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PHYSIO-CHEMICAL, MINERAL COMPOSITION AND ANTIOXIDANT PROPERTIES OF ROSELLE (*HIBISCUS SABDARIFFA* L.) EXTRACT BLENDED WITH TROPICAL FRUIT JUICES

Mgaya Kilima B[1&3*], Remberg SF[2], Chove BE[3] and T Wicklund[1]

Beatrice Kilima Mgaya

*Corresponding author's email: dukile@yahoo.com

[1]Norwegian University of Life Sciences, Department of Chemistry, Biotechnology and Food Science, P.O. Box 5003, 1432 Ås, Norway

[2]Norwegian University of Life Sciences, Department of Plant and Environmental Sciences, P.O. Box 5003, 1432 Ås, Norway

[3]Sokoine University of Agriculture, Department of Food Science and Technology, Faculty of Agriculture P. O Box 3006, Morogoro, Tanzania.

ABSTRACT

Different varieties of fruit juices and beverages are available globally and there has been an increased consumption of fruit juices and beverages due to consumer awareness of nutritional and health benefits. Juice extracts are produced from various parts of plants including leaves, fruits and flowers. *Hibiscus sabdiriffa* (Roselle) is one such plant whose flowers are used to prepare juices. The roselle extract has a unique red colour, good flavour, low sugar and high acidic content. The acidity makes the juice sour hence the need for addition of sweetening products. A study was conducted on the formulation of roselle extract-tropical fruit blends aimed at establishing its physiochemical, mineral and antioxidant composition. Dried roselle calyces at a ratio of 1:10 (dried roselle calyces: water) were extracted at 50^{o}C for 30 minutes. The roselle extracts were blended at various proportions of fruit (mango, papaya and guava) juices. Physiochemical, mineral composition and antioxidant properties were evaluated in all the roselle fruit juice blends The results for all roselle-fruit blends (80% roselle to 20% roselle) showed that pH ranged between (2.35-3.32), total soluble solids (5.6-10.6^o Brix), titratable acidity (1.28-1.92 %), reducing sugars (2.95-5.55) mg/100g, Calcium (555.3-23.4 mg/100g DM), Magnesium (213.8-11.5 mg/100g DM), Phosphorus (39.8-9.0 mg/100g DM), Sodium (2.3-5.47 mg/100g DM), Zinc (5.85-0.69 mg/100g DM), Iron (29.5-1.36 mg/100g DM), monomeric anthocyanin (493.5-118.2 mg cyanidin-3-glucoside/100g), vitamin C (40.0-86.5 mg/100g), total phenol (54.6-10.8 mg gallic acid/ 100 g) and antioxidant activity (1.80-1.37 mmol/L). Blending of tropical fruit juices with roselle extract have improved mineral composition and antioxidant properties of fruit juices as roselle is a good source of calcium, magnesium and iron. Antioxidants acts as free radical scavengers inhibit lipid peroxidation and other free radical mediated process, therefore, consumption of roselle-fruit juices with high anthocyanin will protect human body from several diseases attributed to the reactions of free radicals.

Key words: Roselle, Fruit juice, Antioxidants, Minerals

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INTRODUCTION

There has been a global increase in consumption of fruit juice as consumers became aware of nutritional and health benefits of fruit juices [1]. The increased consumption of fruit juices goes together with increased variety of fruit juices and beverages offered for sale. Juicy extracts are produced from various types of plants especially their leaves, flowers of plants and fruits. *Hibiscus sabdiriffa*, commonly known as Roselle is one such plant whose flowers are used to prepare juices [2]. Roselle juice is also known as hibiscus tea, bissap, agua de Jamaica, Lo-Shen, red sorrel, sudan tea, sour tea or karkadè, is widely grown in Africa, South East Asia, and some tropical countries of America [3, 4]. Roselle produces red edible calyces with unique brilliant red colour, when extracted [3, 5].

Anthocyanins present in roselle are dephinidin 3-sambubioside, cyanidin 3-sambubioside, delphinidin 3-glucoside and cyanidin 3-glucoside [1,6]. They contribute benefit for health as a good source of antioxidants as well as a natural food colorant [7]. Due to high acidity, bitterness and astringency nature, the beverage made from Roselle extract is not well accepted by a large proportion of consumers in Tanzania. Therefore, blending of Roselle extract with other tropical fruit juices such as mango, guava and papaya can improve aroma, taste and nutrients of the beverages.
Guava (*Psidium guajava* L.) belongs to the family Myrtaceae, commonly known as apple of the tropics. It grows well in tropical and subtropical regions. It is rich in ascorbic acid, contains almost five times as much vitamin C as oranges [8, 9, 10]. Most of the guava produced around the world is consumed fresh.

Papaya (*Carica papaya* L.) is grown in every tropical and subtropical country. It has a pulpy flesh yellow or orange coloured with shades of yellow and red, depending on the fruit variety. It has the flavour of a cantaloupe; sweet and juicy with some muskiness [11]. The fruits have high contents of vitamin A, C and iron [12].

Mango is the most important and widely cultivated fruit in tropical and sub-tropical country [13] and is the king of the tropical fruit [14]. The mango fruit is an excellent source of fibre, vitamins A, C and the B complex, iron and phosphorus.

The blending of roselle juice with tropical fruit juices is anticipated to give products with high nutritional value and functional activity. The present study was aimed at assessing the possibility of blending roselle juice with three other fruit juices (guava, papaya and mango) to increase the utilization and establishing the nutritional composition of roselle-fruit juice.

MATERIALS AND METHODS

Raw materials

Dark red dried roselle calyces were purchased from the municipality market in Morogoro. Guava pink variety, papaya yellow variety and mango were purchased from horticulture garden at Sokoine University of Agriculture, Tanzania.

Preparation of roselle extract
Dried roselle calyxes (10% moisture content) were ground for 1 minute using a blender (Kenwood BL 440, France). Grounded roselle calyces at a ratio of 1:10 (dried roselle calyces: water) were extracted using water bath at 50°C for 30 minute [15]. Roselle extracts were filtered with cheesecloth.

Fruit juice preparation
Fully matured and high quality fruits of mango, papaya and guava were used. Fresh mango papaya and guava were thoroughly washed, peeled and cut into small pieces (guava were not peeled). Then the small pieces were transferred to the juice extractor (Kenwood JE 810 UK) to obtain juice.

Preparation of roselle-fruit juice blends
Roselle-mango, roselle-papaya and roselle-guava were formulated in the ratio of 100:0, 80:20, 60:40, 40:60, 20:80 and 0:100 roselle extract: fruit juice pulp respectively. Sodium benzoate (1 g/L) and citric acid (1 g/L) were added to all roselle-fruit blends as preservatives. The juices were filled in 100 mL sterilized plastic bottles, loosely capped and pasteurized in a water bath at a temperature of 82.5°C for 20 min and cooled rapidly to room temperature by immersing the bottles in water bath (28°C). Samples were drawn for chemical analyses.

The pH, titratable acidity and total soluble solids
The pH, titratable acidity (TA) and total soluble solids (TSS) of roselle-fruit blends were determined according to AOAC [16]. The pH was measured using Hanna portable pH meter (HI9125, Romania). TA was determined using 0.1N Sodium Hydroxide and phenolphthalein as an indicator and was expressed as % malic acid and TSS was measured with a hand refractometer (Mettler Toledo, Switzerland) and expressed as Brix.

Colour measurements
The colour for Roselle fruit blends were measured using colour chart (Natural Colour system [NCS],Stockholm Sweden) followed by measuring the standard colour with a Chroma Meter Minolta CR- 400/410 (Minolta Co., Osaka, Japan) with the reflectance mode with D_{65} illuminant and 2° observer angle. Samples were measured against a white ceramic reference plate

C (L* = 94.0, a* = 0.3138, b* = 0.3199) D65 (L* = 94.0, a* = 0.3163, b* = 0.3327).
Colour values were expressed as L* (whiteness or brightness/darkness), a* (redness/greenness) and b*(yellowness/blueness)

Reducing sugars
Reducing sugars were determined by Luff-Schoorl method as described by Egan *et al*. [17]. Sugar content was then determined by interpolation in a table (Egan *et al*.,) after subtracting the blank assay of the volume of sodium thiosulphate of the titration. The results where expressed in mg/100g.

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Minerals analysis

Five gram of roselle-fruit blends were separately weighed into crucibles and dry ashed in muffle furnace maintained at 550°C for 2 hr. The ash was cooled in desiccators and then weighed. After weighing, the ash was dissolved in a solution of 1:1 ratio of H2O: HCl, in which the concentration of the final mixture was 6NHCl. Determination of calcium, magnesium, iron, zinc, phosphorus and copper content of the samples was carried out by AOAC method No 968.08 using atomic absorption spectrophotometer (Shimadzu UNICAM 919, Cambridge, UK) [16]. Two replicates were analyzed for each sample.

Vitamin C assay

Vitamin C content for the roselle fruit juices was determined according to the Folin-Ciocalteu reagent (FCR) method [18]. A 20mL of sample was pipetted into 100 mL volumetric flask followed by 2 mL of 10% TCA solution and diluted to 100 mL with distilled water. The sample was poured into a conical flask, swirled gently for 1 minute and left to stand for 1 minute and filtered with Whatman filter (no 542). One mL of the sample and 1 mL of standard solution (3 mg ascorbic acid in 1 mL distilled water) was pipetted into a test tube followed by 3 mL distilled water and 0.4 mL of Folin reagent. Mixing followed and thereafter the mixture was incubated at room temperature for 10 min. The absorbance was read at 760 nm using Jenway 6405 UV/VIS Spectrophotometer, UK. The results were expressed in mg per 100g fresh weight.

Determination of antioxidant activity

The antioxidant activity for the Roselle fruit blends was determined by the ferric reducing ability of plasma (FRAP) assay with some modifications [19]. Three mL of freshly prepared FRAP solution (0.3 M acetate buffer (pH 3.6) containing 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) in 40 mMol HCl and 20 mM $FeCl_3 .6H_2O$) and 100 µL of sample (standard) was incubated at 37^0C for 4 min and the absorbance was measured at 593 nm using spectrophotometer. An intense blue colour was formed when the ferric-tripyridyltriazine (Fe^{3+}-TPTZ) complex is reduced to the ferrous (Fe^{2+}) form at 593 nm. A range of iron sulphate concentrations from 0.25 to 2.0-mMol/L was used to prepare the calibration curve. The results were expressed as millimoles of (Fe^{2+}) per liter of fresh weight (mg (Fe^{2+})/L FW).

Total phenolics assay

Total polyphenols content (TPC) for the Roselle fruit blends was determined according to the Folin-Ciocalteu method [20] with modifications. An aliquot of 300 µL sample solution was mixed with 1.5 mL of Folin-Ciocalteu's reagent (diluted 10 times), and 1.2 mL of sodium carbonate (7.5% w/v). After incubation at room temperature for 30 min in the dark, the absorbance was measured at 765 nm using spectrophotometer. Gallic acid (0–500 mg/100g) was used for calibration of a standard curve. The results were expressed as milligrams of gallic acid equivalents per 100 g of fresh weight (mg GAE/100 g FW).

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The total monomeric anthocyanin content (TMA)
The total monomeric anthocyanin content for roselle-fruit blends was carried out using the pH differential method [21]. Absorbance was measured at 520 and 700 nm using spectrophotometer.

The absorbance (*A*) of the sample was then calculated according the following formula:

$$\mathbf{A = (A_{520} - A_{700})_{PH\ 1.0} - (A_{520} - A_{700})_{PH4.5}}$$

The monomeric anthocyanin pigment content in the original sample was calculated according the following formula:

$$AC = \frac{A \times MW \times DF \times 1000}{\varepsilon L}$$

Where, A- difference of sample absorbance between pH 1.0 and 4.5, ε- molar extinction coefficient for cyanidin-3-glucoside (26,900); L- path length of the spectrophotometer cell (1.0 cm), DL- dilution factor and molecular weight (MW) of cyanidin-3- glucoside (449.2 g/mol), 1000- factor for conversion from g to mg. The result was expressed as mg cyanidin-3-glucoside equivalent/100 g extract.

Data Analyses
All the results were expressed as mean values ± standard deviation. All statistical analyses were performed using Minitab Statistical Software version 16.0 (Minitab Inc., State College, PA, USA). The results were analyzed by one-way analysis of variance (ANOVA) and the significant means separated by Tukey method ($P<0.05$).

RESULTS

Physiochemical properties of roselle-fruit blends
Table 1 shows the changes in total soluble solids (TSS), pH, titratable acidity (TA) and reducing sugar (RS) in roselle-fruit blends. pH for all roselle-fruit juice blends and TSS for roselle-mango blends decreased significantly ($P<0.05$) with decreasing concentration of roselle extract in all the blends while RS for all roselle-fruit juice blends increased significantly ($P<0.05$) with increased concentration of roselle extract in all the roselle-fruit blends.

Total soluble solids (TSS) for roselle extract, mango, papaya and guava were 5.70, 14.03, 7.88 and 5.88 °Brix. Total soluble solids TSS for roselle-mango, roselle-papaya and roselle-guava blends ranged from 10.62-5.6° Brix (Table 1). The reducing sugars (RS) value for roselle extract, mango, papaya and guava were 2.42, 5.87, 5.73 and 5.55 mg/100g, for roselle-mango roselle-papaya and roselle-guava blends ranged from 5.55-2.95 mg/100g (Table 1).

The pH values for roselle extract, mango, papaya and guava was 2.26, 3.37, 4.48 and 3.72. pH for roselle-mango, roselle-papaya and roselle-guava blends were 3.32-2.35 (Table 1). The titratable acidity (TA) for roselle extract, mango, papaya and guava were 1.92, 0.32, 0.20 and 0.57. TA for roselle-mango, roselle-papaya and roselle-guava blends ranged from 1.92-0.96% (Table 1).

Mineral composition of roselle-fruit blends

The composition of minerals in roselle-fruit blends is showed in Table 2. From the results as amount of roselle extract decrease in all the roselle fruit blends, the quantity of minerals (except sodium) also decreased significantly (P<0.05). Calcium content (Ca) for roselle extract, mango, papaya and guava was 880.8, 4.1, 16.9 and 18.5. Calcium for roselle-fruit juice blends ranged from 555.3-23.4 mg/100g while magnesium (Mg) content for roselle extract, mango, papaya and guava was 316.6, 4.0, 6.6 and 22.4. Magnesium for roselle-fruit juices blends ranged from 213.8-11.5 mg/100g (Table 2).

Phosphorus (P) for roselle extract, mango, papaya and guava was 40.2, 5.0, 36.5 and 36.5. Phosphorus for roselle-fruit juices blends ranged from 39.81-37.8 mg/100g DM and iron (Fe) content for roselle extract, mango, papaya and guava was 37.8, 0.1, 3.2 and 0.4. Iron content for roselle-fruit juices blends ranged from 29.5-1.4 mg/100g (Table 2). Sodium (Na) content for roselle extract, mango, papaya and guava was 6.6, 1.0, 2.2 and 2.2. Sodium content for roselle-fruit juices blends ranged from 5.6-1.0 mg/100g while zinc content (Zn) for roselle extract, mango, papaya and guava was 6.4, 0.1, 0.2 and 0.2. Zinc content for roselle-fruit juices blends ranged from 5.7-0.6mg/100g (Table 2).

Antioxidant properties of roselle-fruit blends

Vitamin C content for roselle extract, mango, papaya and guava were 37.4, 62.2, 73.5 and 92.2 mg/100g. Roselle-fruit juices blends were between 40.0-61.2 mg/100g (Table 3). Total monomeric anthocyanins (TMA) values for roselle extract, mango, papaya and guava were 555.3, 48.0, 46.8 and 62.8 mg/100g. Total monomeric anthocyanins (TMA) for roselle- fruit juice blends were between 493.5-118.2 mg/100g (Table 3). Total phenol content (TPC) for roselle extract, mango, papaya and guava were 54.6, 10.9, 6.8 and 27.3 mg/100g. Roselle-fruit juices blends were between 53.7-10.8 GAE mg/100g (Table 3). Ferric reducing ability of plasma (FRAP) for roselle extract, mango, papaya and guava were 1.87, 1.28, 1.37 and 1.42 mMol/L. Roselle- fruit juice blends was 1.80-1.37 mMol/ L (Table 3). The results showed that as the concentration of roselle in all the blends increased, the quantity of TMA and TPC in the blends also increased significantly (P<0.05) while quantity of vitamin C decreased.

Colour

The lightness (L***)** and the yellowness (b*****) values for all blends increased while the redness (a*****) values decreased significantly (P<0.05) with decreased concentration of roselle extract in the blends (Table 4). The lightness (L***)** values for roselle extract, mango, papaya and guava was 14.3, 42.4, 41.4 and 21.3. Lightness for roselle-fruit juices blends ranged from 15.8 to 18.3 while the redness (a*****) value for roselle extract, mango, papaya and guava was 20.6, 6.6, 14.5 and 15.4. Redness value for roselle-fruit juices blends ranged from 20.0 to 15.4 (Table 4). The yellowness (b*****) value for roselle extract, mango, papaya and guava was 3.9, 43.9, 48.0 and 8.3. The b***** value for roselle-fruit juices blends ranged from 8.5 to 4.5 (Table 4).

DISCUSSION

Physiochemical properties of roselle-fruit blends

Roselle extract is known to be highly acidic with low sugar content [4, 5, 22]. The increase in TSS and RS is due to high sugar content in fruit juices and roselle-mango blends showed highest proportion of sugars among the three fruits used in the blending. The low pH of roselle extract was increased by addition of tropical fruit juices in roselle juice. The reduction of acidity for roselle-fruit blends can be good to people with stomach problems (ulcers) and also increase the shelf life of blends (3).

Mineral composition of roselle-fruit blends

Macro-minerals are needed in large amounts and play major structural roles (such as calcium and phosphorus) and function as electrolytes (such as sodium and potassium). Micro-minerals (trace minerals), often serve as catalysts in enzyme reactions and are only needed in small amounts [3]. Roselle extract is known to be good source of calcium, magnesium, iron and phosphorus [3, 23]. The decrease in mineral composition with decreased concentration on roselle extract in roselle-apple blends was also reported by Fasoyiro *et al*. [22]. The daily recommended Fe requirements for humans are 10-15 mg for children, 18 mg for women and 12 mg for men [24]. The concentrations of Fe in 100%R to 60%R for all the roselle fruit blends provide more than 100% DRI. The roselle-fruit blends can be good source of Fe and can therefore alleviate of iron deficiency.

The vitamin C content of mango, papaya and guava juices were higher than the value of recorded for roselle extract alone. Addition of fruit juice has improved the vitamin C content of the blends. However, all the roselle blends were good source of Vitamin C. The increased vitamin C content with decreased content of sobo (roselle) was also observed in sobo-orange and sobo-pineapple mixture [25].

Anthocyanins are plant pigments responsible for the red, blue, and purple colours of various flowers and plants [21]. The determination of anthocyanins composition in food as well as processed food has been of considerable interest to establish their role as antioxidants in determining their potential health benefits. Roselle extract is a very good source of anthocyanins [5, 6, 21]. Daily intake of anthocyanins is estimated to be 82 mg and 12.5 mg per day per person in Finland and United States [26]. The amount of anthocyanin in the roselle-fruit blends (80%R to 20%R) is equivalent to 6 - 1.4 times, 39 - 9 times) the recommended daily intake for Finland and USA, respectively.

Phenolic compounds including anthocyanins, flavonoids, and phenolic acids are known to be responsible for antioxidant activities in fruits and fruits with higher phenolic contents generally show stronger antioxidant activities [28]. The concept of antioxidant activities which describes the ability of different food antioxidants in scavenging preformed free radicals is a tool for investigating the health effects of antioxidant-rich foods. The reduction in FRAP was due to decreased amount of anthocyanins and total phenol in the blend as concentration of roselle extract is reduced in the blends.

Colour

Colour is the most important quality attribute having influence on consumer acceptability of food as it gives the first impression of food quality [3]. The red colour is due to presence of anthocyanins [3, 6] in the roselle blends. From the results, as the concentration of roselle extract decreased the redness decrease. The yellow colour is due to the presence of carotenoids in (mango, guava and papaya) so as the concentration of fruit juices increased in the blends the yellowness also increased.

CONCLUSIONS

The combinations of roselle extract with fruit juices (mango, papaya and guava) are rich in essential minerals and vitamin C and these blends could replace the existing commercially available non-alcoholic beverages in stores and supermarkets.

Antioxidants act as free radical scavengers, inhibit lipid peroxidation and other free radical mediated process, therefore consumption of roselle-fruit juices with high anthocyanin (493.5-118.2) mg/L will protect human body from several diseases attributed to the reactions of free radicals.

The formulated roselle fruit (mango, papaya and guava) is an ideal low cost blended beverage as the addition of mango, papaya, guava in the roselle extract could bring down cost of production as these tropical fruit are sold at a throw-away price during their seasons and reduce seasonal losses of these fruits.

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Table 1: Physiochemical properties of roselle-fruit blends

Fruits	Blends	TSS °Brix	PH	TA % malic acid	RS mg/100g
Mango	0R	14.03 ^{a} ±0.50	3.37 ^{a} ±0.12	0.32 ^{c} ±0.43	5.87 ^{a} ±0.05
	20R	10.62 ^{a} ±0.49	2.76 ^{b} ±0.14	1.44 ^{b} ±0.00	5.55 ^{b} ±0.00
	40R	9.92 ^{a} ±0.19	2.65 ^{b} ±0.01	1.92 ^{b} ±0.43	5.06 ^{c} ±0.00
	60R	7.48 ^{b} ±0.63	2.40 ^{c} ±0.06	3.12 ^{a} ±0.40	4.51 ^{d} ±0.00
	80R	6.90 ^{b} ±0.20	2.35 ^{c} ±0.01	1.40 ^{b} ±0.00	3.48 ^{e} ±0.0 ^{0}
	100R	5.70 ^{c} ±0.10	2.26 ^{d} ±0.01	1.92 ^{b} ±0.00	2.42 ^{f} ±0.00
Papaya	0R	7.88 ^{a} ±0.45	4.48 ^{a} ±0.03	0.20 ^{d} ±0.00	5.73 ^{a} ±0.02
	20R	6.90 ^{b} ±0,35	3.32 ^{b} ±0.01	1.28 ^{c} ±0.25	5.18 ^{b} ±0.00
	40R	7.60 ^{a} ±0.15	2.94 ^{c} ±0.01	1.36 ^{c} ±0.2	4.87 ^{c} ±0.00
	60R	7.80 ^{a} ± 0.00	2.69 ^{d} ±0.01	1.60 ^{b} ±0.47	3.45 ^{d} ±0.02
	80R	6.75 ^{b} ±0.08	2.54 ^{e} ±0.00	2.00 ^{a} ±0.47	2.95 ^{e} ±0.01
	100R	5.70 ^{c} ±0.37	2.26 ^{f} ±0.02	1.92 ^{a} ±0.00	2.42 ^{f} ±0.00
Guava	0R	5.88 ^{c} ±0.10	3.72 ^{a} ±0.01	0.57 ^{d} ±0.66	5.55 ^{a} ±.01
	20R	5.60 ^{c} ±0.10	3.13 ^{b} ±0.01	1.92 ^{a} ±0.00	4.35 ^{b} ±.01
	40R	5.87 ^{c} ±0.00	2.53 ^{c} ±0.03	1.36 ^{c} ±0.20	4.10 ^{c} ±.02
	60R	6.30 ^{b} ±0.00	2.83 ^{d} ±0.01	0.9 ^{d} 6±0.0	3.88 ^{d} ±.00
	80R	6.70 ^{a} ±0.00	2.41 ^{e} ±0.01	1.68 ^{b} ±0.26	3.23 ^{e} ±.00
	100R	5.70 ^{c} ±0.00	2.26 ^{f} ±0.00	1.92 ^{a} ±0.42	2.42 ^{f} ±.01

Data in columns for each fruit with different superscript are significantly different using Tukey's pair-wise comparison test ($p<0.05$). 100R=100% Roselle; 80R=80% Roselle; 60R=60% Roselle; 40R=40% Roselle; 20R=20% Roselle; 0R=100% Mango or100%Papaya or 100%Guava.

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Table 2: Minerals composition of roselle-fruit blends

Fruit juice	Blend	Calcium	Magnesium	Phosphorus	Iron	Sodium	Zinc
		mg/100 g DW					
Mango	0R	4.1^{f}±0.00	4.0^{f} ±0.00	5.0^{f} ±0.00	0.1^{f} ±0.00	1.0^{f} ±.00	0.1^{f} ±.00
	20R	37.3^{e} ±0.20	11.5^{e} ±0.10	9.0^{e} ±0.00	1.4^{e} ±0.00	6.6^{e} ±.00	0.6^{e} ±.00
	40R	97.5^{d} ±0.00	24.9^{d} ±0.10	14.4^{d} ±0.00	6.4^{d} ±0.00	11.8^{d} ±.01	1.1^{d} ±.01
	60R	255.4^{c} ±3.70	100.4^{c} ±0.40	22.5^{c} ±0.00	15.7^{c} ±0.00	13.7^{c} ±.00	4.9^{c} ±.00
	80R	555.3^{b} ±2.00	213.8^{b} ±0.80	30.8^{b} ±0.00	28.2^{b} ±0.00	15.5^{b} ±.02	5.6^{b} ±.02
	100R	880.8^{a} ±0.01	316.6^{a} ±0.30	40.0^{a} ±0.04	37.8^{a} ±0.00	6.6^{a} ±0.00	6.4^{a} ±0.00
Papaya	0R	16.9^{f} ±0.02	6.6^{f} ±0.31	36.5^{f} ±0.02	3.21^{f} ±0.03	2.2^{a} ±0.01	2.22^{a} ±0.01
	20R	36.2^{e} ±0.02	33.9^{e} ±0.00	36.8^{e} ±0.31	5.3^{e} ±0.00	2.3^{e} ±0.12	2.31^{e} ±0.12
	40R	85.4^{d} ±0.18	88.8^{d} ±0.02	38.8^{d} ±0.05	8.6^{d} ±0.01	2.9^{d} ±0.00	2.94^{d} ±0.00
	60R	148.6^{c} ±0.23	124.4^{c} ±0.07	37.5^{c} ±0.00	19.2^{c} ±0.00	3. 7^{c} ±0.01	3.67^{c} ±0.01
	80R	459.5^{b} ±0.25	190.4^{b} ±0.04	39.3^{b} ±0.03	28.4^{b} ±0.00	5.6^{b} ±0.01	5.6^{b} ±0.01
	100R	880.8^{a} ±0.01	316.6^{a} ±0.30	40.0^{a} ±0.04	37.8^{a} ±0.00	6.6^{a} ±0.00	6.4^{a} ±0.00
Guava	0R	18.5^{f} ±0.16	22.4^{f} ±0.23	36.5^{f} ±0.01	0.4^{f} ±0.01	2.2^{f} ±0.01	0.2^{f} ±0.01
	20R	23.4^{e} ±0.16	54.6^{e} ±0.22	37.8^{e} ±0.01	2.7^{e} ±0.01	3.5^{e} ±0.12	0.7^{e} ±0.12
	40R	58.6^{d} ±0.07	64.5^{d} ±0.35	38.3^{d} ±0.00	6.0^{d} ±0.01	4.8^{d} ±0.00	1.4^{d} ±0.00
	60R	120.5^{c} ±0.43	78.7^{c} ±0.16	39.3^{c} ±0.00	14.1^{c} ±0.01	5.5^{c} ±0.01	2.6^{c} ±0.01
	80R	420.6^{b} ±0.28	116.6^{b} ±0.30	39.8^{b} ±0.00	29.5^{b} ±0.01	6.1^{b} ±0.01	5.6^{b} ±0.01
	100R	880.8^{a} ±0.01	316.6^{a} ±0.27	40.2^{a} ±0.04	37.8^{a} ±0.00	6.6^{a} ±0.00	6.4^{a} ±0.00
DRI(mg/day)		1000	320; 420	700	18;8	1500	8;11

Data in columns for each fruit with different superscript are significantly different using Tukey's pair-wise comparison test ($p<0.05$). Dietary reference intakes (DRI) are established by the US Food and Nutrition Board of the (IMO, 2002, 2004) National Academy of Sciences. Values given are for adult females and males, ages 19–50 years.

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Table 3: Antioxidant properties of roselle-fruit blends

Fruits	Blends	Vitamin C mg/100g	TMA mg/100g	TPC mg/100g	FRAP mMol/L
Mango	0R	62.2 ^{a} ±0.00	48.0 ^{f} ±0.75	10.9 ^{e} ±5.22	1.28 ^{f} ±0.00
	20R	58. 5 ^{b} ±0.00	134.7 ^{e} ±1.50	21.3 ^{d} ±0.01	1.45 ^{e} ±0.00
	40R	53.0 ^{c} ±0.00	282.6 ^{d} ±1.81	28.8 ^{c} ±0.03	1.58 ^{d} ±0.00
	60R	44.4 ^{d} ±0.00	335.2 ^{c} ±1.54	37.9 ^{c} ±0.03	1.66 ^{c} ±0.00
	80R	40.0 ^{e} ±0.00	493.5 ^{b} ±5.15	53.7 ^{b} ±0.02	1.80 ^{b} ±0.00
	100R	37.4 ^{f} ±0.00	555.3 ^{a} ±2.03	54.6 ^{a} ±0.80	1.87 ^{a} ±0.01
Papaya	0R	73.5 ^{a} ±0.00	46.8 ^{f} ±1.00	6.8 ^{f} ±0.06	1.28 ^{f} ±0.02
	20R	61.2 ^{b} ±0.00	146.0 ^{e} ±6.20	10.8 ^{e} ±0.00	1.37 ^{e} ±0.00
	40R	54.3 ^{c} ±0.00	290.2 ^{d} ±8.83	19.8 ^{d} ±0.01	1.59 ^{d} ±0.00
	60R	47.9 ^{d} ±0.00	339.2 ^{c} ±3.31	39.5 ^{c} ±0.01	1.63 ^{c} ±0.00
	80R	41.4 ^{e} ±0.00	454.6 ^{b} ±3.03	51.3 ^{b} ±0.00	1.76 ^{b} ±0.00
	100R	37.45 ^{f} ±0.00	555.3 ^{a} ±0.30	54.6 ^{a} ±0.80	1.87 ^{a} ±0.01
Guava	0R	92.2 ^{a} ±0.01	62.8 ^{f} ±1.38	27.3 ^{f} ±0.00	1.42 ^{f} ±0.03
	20R	44.3 ^{e} ±0.00	118.2 ^{e} ±0.95	47.3 ^{b} ±0.00	1.75 ^{b} ±0.00
	40R	57.3 ^{d} ±0.00	167.8 ^{d} ±2.51	39.9 ^{c} ±0.00	1.62 ^{c} ±0.00
	60R	74.7 ^{c} ±0.00	218.9 ^{c} ±2.15	32.0 ^{d} ±0.01	1.56 ^{d} ±0.00
	80R	86.5 ^{b} ±0.00	373.2 ^{b} ±1.38	29.8 ^{e} ±0.01	1.49 ^{e} ±0.04
	100R	37.4 ^{f} ±0.00	555.3 ^{a} ±0.5	54.6 ^{a} ±0.28	1.87 ^{a} ±0.01

Data in columns for each fruit with different superscript are significantly different using Tukey's pair-wise comparison test ($p<0.05$).

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Table 4: The colour measurements values (L* a* b*) for roselle-fruit blends

Blends	Mango			Papaya			Guava		
	L*	a*	b*	L*	a*	b*	L*	a*	b*
0R	42.4 a ±0.7	06.6e ±0.3	43.9a±0.6	41.4a±0.2	14.5f±0.1	48.0a ±0.3	21.3a±0.1	15.4e±0.4	8.3a ±0.1
20R	18.6b ±0.4	16.4 d ±0.2	8.5 b ±0.2	18.3b±0.1	15.4e±0.3	7.4b ±0.1	19.6b±0.4	16.5d ±0.2	7.3b ±0.4
40R	17.6c±0.3	18.1 c ±0.7	7.7c±0.5	17.8c±0.4	16.2d±0.4	6.4d ±0.1	16.3c±0.0	17.2c ±0.4	6.4c ±0.0
60R	16.1d ±0.2	19.2a ±0.4	5 6d±0.2	16.6d±0.0	18.8c±0.1	6.0c±0.0	15.8d±0.1	17.6c ±0.0	5.3d ±0.0
80R	14.7 e ±0.1	20.0a ±0.5	4.7e ±0.6	15.8e ±0.0	19.9b±0.1	5.7c ±1.8	15.1e±0.0	19.5b±0.1	4.5e ±0.2
100R	14.3 e ±0.0	20.6a ±0.0	3.9f ±0.0	14.3f±0.0	20.6a±0.0	3.9d±0.0	14.3f±0.0	20.6a±0.0	3.9f ±0.0

Data in columns for each fruit with different superscript are significantly different using Tukey's pair-wise comparison test ($p<0.05$).

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