NUTRITIONAL QUALITY AND ANTIOXIDANT PROPERTIES OF ROSELLE-TIGER NUT BEVERAGE

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 antioxidants 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 elevation in protein (16.29 ± 2.39^{a}) carbohydrates (11.08 ± 9.59^{a}), crude protein (16.29 ± 2.39^{a}) and ash contents (3.09 ± 0.56^{a}) but a decrease in moisture and crude fats contents. Also, an increase in Sodium(0.09 ± 0.01^{b}), Zinc(0.21 ± 0.00^{b}) and Copper(0.09 ± 0.00^{b}) contents 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 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: 20% substitution of roselle beverage with tiger nut beverage improve the nutritional quality of the beverage.

Keywords: Antioxidants, Roselle, Tiger nut, beverage and Nutritional.

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 these is further classified into industrial and homemade beverages (Sayed, 2018). Global beverage consumption stands at about 145 billion liters in 2017 for alcoholic beverages and 812 billion liters for non-alcoholic beverages (Conway, 2020). 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 (Yue *et al.,* 2016). High levels of non-alcoholic beverage intake are associated with weight gain, diabetes mellitus and obesity.

Habiscus sabdariffa Linn is a tropical plant that belongs to the super order Malvaceae. The plants are cultivated and consumed as vegetable and beverages, whereas other Habiscus varieties are cultivated because of their rich fiber content. *Habiscus sabdariffa* is commonly known as Roselle or Sorrel, Zobo (Hausa), Ashwe (Tiv) in Nigeria (Nwosu *et al.,* 2015). Various types of highly valued food and medicinal products have been produced from *Habiscus sabdariffa*. Roselle beverage made from the calyxes of *Habiscus 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. (Muhammad *et. al.,* 2022). 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 (WHO, 2013). 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 *Habiscus 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 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 (El-Adawy and Khalil, 1994).

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 (Oladele and Aina, 2007). It has been reported to contain appreciable quantities of myristic, oleic and linoleic fatty acids (Ezeh *et al.,* 2017). 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 nuts are rich in energy content (starch, fat, sugars and protein), mineral (phosphorus, potassium) and vitamins E and C (Manek *et al*., 2012).

METHODOLOGY
Roselle (*Habiscus 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.

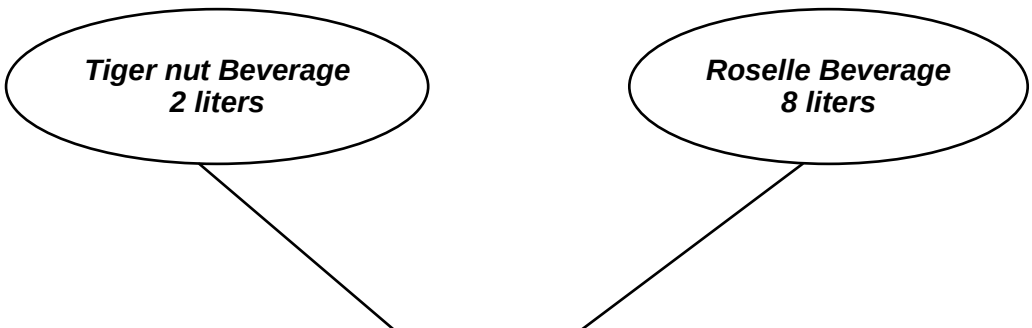

Figure 1: Flowchart of Roselle Tiger nut beverage production
Source: Abiodun, *et. al*., 2019 (Modified)

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 (Kibui *et al*., 2018).

Moisture content determination
The oven drying method was used as described by AOAC (2012). The samples (2g) were accurately weighed into moisture dishes. The moisture dishes were washed, dried in the oven at 85^{o}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^{o}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:

$$\%Moisture\ Content = \frac{W2-W3}{W2-W1}\ X\ 100 \quad = \frac{Weight\ loss\ after\ drying}{Weight\ of\ sample\ \ before\ \ drying}\ X\ 100 \qquad (1)$$

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

Nitrogen and crude protein determination

Protein Digestion

The method of Onyeike and Osuji (2003) was used. Exactly 1.5g of the defatted sample in an ashless filter paper was dropped into 300mL Kjeldahl flask. 25mL of H_2SO_4 and 3g of digested mixed catalyst (weighed separately into an ashless filter paper) was dropped 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 100mL with distilled water.

Distillation of the Digest

20mL of the diluted digest was measured into a 500mL Kjeldahl flask containing antibumping chips and 40mL of 40% NaOH was slowly added by the side of the flask. A 250mL conical flask containing a mixture of 50mL 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 Kjedahl distillation apparatus, with the tubes inserted into the conical flask and the Kjedahl flask. The flask was heated to distill out NH_3 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:

$$\%Nitrogen\ (N) = \frac{14\ x\ M\ x\ Vt\ x\ Tv\ x\ 100}{Weight\ of\ Sample\ (mg)\ x\ Va} \quad (2)$$

Therefore;

%Crude Protein = % Nitrogen (N) x 6.25 (conversion factor) (3)

Where;

M = Actual molarity of acid
Tv = Titre volume of HCl used
Vt = Total volume of diluted digest
Va = Aliquot volume distilled

Crude fat (lipid) determination

The Soxhlet fat extraction methods as described by AOAC (2012) was used. 250mL boiling flask was cleaned dried in an oven at 105^{o}C for 30 minutes. The flask will then be transferred into a desiccator and allowed to cool. The flask will then be labelled (W_1), weighed and then filled with 30mL petroleum ether. Then 20g 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^{o}C for 20 minutes, then transferred from the oven into a desiccator and allowed to cool and then weighed and labelled (W_2). The percentage fat was calculated as follows.

$$\%Crude\ Fat\ (Lipid) = \frac{Weight\ of\ Fat\ (W2-W1)}{Weight\ of\ Sample}\ X\ 100 \quad (4)$$

Ash determination

The ash content was determined using the official method of the AOAC (2012). The porcelain crucibles were washed, dried in hot air oven at 105^{o}C for 10 minutes and cooled in desiccators and then weighed (W_1). Then 2g of each sample was transferred into the crumble (W_2) on a heater inside a fume cupboard. The sample was transferred into a preheated muffle furnace at 550^{o}C and left at this temperature for 8 hours to ensure proper ashing. The residue will then be cooled in a desiccator and weighed (W_3). The percentage of ash was calculated as follows:

$$\%\text{Ash} = \frac{W3-W1}{W2-W1} X\ 100 \quad (5)$$

Where;

W_1 = Weight of the empty crucible
W_2 = Weight of crucible +Sample before ashing

W_3 = Weight of crucible + Sample after ashing

Crude fiber determination

The method described by AOAC (2012) was used. Two grams of sample was weighed out into a round bottom flask. 100mL of 0.25M Sulphuric acid Solution was added and the mixture boiled under reflux for 30mins. The hot solution was quickly filtered under sunction. 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 0.31M Sodium hydroxide solution was added and the mixture boiled again under reflux for 30 minutes and quickly filtered under sunction. The insoluble residue was washed with boiling water until it was based free. It was dried to constant weight in the oven at 100^0C, cooled in a desiccator and weighed (C_1) was then incinerated in a muffle furnace at 550^0C for 2 hours, cooled in the desiccator and reweighed (C_2). It can be calculated using the following expression:

$$\%Crude\ Fibre = \frac{Loss\ in\ weig\,ht\ on\ iincriation\ \ (C1-C2)}{Weig\,ht\ of\ \ Original\ \ Sample}\ X\ 100 \quad (6)$$

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 (Muller and Tobin, 1980). The value obtained was the percentage carbohydrate constituent of the sample. Thus,

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

Minerals Determination

2mL of each sample was weighed using weighing balance and then transferred to a beaker. 15mL of Hydrochloric acid (HCl) and 5mL 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 100^0C 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 60mLof samples which were used for mineral analysis.

0.5mL was aspirated to the nebulizer via nebulizer rubber to burner, 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). The detector then captured the concentration in the vapour in ppm (parts per minute) as well as the beam of cathode lamp. The analysis was done in triplicates and data was printed for use. Model AA 6800 Shimadzu Japan AAS was used for mineral analysis in the AAS/Ultra Violet Visible Spectrophotometer Laboratory.

Phytochemical Determination

Phytochemical composition refers to the chemical compounds found in plants that have potential therapeutic benefits and can be obtained through extraction processes (Abdullah *et al*., 2022).

Alkaloid determination

Harborne (1973) method was used for the determination alkaloid. 5g of the sample was weighed into a 250mL beaker and 80mL 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.

Flavonoid determination

10g of the plant sample was extracted repeated with 100mL of 80% aqueous methanol at room temperature. The whole solution was filtered through whatman

filter paper No 42 (125mm). The filtrate was later transferred into a crucible and evaporated into dryness over a water bath ad weighed to a constant weight. Bohm and Kocipai-Abyazan (1994) method was used.

Phytate determination
The level of phytate was determined by spectrophotometric method (Kirk and Sawyer 1991). The sample was extracted with 100mL of 0,02N HCl with vigorous shaking for 30 minutes. Exactly 1mL of the supernatant was treated with 1.5mL bipyridine solution. The absorbance was measured in a spectrophotometer at 519nm. The amount of phytate was extrapolated from the standard curve prepared from phytic acid.

Tannin determination
Van-Burden and Robinson (1981) method was used in this determination. 500mg of the sample was weighed into a 50mL plastic bottle. 50mL of distilled water was added and shaken for 1 hour in a mechanical shaker. This was filtered into a 50mL volumetric flask and made up to the mark. Then 5mL of the filtrate was pipetted out into a test tube and mixed with 2mL of 0.1M $FeCl_3$ in 0.1N HCl and 0.008 M Potassium Ferro cyanide. The absorbance was measured at 620nm within 10 minutes.

Oxolate determination
The titration method described by Day and Underwood (1986) was used to determine the oxalate content. One gram of the sample was weighed into 100mL conical flask where 75mL of 3N H_2SO_4 was added and stirred for 1 hour. It was then filtered using Whatman No I filter paper. From the filtrate, 25mL was taken and titrated while hot (80-90^{0}C) against 0.1N $KMnO_4$ solution, until a faint pink colour persisted for at least 30 seconds. The overall redox reaction was $MnO_4^- + C_2O_4^{2} + 8H^+ \rightarrow Mn^{2+} + 4H_2O + 2CO_2$
Oxalate can be determined by using the expression below;

$$Oxolate\ (mg/100mg) = \frac{T\ x\ Vme\ x\ Df}{ME\ x\ MS\ (g)}\ X\ 100 \quad (8)$$

Where;
T = Titre value of KMnO4 (mL)
V_{me} = Volume mass equivalent (1 cm^3 of 0.05M $KMnO_4$ solution is equivalent to 0.00225 anhydrous Oxalic acid.
Df = Dilution factor (Vt/A = 75/25 = 3) where Vt is the total volume of titrate (filtrate, 75mL) and A is the aliquot used (25mL).
M_E = Molar equivalent of KMnO4 redox reaction and
M_S = Mass of sample used.

Saponin determination
The method of Obadoni and Ochuko (2001) was used. The samples were ground and 20g of each were put into a conical flask and 100 cm^3 of 20% aqueous ethanol were added. The samples were heated over a hot water bath for 4 hours with continuous stirring at 55^{0}C. The mixture was filtered and the residue re-extracted with another 200 mL 20% ethanol. The combined extracts were reduced into 40mL over water bath at about 90^{0}C. The concentrate was transferred into a 250mL separator funnel 20mL 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.

Total phenol determination

The fat free sample was boiled with 50mL of ether for the extraction of the phenolic component for 15minutes. 5mL of the extract was pipetted into a 50mL flask, then 10mL 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.

Antioxidant Properties Determination
An aqueous extract of sample was obtained by weighing 10g 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.

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 (2011).The scavenging activity was estimated based on the percentage of DPPH radical scavenged in the following equation:

$$DDPH\ Radical\ Scavenging\ Activity(\%) = \frac{[(Acontrol - Atest)]}{Acontrol}\ X\ 100 \quad (9)$$

Where;
$A_{control}$ = Absorbance of the control reaction
A_{test} = Absorbance in the presence of the sample of the extracts.

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.,* (2011). The reaction mixture contained deoxyribose 1 mL (2.8 mM), KH_2PO4-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:

$$Hydroxyl\ Radical\ Scavenging\ Activity(\%) = \frac{1 - Atest}{Acontrol}\ X\ 100 \quad (10)$$

Where;
$A_{control}$ = Absorbance of the control reaction
A_{test} = Absorbance in the presence of the sample of the extracts.

Ferric reducing antioxidant power (FRAP)
The reducing power of the extracts of Roselle-Tiger nut beverage was determined by the method described by Oyaizu (1986). Aliquots of 1 mL of methanol extract of the sample at 4 different concentrations 0.1, 0.5, 1and 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 a 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% $FeCl_3$.

Chelation of metal ions
The metal chelating activity was measured by adding 0.1mM FeSO4 (0.2mL) and 0.25mM ferrozine (0.4mL) subsequently into 0.2mL of plant extract (Chew *et. al,* 2009). After incubating at room temperature for 10 minutes, the absorbance of the mixture was recorded at 562nm. The chelating activity was calculated using the following formula:

$$Metal\ Chelating\ Effect(\%) = \frac{[(Acontrol - Atest)]}{Acontrol}\ X\ 100 \quad (11)$$

Where;
Ac = Absorbance Values of the control
As = Absorbance Values of the samples

Sensory Analysis
Sensory evaluation of the beverage was carried out using 21 panelists. Testing was conducted in the Sensory Laboratory of the department of Home Science and Management, Joseph Sarwuan Tarka University, Makurdi. Panelists evaluated the taste, flavor, mouth feel, appearance and overall acceptability of the beverage using a 9-point Hedonic Scale with 1=Dislike Extremely, 2=Dislike Very Much, 3=Dislike Moderately, 4=Dislike Slightly, 5=Neither Like nor Dislike, 6=Like Slightly, 7=Like Moderately, 8=Like Very Much, and 9=Like Extremely. (Eke and Beleya, 2018). Six samples of beverages namely MNP834, MNP835, MNP836, MNP837, MNP838 and MNP839 were subjected to sensory evaluations. The most acceptable blend after sensory evaluation was sample MNP836 which was composed of 80% Roselle beverage and 20% Tiger nut beverage.

Data Analysis Techniques
Analytical determination was conducted in triplicates. Data was subjected to Analysis of Variance (ANOVA) and Sampled Paired T-Test for comparison. Difference was considered significant at 95% ($p \leq 0.05$) using the Statistical Package for Social Sciences (SPSS V23 software) (Coenen, *et al.,* 2021).

RESULTS AND DISCUSSION

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 are considered statistically significant ($p \leq 0.05$).While values with the same small letters 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 Nguhemen Patience

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 ($p \geq 0.05$).
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 (Fasoyiro *et al.,* 2005). 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 (2005). 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 (Fasoyiro *et al.,* 2005).

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 are considered statistically significant (p≤0.05).While values with the same small letters across the rows were considered not significant (p≥0.05).
RDA = Recommended Dietary Allowances

Table 2, revealed that the Sodium (0.09mg/L), Zinc (0.21mg/L) and Copper (0.09mg/L) significantly (p≤0.05) increased in Roselle-Tiger nut beverage relative to Roselle beverage control. The Potassium (0.53mg/L), Phosphorus (0.05mg/L), Manganese (0.53mg/L), Magnesium (3.56mg/L), Calcium (4.33mg/L) and Iron (2.67mg/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 (Celso *et al*., 2021).Minerals are essential nutrients as they cannot be synthesized by the human body and must be obtained from the diet (Abou-Arab *et al*., 2001). 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 (Adeniji, 2017). Roselle extract has been reported to be a good source of calcium, magnesium, iron and phosphorus (Abou-Arab *et al.,* 2001; Babalola *et al.,* 2001). 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*. (2005) 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 (Turra *et al*., 2011). Iron and zinc perform important physiological roles in the human body, with prevalent nutritional deficiency problems globally (Platel and Srinivasan 2016). Iron participates in a wide variety of metabolic processes, including respiration, energy production, DNA synthesis, and cell

proliferation (Tapiero and Tew, 2003; Belitz, Grosch and Schieberle 2009; Hentze *et al.,* 2010). 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 (National Academies Press, 2019). The daily recommended Fe requirements for humans are 10-15 mg for children, 18 mg for women and 12 mg for men (National Academies Press, 2019). The RDI is set assuming a 10% rate of intestinal absorption (Gaiina and Daina, 2013). Absorption of iron and zinc mainly takes place in the small intestine (Sitrin 2014). Deficiency of iron leads to aneamia. 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 (Platel and Srinivasan 2016). 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 (National Academies Press, 2019). 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 aneamia. 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 (Food and Nutrition Board, 2000). 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 (Underwood, 1971). 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 (Tapiero and Tew, 2003; Belitz, Grosch and Schieberle 2009; Hentze *et al.,* 2010). Therefore, sufficient intake of zinc is important for ensuring optimal health, growth, and development of humans (Untoro *et al.,* 2005). 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 (NAS, 1976). Magnesium is an activator of many enzyme systems and maintains the electrical potential in nerves (Shills and Young, 1992). The mineral content of plants can be significantly influenced by variety, location, and environmental conditions (Rao, 1996). 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 (Fleck, 1976).

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 are considered statistically significant (p≤0.05).While values with the same small letters across the rows were considered not significant (p≥0.05).

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 (Abdullah *et al*., 2022). Roselle *(Habiscus 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 (Tahmina *et al.*, 2018). Roselle petals also contain alkaloids, anthocyanins, flavonoids, saponins, steroids, sterols, and tannins, with high anthocyanin content and low phenol and flavonoid contents.

Table 4: Effect of 20% Substitution of Roselle Beverage withTiger 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}	$\mathbf{0.36\pm0.00^{ab}}$
Hydroxyl Radial Scavenging	1.63 ± 0.11^{c}	0.78 ± 0.01^{a}	$\mathbf{1.28\pm0.00^{b}}$
Ferric Reducing Antioxidant Power	0.31 ± 0.00^{a}	0.38 ± 0.00^{c}	$\mathbf{0.32\pm0.00^{c}}$
Chelation of Metal Ion	1.63 ± 0.10^{c}	0.78 ± 0.01^{a}	$\mathbf{1.28\pm0.00^{b}}$

Values were expressed as mean ± S.M.E; n=3.Values with different superscript across the rows are considered statistically significant (p≤0.05).While values with the same small letters across the rows were considered not significant (p≥0.05).

20% substitution of Roselle beverage with tiger nut bevrage significantly (p≤0.05) elevated the DPPH (0.36mg/mL) and Ferric reducing power (0.32mg/mL) of Roselle-Tiger nut beverage and lowered its hydroxyl (1.28mg/mL) and metal ions chelating (1.28mg/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 cholesterols. Besides, phytate, tannins, saponins, protease inhibitor and oxalate have been associated with reduced cancer risks (Habtamu and Negussie, 2014). 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 5: The effect of Substitution of Roselle Beverage with Tiger nu Beverage on the Mean Sensory Scores of Panelists

Sample/Attribute	Appearance	Taste	Flavour	Mouth Feel	Overall Acceptability
Sample MNP834	$8.52^{a}\pm0.60$	$7.90^{ab}\pm0.77$	$7.71^{a}\pm0.78$	$7.90^{a}\pm0.70$	$8.43^{a}\pm0.68$
Sample MNP835	$7.90^{ab}\pm1.14$	$6.24^{c}\pm1.58$	$6.38^{b}\pm1.63$	$6.62^{b}\pm1.91$	$7.76^{b}\pm1.18$
Sample MNP836	$8.10^{ab}\pm1.26$	$8.24^{adf}\pm0.83$	$8.38^{a}\pm0.67$	$8.38^{a}\pm0.74$	$8.48^{ad}\pm0.81$
Sample MNP837	$7.76^{b}\pm1.41$	$7.71^{bde}\pm1.49$	$7.62^{ac}\pm1.28$	$8.00^{a}\pm0.89$	$8.05^{ab}\pm0.74$
Sample MNP838	$8.05^{ab}\pm1.02$	$7.76^{bef}\pm1.09$	$8.05^{a}\pm1.12$	$7.57^{ac}\pm1.16$	$8.05^{ab}\pm1.07$
Sample MNP839	$7.67^{b}\pm1.24$	$7.10^{e}\pm1.48$	$7.43^{ad}\pm1.33$	$7.48^{ad}\pm1.86$	$7.81^{bc}\pm1.25$
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).

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

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