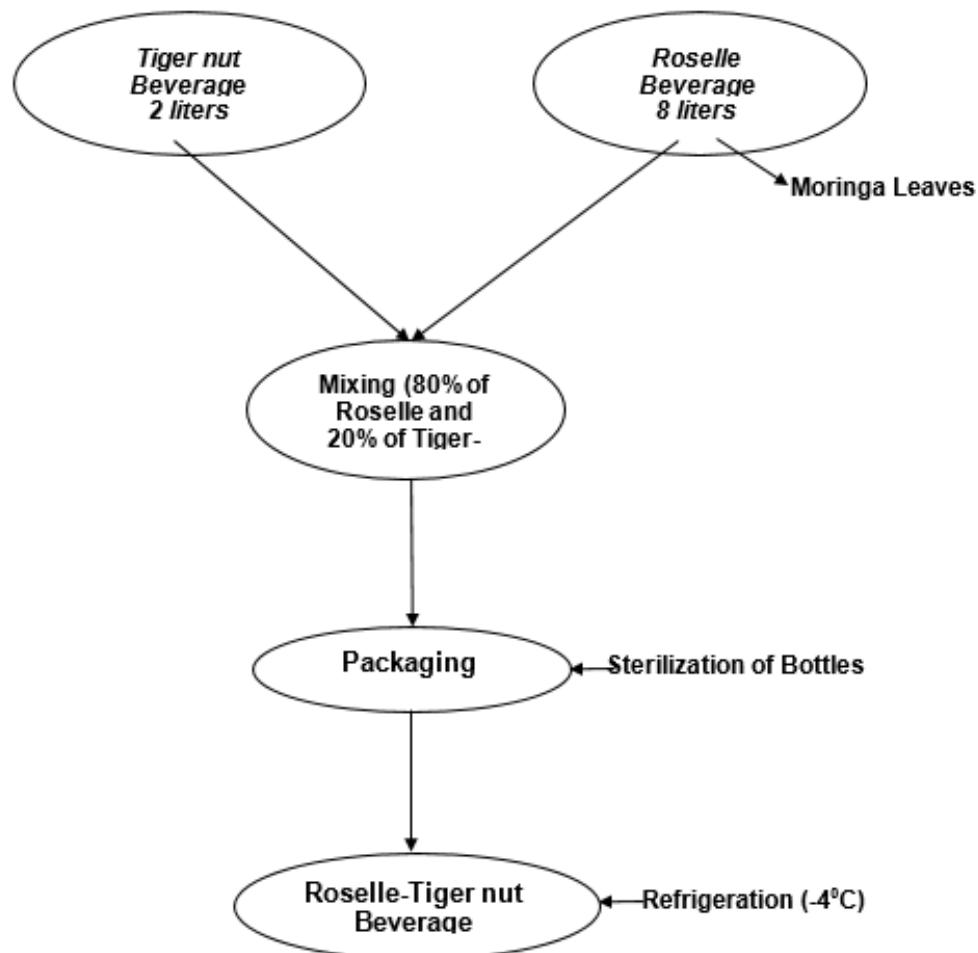


Fig. 1. Flowchart of roselle tiger nut beverage production

Source: Abiodun, et al. [12] (Modified)

2.3 Nitrogen and Crude Protein Determination

2.3.1 Protein digestion

The method of AOAC [13] was used. Exactly 1.5 g of the defatted sample in an ashless filter paper was put into 300mL Kjeldahl flask. 25 mL of H₂SO₄ and 3 g of digested mixed catalyst (weighed separately into an ashless filter paper) was poured into the Kjeldahl flask. The flask was then transferred to the Kjeldahl digestion apparatus. The sample was digested until a clear green colour was obtained. The digest was cooled and diluted to 100 mL with distilled water.

2.3.2 Distillation of the digest

20mL of the diluted digest was measured into a 500mL Kjeldahl flask containing antibumping chips and 40 mL of 40% NaOH was slowly added by the side of the flask. A 250 mL conical flask containing a mixture of 50 mL of 2% Boric acid and 4 drops of mixed indicator was used to trap the ammonia liberated. The conical flask and the Kjeldahl flask were then placed on the Kjeldahl distillation apparatus, with the tubes inserted into the conical flask and the Kjeldahl flask. The flask was heated to distill out NH₃ evolved. The distillate was collected into the boric acid solution. From the point when the boric acid turned green, 10 minutes were allowed for complete distillation of the ammonia present in the digest. The distillate was then titrated with 0.1M HCl. Percent Nitrogen can be obtained using the following expression:

$$\% \text{Nitrogen (N)} = \frac{14 \times M \times V_t \times T_v \times 100}{\text{Weight of Sample (mg)} \times V_a} \quad (2)$$

Therefore:

$$\% \text{Crude Protein} = \% \text{Nitrogen (N)} \times 6.25 \quad (3)$$

(conversion factor)

Where:

M = Actual molarity of acid

Tv = Titre volume of HCl used

Vt = Total volume of diluted digest

Va = Aliquot volume distilled

2.4 Crude Fat (Lipid) Determination

The soxhlet fat extraction methods as described by AOAC [13] was used. 250 mL boiling flask was cleaned and dried in an oven at 105°C for 30 minutes. The flask will then be transferred into

a desiccator and allowed to cool. The flask will then be labelled (W₁), weighed and then filled with 30 mL petroleum ether. Then 20 g of the sample was weighed into a correspondingly labelled thimble. The extraction thimble was tightly plugged with cotton wool. The soxhlet apparatus was assembled and allowed to reflux and ether collected at the top of the container in the set-up and drained into another container for re-use. The flask was removed and dried at 103°C for 20 minutes, then transferred from the oven into a desiccator and allowed to cool and then weighed and labelled (W₂). The percentage fat was calculated as follows:

$$\% \text{Crude Fat (Lipid)} = \frac{\text{Weight of Fat (W}_2 - \text{W}_1)}{\text{Weight of Sample}} \times 100 \quad (4)$$

2.5 Ash Determination

The ash content was determined using the official method of the AOAC [13]. The porcelain crucibles were washed and dried in hot air oven at 105°C for 10 minutes and cooled in desiccators and then weighed (W₁). Then 2 g of each sample was transferred into the crumble (W₂) on a heater inside a fume cupboard. The sample was transferred into a preheated muffle furnace at 550°C and left at this temperature for 8 hours to ensure proper ashing. The residue will then be cooled in a desiccator and weighed (W₃). The percentage of ash was calculated as follows:

$$\% \text{Ash} = \frac{W_3 - W_1}{W_2 - W_1} \times 100 \quad (5)$$

Where:

W₁ = Weight of the empty crucible

W₂ = Weight of crucible + Sample before ashing

W₃ = Weight of crucible + Sample after ashing

2.6 Crude Fiber Determination

The method described by AOAC [13] was used. Two grams of sample was weighed out into a round bottom flask. 100 mL of 0.25 M sulphuric acid solution was added and the mixture boiled under reflux for 30 min. The hot solution was quickly filtered under suction. The insoluble matter was washed several times with hot water until it was acid free. It was quantitatively transferred into the flask and 100mL of hot 0.31 M Sodium hydroxide solution was added and the mixture boiled again under reflux for 30 minutes and quickly filtered under suction. The insoluble residue was washed with boiling water until it

was based free. It was dried to constant weight in the oven at 100°C, cooled in a desiccator and weighed (C_1) was then incinerated in a muffle furnace at 550°C for 2 hours, cooled in the desiccator and reweighed (C_2). It can be calculated using the following expression:

$$\%Crude\ Fibre = \frac{Loss\ in\ weight\ on\ incineration\ (C_1 - C_2)}{Weight\ of\ Original\ Sample} \times 100 \quad (6)$$

2.7 Carbohydrate Determination (By Difference)

The total carbohydrate content was determined by difference. The sum of the percentage moisture, ash, crude lipid, crude protein and crude fibre was subtracted from 100 [14]. The value obtained was the percentage carbohydrate constituent of the sample. Thus,

$$\%Carbohydrate = 100 - (\%Moisture + \%Crude\ Fibre + \%Protein + \%Fat + \%Ash) \quad (7)$$

2.8 Minerals Determination

2 mL of each sample was weighed using weighing balance and then transferred to a beaker. 15mL of Hydrochloric acid (HCl) and 5 mL of Nitric acid (HNO_3) were also added to the mixture respectively. The mixtures were then heated with a magnetic stirrer regulator hot plate under a fume hood cupboard at 100°C to produce fumes for 1 hour and then allowed to cool for 20 minutes. Spatula was then used to stir the digested solutions ready for filtration. Distilled water was added to get 60 mL of samples which were used for mineral analysis. 0.5 mL was aspirated to the nebulizer via nebulizer rubber to burner, and then flame photometer was also burnt to the aspirated drop of digested samples to vapour within the mobile phase of the AAS (Atomic Absorption Spectroscopy), model AA 6800 Shimadzu, Japan. The detector then captured the concentration in the vapour in ppm (parts per million) as well as the beam of cathode lamp. The analysis was done in triplicates and data was printed for use.

2.9 Phytochemical Determination

"Phytochemical composition refers to the chemical compounds found in plants that have potential therapeutic benefits and can be obtained through extraction processes" [15,16].

2.10 Alkaloid Determination

[17] method was used for the determination alkaloid. 5 g of the sample was weighed into a

250 mL beaker and 80 mL of 10% acetic acid in ethanol was added and covered and allowed to stand for 4 hours. This was filtered and the extract was concentrated on a water bath to one quarter of the original volume. Concentrated ammonium hydroxide was added drop-wise to the extract until precipitation was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed.

2.11 Flavonoid Determination

Ten grammes of the plant sample were extracted with 100 mL of 80% aqueous methanol at room temperature. The solution was filtered through whatman filter paper No 42 (125 mm). The filtrate was later transferred into a crucible and evaporated into dryness over a water bath and weighed to a constant weight [18].

2.12 Phytate Determination

Phytate content was determined according to Kirk and Sawyer [19] method. The sample was extracted with 100 mL of 0.02N HCl with vigorous shaking for 30 minutes. Exactly 1 mL of the supernatant was treated with 1.5 mL bipyridine solution. The absorbance was measured in a spectrophotometer at 519 nm. The amount of phytate was extrapolated from the standard curve prepared from phytic acid.

2.13 Tannin Determination

Van-Burden and Robinson [20] method was used in tannin determination. 500 mg of the sample was weighed into a 50 mL plastic bottle. 50 mL of distilled water was added and shaken for 1 hour in a mechanical shaker. This was filtered into a 50 mL volumetric flask and made up to the mark. Then 5 mL of the filtrate was pipetted out into a test tube and mixed with 2 mL of 0.1M $FeCl_3$ in 0.1N HCl and 0.008 M potassium ferro cyanide. The absorbance was measured at 620 nm within 10 minutes.

2.14 Oxalate Determination

The titration method described by Day and Underwood [21] was used to determine the oxalate content. One gram of the sample was weighed into 100 mL conical flask where 75 mL of 3N H_2SO_4 was added and stirred for 1 hour. It was then filtered using Whatman No 1 filter paper. From the filtrate, 25 mL was taken and titrated while hot (80-90°C) against 0.1N $KMnO_4$

solution, until a faint pink colour persisted for at least 30 seconds. The overall redox reaction was $\text{MnO}_4^- + \text{C}_2\text{O}_4^{2-} + 8\text{H}^+ \rightarrow \text{Mn}^{2+} + 4\text{H}_2\text{O} + 2\text{CO}_2$. Oxalate can be determined by using the expression below:

$$\text{Oxalate (mg/100mg)} = \frac{T \times V_{\text{me}} \times D_f}{M_E \times M_S (g)} \times 100 \quad (8)$$

Where:

T = Titre value of KMnO_4 (mL)

V_{me} = Volume mass equivalent (1 cm³ of 0.05M KMnO_4 solution is equivalent to 0.00225 anhydrous Oxalic acid).

D_f = Dilution factor ($V_t/A = 75/25 = 3$) where V_t is the total volume of titrate (filtrate, 75mL) and A is the aliquot used (25mL).

M_E = Molar equivalent of KMnO_4 redox reaction and

M_S = Mass of sample used.

2.15 Saponin Determination

The method of Obadoni and Ochuko [22] was used. The samples were ground and 20 g of each were put into a conical flask and 100 cm³ of 20% aqueous ethanol were added. The samples were heated over a hot water bath for 4 hours with continuous stirring at 55°C. The mixture was filtered and the residue re-extracted with another 200 mL 20% ethanol. The combined extracts were reduced into 40 mL over water bath at about 90°C. The concentrate was transferred into a 250 mL separator funnel 20 mL of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60 mL of n-butanol was added. The combined n-butanol extracts were washed twice with 10 mL of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the samples were dried in the oven to a constant weight; the saponin content was calculated as percentage.

2.16 Total Phenol Determination

The fat free sample was boiled with 50 mL of ether for the extraction of the phenolic component for 15 minutes. 5 mL of the extract was pipetted into a 50 mL flask, then 10 mL of distilled water was added. 2 mL of ammonium hydroxide solution and 5 mL of concentrated amyl alcohol were also added. The samples were made up to mark and left react for 30 minutes for colour development.

2.17 Antioxidant Properties Determination

An aqueous extract of sample was obtained by weighing 10 g of powdered sample and extracted with 300 mL of 5% ethanol by boiling for 15 minutes. After filtration, the filtrate was then evaporated to get the extract. Extract was then stored in the refrigerator for further analysis.

2.18 DPPH Radical Scavenging Activity

The free radical scavenging activity of Roselle-Tiger nut beverage was measured by diphenyl 1-picrylhydrazyl (DPPH) assay according to the method described by Bursal and Gulcin [23]. The scavenging activity was estimated based on the percentage of DPPH radical scavenged in the following equation:

$$\text{DDPH Radical Scavenging Activity(\%)} = \frac{[(A_{\text{control}} - A_{\text{test}})]}{A_{\text{control}}} \times 100 \quad (9)$$

Where:

A_{control} = Absorbance of the control reaction

A_{test} = Absorbance in the presence of the sample of the extracts.

2.19 Hydroxyl Radical Scavenging Activity

The scavenging activity of the extracts of Roselle-Tiger nut beverage (1, 2, 3 and 4 mg/mL) on hydroxyl radical activity was measured according to the method of Ilavarasan *et al.*, [24]. The reaction mixture contained deoxyribose 1 mL (2.8 mM), KH_2PO_4 -NaOH buffer, pH 7.4 (0.05 M), 0.4 mL FeCl_3 (0.1 mM) and EDTA (0.1 mM), 0.2 mL H_2O_2 (1 mM) and different concentrations of the sample extracts in a final volume of 2 mL. The mixture was incubated at 37°C for 30 minutes followed by the addition of 2 mL of trichloroacetic acid (2.8% w/v) and thiobarbituric acid. Thereafter, it was kept for 30 minutes in a boiling water bath and then cooled. The absorbance was recorded at 532 nm in a UV-VIS spectrophotometer. The hydroxyl radical scavenging activity of the sample extract was evaluated using the following equation:

$$\text{Hydroxyl Radical Scavenging Activity(\%)} = \frac{1 - A_{\text{test}}}{A_{\text{control}}} \times 100 \quad (10)$$

Where:

A_{control} = Absorbance of the control reaction

A_{test} = Absorbance in the presence of the sample of the extracts.

2.20 Ferric Reducing Antioxidant Power (FRAP)

The reducing power of the extracts of Roselle-Tiger nut beverage was determined by the method described by Oyaizu [25]. Aliquots of 1 mL of methanol extract of the sample at 4 different concentrations 0.1, 0.5, 1 and 2 mg/mL (in three replicates) were mixed with 2.5 mL of 0.2 mM phosphate buffer solution at pH 6.6 and 2.5 mL of 1% potassium ferrocyanide. The mixture was incubated for 20 min at 50 °C in a water bath. Then, 2.5 mL of 10% trichloroacetic acid was added, and the mixture was centrifuged at 3000 rpm for 10 minutes. After centrifugation, 2.5 mL of the supernatant was mixed with 2.5 mL of distilled water and 0.5 mL of 0.1% $FeCl_3$.

2.21 Chelation of Metal Ions

The metal chelating activity was measured by adding 0.1 mM $FeSO_4$ (0.2 mL) and 0.25 mM ferrozine (0.4 mL) subsequently into 0.2 mL of plant extract [26]. After incubating at room temperature for 10 minutes, the absorbance of the mixture was recorded at 562 nm. The chelating activity was calculated using the following formula:

$$\text{Metal Chelating Effect(\%)} = \frac{[(A_{control} - A_{test})]}{A_{control}} \times 100 \quad (11)$$

Where:

A_c = Absorbance Values of the control
 A_s = Absorbance Values of the samples

2.22 Sensory Analysis

Sensory evaluation was carried out on six samples of the beverage namely; samples MNP834, MNP835, MNP836, MNP837, MNP838 and MNP839 using 21 panelists. Testing was conducted in the Sensory Laboratory of the Department of Home Science and Management, Joseph Sarwuan Tarka University, Makurdi, Nigeria. Panelists evaluated the taste, flavor, mouth feel, appearance and overall acceptability of the beverages using a 9-point Hedonic Scale with 1 = Dislike Extremely, 2 = Dislike Very

Much, 3 = Dislike Moderately, 4 = Dislike Slightly, 5 = Neither Liked nor Disliked, 6 = Like Slightly, 7 = Like Moderately, 8 = Like Very Much, and 9 = Liked Extremely [27]. Three samples namely; Samples MNP834, MNP835 and MNP836 were chosen after sensory evaluation by the panelists for this research.

2.23 Design of the Study/Sample Size

The research design adopted was Quasi-Experimental Design. It is a design used when it is not ethical to conduct a randomized controlled trial. Similarly, it is a design where a researcher is only able to use partial control instead of full control over the subjects and the time, he can expose them to the experimental stimuli.

Three departments namely Agricultural Economics, Home Science and Management and Soil Science were used for the study and the total sample size was 357 students.

2.24 Data Analysis Techniques

Analytical determination was conducted in triplicates. Data entry was performed using the Statistical Package for Social Sciences (SPSS V23) software while data was subjected to Analysis of Variance (ANOVA) and pairwise T-Test was used to compare means. Differences were considered significant at 95% ($p \leq 0.05$) [28].

3. RESULTS AND DISCUSSION

In Table 1, the proximate properties of the beverage ranges between 65.42(MNP834) to 59.03% (MNP836) for moisture, crude protein 12.68 (MNP834) to 16.29% (MNP836), crude lipid 8.73% (MNP835) to 6.10% (MNP836), crude fibre 3.97% (MNP834) to 5.05% (MP835), ash 1.78% (MNP834) to 3.09% (MNP836) and carbohydrates 7.43% (MNP834) to 11.08% (MNP836). Substitution of Roselle beverage with Tiger nut beverage elevated the protein (12.68 to 16.29%), fibre (3.97 to 4.88%), ash (1.78 to 3.09%) and carbohydrates (7.43 to 11.08%). Contents of its blends related to roselle beverage alone but lowered moisture contents of its blend with tiger nut beverage. The elevations of decreases in the proximate parameters were not significant ($p \geq 0.05$).

Table 1. The Effect of Substitution (v/v) of Roselle Beverage with Tiger nut Beverage on the Proximate Composition of Roselle-Tiger nut Beverage (%)

Parameter	Sample MNP834	Sample MNP835	Sample MNP836
Moisture Content	65.42±2.92 ^a	61.00±3.06 ^a	59.03±0.72 ^a
Ash	1.78±0.34 ^a	1.95±0.31 ^a	3.09±0.56 ^a
Crude Lipid	8.31±2.01 ^a	8.73±3.06 ^a	6.10±0.15 ^a
Crude Protein	12.68±2.59 ^a	13.44±2.64 ^a	16.29±2.39 ^a
Crude Fibre	3.97±2.17 ^a	5.05±3.78 ^a	4.88 ±2.75 ^a
Carbohydrates	7.43±0.94 ^a	10.25±0.12 ^a	11.08±9.59 ^a

Values were expressed as mean ± S.M.E; n=3. Values with different superscript across the rows were considered statistically significant ($p \leq 0.05$) while values with the same superscript across the rows were considered not significant ($p \geq 0.05$)

KEY:

MNP834 = 100% Roselle beverage (Control)

MNP835 = 100% Tiger nut beverage (Control)

MNP836 = 80% Roselle beverage + 20% Tiger nut beverage (Blend)

MNP = Mtam Nguhemmen Patience

Table 2. The effect of 20% tiger nut beverage substitution of roselle beverage on the mineral elements composition of roselle-tiger nut beverage (mg/L)

Mineral	Sample MNP834	Sample MNP835	Sample MNP836	RDA
Sodium	0.03 ±0.01 ^a	0.03 ±0.01 ^a	0.09±0.01 ^b	2300
Potassium	0.66±0.01 ^c	0.28±0.00 ^a	0.53±0.00 ^b	4700
Phosphorus	0.14±0.02 ^a	0.13±0.02 ^a	0.05±0.04 ^a	1250
Manganese	0.66±0.01 ^c	0.28±0.00 ^a	0.53±0.00 ^b	2.3
Zinc	0.19±0.00 ^a	0.30±0.00 ^c	0.21±0.00 ^b	11
Copper	0.08±0.00 ^a	0.08±0.00 ^a	0.09±0.00 ^b	0.9
Magnesium	3.58±0.00 ^b	3.54±0.01 ^a	3.56±0.00 ^b	420
Calcium	6.61±0.22 ^c	0.88±0.02 ^a	4.33±0.08 ^b	1300
Iron	2.77±0.04 ^a	3.93±0.04 ^b	2.67±0.00 ^a	18

Values were expressed as mean ± S.M.E; n=3. Values with different superscript across the rows were considered statistically significant ($p \leq 0.05$) while values with the same superscript across the rows were considered not significant ($p \geq 0.05$)

KEY:

MNP834 = 100% Roselle beverage (Control)

MNP835 = 100% Tiger nut beverage (Control)

MNP836 = 80% Roselle beverage + 20% Tiger nut beverage (Blend)

MNP = Mtam Nguhemmen Patience

RDA = Recommended Dietary Allowances

The moisture content of 65.42% (Roselle beverage) and 61.00% (Tiger nut beverage) as well as ash 1.78% (Roselle beverage) and 1.95% (Tiger nut beverage) content of this study were lower than 89.63% and 2.31%, respectively reported for Roselle-Apple beverage [29]. The carbohydrate, protein, crude fibre and crude fats of Roselle and Tiger nut beverage of this study were however, higher than 6.31%, 0.36%, 0.24% and 1.14%, respectively reported for Roselle-Apple beverage by Fasoyiro and Coworkers [29]. Roselle-Tiger nut beverage had lower moisture (59.03%) and carbohydrate (11.08%) contents when compared to 80.13-88.21% and 11.70-14.60% respectively reported for Roselle-Apple beverage [29].

Table 2, revealed that the Sodium (0.09mg/L), Zinc (0.21 mg/L) and Copper (0.09 mg/L) significantly ($p \leq 0.05$) increased in Roselle-Tiger nut beverage relative to Roselle beverage control. The Potassium (0.53 mg/L), Phosphorus (0.05 mg/L), Manganese (0.53 mg/L), Magnesium (3.56mg/L), Calcium (4.33 mg/L) and Iron (2.67 mg/L) of Roselle-Tiger nut beverage decreased following 20% substitution of Roselle beverage with Tiger nut beverage.

Mineral elements play crucial roles in human health and well-being. They are essential for various physiological and metabolic processes in the body, such as enzyme function, bone maintenance, immune responses, and nerve impulse transmission [30]. Minerals are essential

nutrients as they cannot be synthesized by the human body and must be obtained from the diet [30]. The potassium, phosphorus, manganese, copper and calcium content of Roselle-Tiger nut beverage of this study was higher than 2.0-2.6 mg/100g, 1.7-2.0 mg/100g, 1.2-1.8 mg/100g, 0.2-0.5 mg/100g and 2.8-3.5 mg/100g of local and commercial Roselle as well as soy fortified Roselle beverages [31]. Roselle extract has been reported to be a good source of calcium, magnesium, iron and phosphorus [30]. However, a 20% substitution of its beverage with Tiger nut beverage significantly ($p<0.05$) elevated the sodium, zinc and copper contents, but significantly ($p<0.05$) lowered the potassium, manganese and calcium contents while having no significant effect on the phosphorus, magnesium and iron levels. The decrease in mineral elements composition with decreased concentration of Roselle extract was in conformity with Fasoyiro *et al.* [29] findings for Roselle-Apple beverages. The mineral elements content of Roselle calyxes and Tiger nuts used in beverage production may have been influenced by fruit variety, soil, irrigation, water, weather conditions, and types and amounts of fertilizers used [31]. Iron and zinc perform important physiological roles in the human body, with prevalent nutritional deficiency problems globally [32]. Iron participates in a wide variety of metabolic processes, including respiration, energy production, DNA synthesis, and cell proliferation [33,34,35]. The Iron content (2.67 mg/l) of Roselle-Tiger nut beverage may provide about 14.83% of the 18 mg Recommended Daily Intake of iron for 19-50 years female [36]. The daily recommended Fe requirements for humans are 10-15 mg for children, 18 mg for women and 12 mg for men [36]. The RDI is set assuming a 10% rate of intestinal absorption [37]. Absorption of iron and zinc mainly takes place in the small intestine [38]. Deficiency of iron leads to anemia. High consumption of plant-based foods could have consequences for human health. Even though Roselle-Tiger nut beverage may be intrinsically rich in minerals such as iron and zinc (Table 3), it has been reported that their release from the food matrix during digestion might be limited due to anti-nutritional factors that bind them, thereby reducing the amount available for absorption [32]. "The Roselle-tiger nut beverage could be a good source of Fe, if its anti-nutrient content is low and could therefore alleviate of iron deficiency. The daily recommended Fe requirements for humans are 10-15 mg for children, 18 mg for women and 12 mg for men" [36]. This implies that Roselle-Tiger nut beverage

could provide 14.83% of dietary iron requirements of women. Roselle-Tiger nut beverage could be a good source of Fe, if its anti-nutrient content is low and could therefore be used to alleviate iron deficiency anemia. Potassium and sodium are required to maintain osmotic balance and the pH of the body, muscle regulation and nerve irritability, glucose absorption control and enhancement of normal retention of protein during growth [37]. The rich phosphorous and potassium content of the beverage may help prevent heart attacks and thrombosis, when consumed in the right quantities. Zinc plays an important role in human nutrition. Zinc deficiency results in retarded growth and delayed sexual maturation because of its role in nucleic acid metabolism and protein synthesis [39]. Zinc has been shown to be essential to the structure and function of a large number of macromolecules and for more than 300 enzymatic reactions [33,34,35]. Therefore, sufficient intake of zinc is important for ensuring optimal health, growth, and development of humans [40]. Copper is important in many metabolic activities of the body. The 0.09 mg/l copper content of Roselle-Tiger nut beverage may provide 10% of the 0.9 mg daily requirement. Copper and iron present in cytochrome oxidase (enzyme) which is involved in energy metabolism [41]. Magnesium is an activator of many enzyme systems and maintains the electrical potential in nerves [42]. The mineral content of plants can be significantly influenced by variety, location, and environmental conditions [43]. Calcium is a coordinator among inorganic elements, for example excess amount of K, Mg or Na in the body can be corrected by Ca and also adequate quantity of Ca in the diet assist in Fe utilization [44].

Table 3 showed that 20% substitution of Roselle beverage with Tiger nut beverage increased the tannins (0.79mg/g), flavonoids (1.73mg/g), saponins (1.25mg/g) and alkaloids (1.36mg/g) contents of Roselle-tiger nut beverage but lowered the oxolates (0.10mg/g) and phytate (0.02mg/g) contents of Roselle-Tiger nut beverage relative to the content of Roselle beverage alone. Among the three beverages Tiger nut beverage had the highest content of tannins (1.69mg/g), flavonoids (2.00mg/g), saponins (5.02mg/g) and alkaloid (3.86mg/g) while Roselle beverage had the highest contents of oxolates (1.02mg/g) and phytate (0.27mg/g).

Phytochemical composition refers to the chemical compounds found in plants that have potential therapeutic benefits and could be

obtained through extraction processes [45]. Roselle (*Hibiscus sabdariffa*) has a rich phytochemical composition, including carotenoids, flavonoids, phenols, anthocyanins, and vitamin C. The calyx extract of roselle genotypes contains high amounts of these phytochemicals, making it an excellent source of natural antioxidants [46]. Roselle petals also contain alkaloids, anthocyanins, flavonoids, saponins, steroids, sterols, and tannins, with high anthocyanin content and low phenol and flavonoid contents.

Twenty percent substitution of Roselle beverage with tiger nut beverage significantly ($p \leq 0.05$) elevated the DPPH (0.36 mg/mL) and Ferric reducing power (0.32 mg/mL) of Roselle-Tiger nut beverage and lowered its hydroxyl (1.28 mg/mL) and metal ions chelating (1.28 mg/mL) power relative to Roselle beverage.

Anti-nutrients are secondary metabolites, which have been evolved by plants for their defense mechanisms against pathogenic organisms (among other biological functions). In man, these secondary metabolites of plant food materials confer health benefits on man and animals if consumed at the appropriate amount in human food but hazardous at high quantity. Anti-nutrients have been shown to reduce blood glucose and but improve insulin responses to starchy foods and/or cholesterol. Besides, phytate, tannins, saponins, protease inhibitor and oxalate have been associated with reduced cancer risks [47]. At relatively high amount, they reduce the maximum utilization of nutrients especially proteins, vitamins and minerals and thus prevent the optimal exploitation of the nutrients present in food and decreases the food nutritive values.

Table 3. Phytochemical Compositions of Beverages (mg/g)

Parameter	Sample MNP834	Sample MNP835	Sample MNP836
Tannins	0.11±0.06 ^a	1.69±1.35 ^a	0.79±0.51^a
Flavonoid	1.27±0.70 ^a	2.00±0.82 ^a	1.73±0.43^a
Saponins	0.06±0.02 ^a	5.02±2.43 ^a	1.25±0.75^a
Alkaloids	0.80±0.44 ^a	3.86±1.62 ^a	1.36±0.13^a
Oxolates	1.02±0.30 ^b	0.20±0.00 ^a	0.10±0.00^a
Phytate	0.27±0.00 ^b	0.10±0.00 ^{ab}	0.02±0.00^a

Values were expressed as mean ± S.M.E; n=3. Values with different superscript across the rows were considered statistically significant ($p \leq 0.05$) while values with the same superscript across the rows were considered not significant ($p \geq 0.05$)

KEY:

MNP834 = 100% Roselle beverage (Control)

MNP835 = 100% Tiger nut beverage (Control)

MNP836 = 80% Roselle beverage + 20% Tiger nut beverage (Blend)

MNP = Mtam Nguhemem Patience

Table 4. Effect of 20% Substitution of Roselle Beverage with Tiger nut Beverage on the Antioxidant Properties of Roselle-Tiger nut Beverage (mg/mL)

Parameter	Sample MNP834	Sample MNP835	Sample MNP836
DPPH Scavenging Activity	0.29±0.04 ^a	0.41±0.00 ^b	0.36±0.00 ^{ab}
Hydroxyl Radical Scavenging	1.63±0.11 ^c	0.78±0.01 ^a	1.28±0.00 ^b
Ferric Reducing Antioxidant Power	0.31±0.00 ^a	0.38±0.00 ^c	0.32±0.00 ^c
Chelation of Metal Ion	1.63±0.10 ^c	0.78±0.01 ^a	1.28±0.00 ^b

Values were expressed as mean ± S.M.E; n=3. Values with different superscript across the rows were considered statistically significant ($p \leq 0.05$) while values with the same superscript across the rows were considered not significant ($p \geq 0.05$)

KEY:

MNP834 = 100% Roselle beverage (Control)

MNP835 = 100% Tiger nut beverage (Control)

MNP836 = 80% Roselle beverage + 20% Tiger nut beverage (Blend)

MNP = Mtam Nguhemem Patience

Table 5. The effect of Substitution of Roselle Beverage with Tiger nut Beverage on the Mean Sensory Scores of Panelists

Sample/Attribute	Appearance	Taste	Flavour	Mouth Feel	Overall Acceptability
Sample MNP834	8.52 ^a ±0.60	7.90 ^{ab} ±0.77	7.71 ^a ±0.78	7.90 ^a ±0.70	8.43 ^a ±0.68
Sample MNP835	7.90 ^{ab} ±1.14	6.24 ^c ±1.58	6.38 ^b ±1.63	6.62 ^b ±1.91	7.76 ^b ±1.18
Sample MNP836	8.10 ^{ab} ±1.26	8.24 ^{ad} ±0.83	8.38 ^a ±0.67	8.38 ^a ±0.74	8.48 ^{ad} ±0.81
Sample MNP837	7.76 ^b ±1.41	7.71 ^{bde} ±1.49	7.62 ^{ac} ±1.28	8.00 ^a ±0.89	8.05 ^{ab} ±0.74
Sample MNP838	8.05 ^{ab} ±1.02	7.76 ^{bef} ±1.09	8.05 ^a ±1.12	7.57 ^{ac} ±1.16	8.05 ^{ab} ±1.07
Sample MNP839	7.67 ^b ±1.24	7.10 ^e ±1.48	7.43 ^{ad} ±1.33	7.48 ^{ad} ±1.86	7.81 ^{bc} ±1.25
LSD	0.287	0.056	0.124	0.244	0.050

Mean ± SD are values of twenty-one panelist ratings. Mean values in a column with same superscript are significantly ($p \leq 0.05$) not different from each other

KEY:

MNP834 = 100% Roselle beverage (control)

MNP835 = 100% Tiger nut beverage (control)

MNP836 = 80% Roselle Calyxes beverage + 20% Tiger nut beverage (v/v)

MNP837 = 60% Roselle Calyxes beverage + 40% Tiger nut beverage (v/v)

MNP838 = 40% Roselle Calyxes beverage + 60% Tiger nut beverage (v/v)

MNP839 = 20% Roselle Calyxes beverage + 80% Tiger nut beverage (v/v)

Table 5 showed that progressive 20% substitution of Roselle with Tiger nut beverage significantly ($p \leq 0.05$) lowered the appearances, taste, flavor, mouth feel and over acceptability of Roselle-Tiger nut beverage relative to Roselle beverage. Among the Roselle-Tiger nut beverage samples, MNP834 was the most preferred for appearance (8.10) while MNP836 was the most preferred by panelists for taste (8.24), flavor (8.38), mouth feel (8.38), mouth feel (8.38) and over all acceptability (8.48).

(8.24), flavor (8.38), mouth feel (8.38) and overall acceptability (8.48).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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4. CONCLUSION

In conclusion, it was observed that a twenty percent substitution of Roselle Calyxes beverage with Tiger nuts beverage significantly (0.05):

1. Elevated the protein, carbohydrates, crude fibre and ash contents but lowered its moisture and crude fats contents.
2. Increased its Sodium, Zinc and Copper contents but lowered its Potassium, Phosphorus, Manganese, Magnesium, Calcium and Iron levels.
3. Increased its flavonoids, saponins and alkaloids contents but decreased its tannins, oxalates and phytate contents.
4. Elevated its free radical scavenging activity and ferric reducing power but lowered its hydroxyl scavenging activity and metal chelating ability.
5. Sample MNP836 of Roselle-tiger nut beverage was the most preferred by panelists for appearance (8.10), taste

- (*Cyperus esculentus*) Milk flavoured with Moringa oleifera leaf extract. Current Research in Nutrition; 2019.
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