Physiochemical and antioxidant properties of roselle-mango juice blends; effects of packaging material, storage temperature and time

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Abstract
A study was conducted to determine the effects of packaging materials, seasonality, storage temperature and time on physiochemical and antioxidant properties of roselle-mango juice blends. Roselle extract (20%, 40%, 60%, and 80%) was mixed with mango juice and stored in glass and plastic bottles at 4°C and 28°C. Total soluble solids, pH, titratable acidity, reducing sugar, color, vitamin C, total monomeric anthocyanins, total phenols, and antioxidant activity (FRAP) were evaluated in freshly prepared juice, and after, 2, 4, and 6 months of storage. The results showed that total soluble solids, reducing sugars, and pH increased with storage times under different storage time, irrespective of packaging materials. The acidity, color, total monomeric anthocyanin, vitamin C, total phenols, and antioxidant activity decreased during storage irrespective of storage temperature and packaging material. Loss of anthocyanins, total phenols, and vitamin C content were higher in blends stored at 28°C than 4°C.

Introduction
Hibiscus sabdariffa L. (family Malvaceae), commonly known as roselle, red sorrel, or Karkadé, is widely grown in Africa, South East Asia, and some tropical countries of America (Abou-Arab et al. 2011; Sagayo-Ayerdi et al. 2007; Amor and Allaf 2009; Cisse et al. 2011) Roselle produces red edible calyces with unique brilliant red color and flavor. The calyces are commonly used in the production of jelly, juice, jam, wine, syrup, gelatine, pudding, cake, ice cream, and flavoring (Tsai et al. 2002; Tsai and Huang 2004; Duangmal et al. 2008 and Hussein et al. 2010). The beverages produced by Hibiscus sabdariffa calyces are called hibiscus tea, bissap, roselle, red sorrel, agua de Jamaica, Lo-Shen, Sudan tea, or karkade (McKay et al. 2010).

Two anthocyanins are dominant in roselle calyces, delphinidin-3-sambubioside, also known as delphinidin-3-xylosylglucoside or hibiscin, and cyanidin-3-sambubioside, also known as cyanidin-3-xylosylglucoside or gossypicyanin. In addition, two minor anthocyanins, delphinidin-3-glucoside, and cyanidin-3-glucoside are present (Wong et al. 2002; Tsai and Huang 2004; Duangmal et al. 2008 and Hussein et al. 2010). The

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colorant (Tsai et al. 2002; Duangmal et al. 2008). Anthocyanins possess antioxidative, antitumor, and anticarcinogenic activity (Fasoyiro et al. 2005; González-Molina et al. 2009). They are derivatives of the basic flavlyium cation structure with electron-deficient nucleus which make them highly reactive and their reaction involves discoloration of the anthocyanin pigments. Factors like light, pH, temperature, oxygen, ascorbic acid, and sugar are contributing factors in degradation or stability of anthocyanins (Tsai and Huang 2004; Chumsri et al. 2008; Cisse et al. 2011).

Most people do not prefer beverages made from pure roselle as it has an acidic and bitter taste (Wong et al. 2002). Blending of the extract with juice from sweet tropical fruits such as mango can improve the aroma, taste and nutritional, and antioxidant properties of the juice blends. The choice of mango fruits in this study was due to an abundance seasonal availability, which normally led to high postharvest losses due to lack of sufficient market outlets, transport, storage facilities, and commercial fruit processing industries.

Packaging is an important aspect in the food processing industry as it serves the important functions of containing the food, protecting against chemical and physical damage whilst providing information on product features, nutritional status, and ingredient information (Anin et al. 2010). Various packaging materials such as high-density polyethylene (HDPE), polypropylene (PP), and glass are commonly used for packaging of fruit juice (Marsh and Bugusu 2007). Different packaging materials influence the quality of the stored products differently. Therefore, the study of the effect of packaging material on the quality parameters during storage is essential. In this study, roselle-mango juice blends were stored in plastic and glass 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 cold water bath (Ndabikunze et al. 2010). The bottles were tightly capped, labeled and stored at 4 °C and 28 °C for 6 months. Samples were drawn for chemical analyses at 0, 2, 4, and 6 months of storage.

**Determination of pH, titratable acidity, and total soluble solids**

The pH, titratable acidity (TA), and total soluble solids (TSS) of roselle-mango blends were determined according to AOAC (1995). The pH was measured using Hanna portable pH meter (Hanna, Cluj-Napoca, Romania). TA was determined titrimetrically using 0.1N sodium hydroxide and phenolphthalein as an indicator and was expressed as % malic acid, while TSS (°Brix) was measured with a hand refractometer (Mettler Toledo, Schwerzenbach, Switzerland) and expressed as %.

**Color measurements**

The color for roselle-mango blends were measured using color chart (Natural Color system [NCS], Stockholm Sweden) followed by measuring the standard color with a Chroma Meter Minolta CR-400/410 (Minolta Co., Osaka, Japan) with the reflectance mode with D65 illuminant and 2° observer angle. Samples were measured against a white ceramic reference plate.

\[
C(L^* = 94.0, a^* = 0.3138, b^* = 0.3199) \text{ D65} \\
(L^* = 94.0, a^* = 0.3163, b^* = 0.3327)
\]

Color values were expressed as \(L^*\) for lightness, \(a^*\) for redness, and \(b^*\) for yellowness.

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**Materials and Methods**

**Raw material and preparation of extract**

Dark red dried roselle calyces were purchased from the Morogoro municipality market in Tanzania. Mango fruits (cv. "Dodo") were purchased from the horticulture unit at Sokoine University of Agriculture, Tanzania.

Dried roselle calyces (10% moisture content) were ground for 1 min using a blender (Kenwood BL 440, Boulogne, France). The ground calyces were mixed with water (1:10 w/v) and extracted using a water bath at 50°C for 30 min (Chumsri et al. 2008). The extract was filtered with a cheese cloth.

**Mango juice preparation**

Fully matured and high-quality fruits of mango were used. Fresh fruits were thoroughly washed, peeled and cut into small pieces, and transferred to a juice extractor (Kenwood JE 810, Edinburgh, UK).

**Preparation of roselle-mango juice blends**

Roselle-mango juice blends were formulated in the ratio of (80:20, 60:40, 40:60, and 20:80) roselle extract: mango 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 sterile plastic and glass 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 cold water bath (Ndabikunze et al. 2010). The bottles were tightly capped, labeled and stored at 4 °C and 28 °C for 6 months. Samples were drawn for chemical analyses at 0, 2, 4, and 6 months of storage.
Reducing sugars
Reducing sugars (RS) were determined by the Luff–Schoorl method as described by Egan et al. (1981). Two grams of sample were weighed into a 100 mL Erlenmeyer cylinder and 90 mL distilled water, 5 mL Carrez I, and 5 mL Carrez II solution were added. The solution was mixed and filtered with Whatman filter (no. 542), and 10 mL of filtrate was transferred into a 250 mL Erlenmeyer flask, 10 mL of copper reagent was added, and then swirled. The solution was boiled in a direct flame for 3 min, cooled with tap water, and 1 g potassium iodide and 10 mL 6N HCl were added. This mixture was titrated with 0.1N Na2S2O3 until a yellow color appeared followed by adding a few drops of starch solution and titrated continuously until the blue color disappeared. Sugar content was then determined by interpolation in a table (Egan et al. 1981) after subtracting the blank assay.

The results are expressed in mg/100 g fresh weight (FW).

Determination of Vitamin C
Vitamin C content for the roselle-mango juice blends were determined according to the Folin–Ciocalteu reagent (FCR) method with modifications (Dashman et al. 1996), where 20 mL of sample was pipetted into a 100 mL volumetric flask followed by 2 mL of 10% tetrachloroacetic acid (TCA) solution and diluted to the 100 mL with distilled water. The sample was poured into a conical flask, swirled gently for 1 min, and left to stand for 1 min and filtered (Whatman filter no 542). One mL of the sample or 1 mL of standard solution (20 mg/100 mL) was pipetted into a test tube followed by 3 mL distilled water and 0.4 mL (1:10) Folin reagent. Mixing followed and thereafter the mixture was incubated at room temperature for 10 min. The absorbance was read at 765 nm using a Jenway 6405 UV/VIS Spectrophotometer (Jenway, Essex, UK). The results are expressed in mg/100 g FW.

Determination of antioxidant activity
Antioxidant activity for the roselle-mango blends was determined by the ferric-reducing ability of plasma (FRAP) assay (Benzie and Strain 1996) with some modifications. Three milliliters of freshly prepared FRAP solution (0.3 mol/L acetate buffer (pH 3.6) containing 10 mmol/L 2,4,6-tripyridyl-s-triazine (TPTZ) in 40 mmol/L HCl and 20 mmol/L FeCl3-6H2O) and 100 mL of sample (standard) was incubated at 37°C for 4 min, absorbance was measured at 593 nm using a spectrophotometer. An intense blue color was formed when the ferric-tripyridyltriazine (Fe3+–TPTZ) complex reduced to the ferrous (Fe2+) form. A range of FeSO4.7H2O concentrations from 0.25 to 2.0 mmol/L was used to prepare the calibration curve. The results are expressed as millimoles of (Fe2+) per liter of FW (mmol (Fe2+)/L FW).

Total phenolic content
Total phenolic content (TPC) for the roselle-mango blends was determined according to the Folin-Ciocalteu method with modifications (Singleton et al. 1999). 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. Gallic acid (0–5 mmol/L/100 mL) was used for calibration of a standard curve. The results are expressed as milligrams of gallic acid equivalents per 100 g of FW (mg GAE/100 g FW).

The total monomeric anthocyanin content
The total monomeric anthocyanin (TMA) content for roselle-mango blends was carried out using the pH differential method (Lee et al. 2005). Absorbance was measured at 520 and 700 nm using a spectrophotometer. The absorbance (A) of the sample was then calculated according to the following formula:

\[ A = (A_{520} - A_{700})_{pH 1.0} - (A_{520} - A_{700})_{pH 4.5} \]

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

\[ AC = \frac{A \times MW \times DF \times 1000}{eL} \]

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

Statistical analyses
All the tests were performed in triplicate and the results averaged (n = 3). Similar trends were observed in all the roselle-mango juice blends hence only one blend (40% roselle) was used in analysis of variance (ANOVA) using Minitab statistical software (Release 16.1 Minitab Inc., State College, PA). Multifactorial analysis of variance
Table 1. Initial physiochemical and antioxidant properties of roselle extract, mango juice, and roselle-mango juice blends (2011, 2012).

| Year Blends | TSS | pH | TA | RS | L* | a* | b* | Vit C | TMA | TPC | FRAP |
|-------------|-----|----|----|----|----|----|----|-------|-----|-----|------|
| 2011 OR     | 14.0a±0.50 | 3.4a±0.12 | 0.3c±0.43 | 5.9a±0.05 | 42.4a±0.7 | 14.3e±0.3 | 43.9a±0.6 | 62.2a±0.00 | 48.0f±0.75 | 10.9e±5.22 | 1.28f±0.00 |
| 20R         | 10.6b±0.49 | 2.6b±0.14 | 1.4b±0.00 | 5.6b±0.00 | 18.6b±0.4 | 16.4d±0.2 | 8.5b±0.2 | 58.5b±0.00 | 134.7e±1.50 | 21.3d±0.01 | 1.45e±0.00 |
| 40R         | 9.9b±0.19 | 2.7b±0.01 | 1.9a±0.35 | 5.1b±0.00 | 17.6b±0.3 | 18.1c±0.7 | 7.7c±0.5 | 53.0c±0.00 | 282.6d±1.81 | 28.8c±0.03 | 1.58d±0.00 |
| 60R         | 7.5c±0.63 | 2.4c±0.06 | 1.6b±0.00 | 4.5d±0.00 | 16.1d±0.2 | 19.2a±0.4 | 5.6d±0.2 | 44.4d±0.00 | 335.2c±1.54 | 37.9c±0.03 | 1.66c±0.00 |
| 80R         | 6.9c±0.20 | 2.6c±0.01 | 1.4b±0.00 | 3.5e±0.00 | 14.7e±0.1 | 20.0a±0.5 | 4.7e±0.6 | 40.0e±0.00 | 493.5b±5.15 | 53.7b±0.02 | 1.80b±0.00 |
| 100R        | 7.0d±0.10 | 2.3d±0.01 | 1.9a±0.00 | 2.4f±0.00 | 13.8e±0.0 | 20.6a±0.0 | 3.9f±0.0 | 37.4f±0.00 | 555.3a±2.03 | 54.6a±0.80 | 1.87a±0.00 |
| 2012 OR     | 15.5a±0.13 | 3.1a±0.01 | 0.1f±0.02 | 5.2a±0.00 | 42.4a±0.80 | 14.6e±0.0 | 45.5a±0.05 | 65.3a±0.01 | 32.9f±0.11 | 14.5f±0.10 | 1.42f±0.00 |
| 20R         | 13.8b±0.07 | 2.9b±0.01 | 0.8e±0.02 | 4.4b±0.00 | 18.6b±0.3 | 16.7d±0.4 | 8.5b±0.6 | 60.5b±0.00 | 82.4e±0.05 | 23.4e±0.01 | 1.48d±0.02 |
| 40R         | 11.2c±0.12 | 2.5c±0.01 | 1.3d±0.03 | 3.6c±0.01 | 17.9b±0.0 | 18.3c±0.49 | 7.8c±0.07 | 55.1c±0.02 | 236.3d±0.38 | 30.8d±0.01 | 1.58c±0.01 |
| 60R         | 10.0d±0.13 | 2.4d±0.01 | 1.5c±0.03 | 2.9d±0.01 | 16.3c±0.36 | 19.8b±0.03 | 5.7d±0.07 | 47.2d±0.03 | 280.5c±0.01 | 38.4c±0.12 | 1.60b±0.00 |
| 100R        | 8.0e±0.10 | 2.2e±0.01 | 1.7b±0.01 | 2.5e±0.00 | 14.9d±0.03 | 20.7a±0.02 | 4.7e±0.09 | 42.7e±0.05 | 464.2b±0.00 | 54.6b±0.12 | 1.86a±0.02 |
| 100R, 100% roselle; 80R, 80% roselle; 60R, 60% roselle; 40R, 40% roselle; 20R, 20% roselle; 0, 0% roselle. TSS, total soluble solids (°Brix); TA, titratable acidity (%); RS, reducing sugars (mg/100 g FW); Vit C, vitamin C (mg/100 g FW); FRAP, ferric-reducing ability of plasma (mmol/100 g FW); TMA, total monomeric anthocyanins (mg/L FW); TPC, total phenolic content (mg/100 g GAE FW); L*, lightness; a*, redness; b*, yellowness. Data in columns for each year with different superscript are significantly different using Tukey’s pairwise comparison test (P < 0.05).

Results and Discussion

The initial physiochemical properties of roselle-mango juice blends and mango juice (2011 and 2012) are shown in Table 1. The pH of roselle extract in the blends as roselle extract is known to contain high pectin content (Wong et al. 2002). The pH of the roselle-mango juice was increased with increasing concentration of roselle extract as roselle is a good source of anthocyanins (Wong et al. 2002). Treatment 1, 2, and 3 of the blends (Table 2) were stored for 0, 2, 4, 6 months (Wong et al. 2002).

(Continued...
Table 2. Initial and final total soluble solids (TSS) and reducing sugar (RS) of roselle-mango juice blends stored in glass and plastic bottles for 6 months.

| Packaging material | TSS (%) | Glass | Plastic | RS (mg/100 g FW) | Glass | Plastic |
|--------------------|---------|-------|---------|-----------------|-------|---------|
| Storage month      |         |       |         |                 |       |         |
| 0                  | 6       | 6     | 6       | 6               | 6     | 6       |
| Storage temperature| BS      | 28°C  | 4°C     | 28°C            | 4°C   | 28°C    | 4°C    |
| 80R                | 8.0 ± 0.10 | 8.4 ± 0.12 | 8.5 ± 0.09 | 8.4 ± 0.10 | 8.5 ± 0.09 | 2.5 ± 0.01 | 3.7 ± 0.01 | 3.2 ± 0.01 | 3.7 ± 0.01 | 3.2 ± 0.01 |
| 60R                | 10.0 ± 0.15 | 11.9 ± 0.2 | 12.2 ± 0.42 | 11.9 ± 0.05 | 12.2 ± 0.42 | 2.9 ± 0.01 | 4.1 ± 0.01 | 4.0 ± 0.01 | 4.0 ± 0.01 | 4.0 ± 0.01 |
| 40R                | 11.2 ± 0.14 | 11.2 ± 0.17 | 11.5 ± 0.15 | 11.4 ± 0.04 | 11.5 ± 0.15 | 3.6 ± 0.01 | 4.9 ± 0.00 | 4.2 ± 0.01 | 4.9 ± 0.00 | 4.2 ± 0.01 |
| 20R                | 13.8 ± 0.08 | 13.7 ± 0.11 | 14.1 ± 0.08 | 13.7 ± 0.11 | 14.1 ± 0.08 | 4.4 ± 0.01 | 5.7 ± 0.01 | 5.1 ± 0.01 | 5.7 ± 0.01 | 5.1 ± 0.01 |

TSS, total soluble solids; RS, reducing sugar; M, months; 80R, 80% roselle; 60R, 60% roselle; 40R, 100% roselle; 20R, 20% roselle.

Table 3. Initial and final pH and titratable acidity (TA) of roselle-mango juice blends stored in glass and plastic bottles at 28°C and 4°C.

| Packaging material | pH | Glass | Plastic | TA (%) | Glass | Plastic |
|--------------------|----|-------|---------|--------|-------|---------|
| Storage month      |    |       |         |        |       |         |
| 0                  | 6  | 6     | 6       | 6      | 6     | 6       |
| Storage temperature| BS | 28°C  | 4°C     | 28°C   | 4°C   | 28°C    | 4°C    |
| 80R                | 2.2 ± 0.01 | 2.7 ± 0.01 | 3.0 ± 0.02 | 2.5 ± 0.01 | 2.6 ± 0.01 | 1.70 ± 0.02 | 1.57 ± 0.02 | 1.6 ± 0.09 | 1.58 ± 0.05 | 1.57 ± 0.02 |
| 60R                | 2.4 ± 0.01 | 2.8 ± 0.01 | 3.0 ± 0.03 | 2.6 ± 0.01 | 2.7 ± 0.01 | 1.50 ± 0.03 | 1.51 ± 0.02 | 1.5 ± 0.02 | 1.51 ± 0.02 | 1.52 ± 0.02 |
| 40R                | 2.5 ± 0.01 | 2.9 ± 0.01 | 3.0 ± 0.01 | 2.8 ± 0.01 | 2.9 ± 0.15 | 1.37 ± 0.01 | 1.3 ± 0.02 | 1.4 ± 0.02 | 1.34 ± 0.02 | 1.35 ± 0.02 |
| 20R                | 2.9 ± 0.02 | 3.1 ± 0.01 | 3.2 ± 0.01 | 3.1 ± 0.01 | 3.1 ± 0.01 | 0.83 ± 0.02 | 0.79 ± 0.02 | 0.8 ± 0.02 | 0.80 ± 0.02 | 0.79 ± 0.02 |

BS, before storage; TA, titratable acidity; M, months; 80R, 80% roselle; 60R, 60% roselle; 40R, 100% roselle; 20R, 20% roselle.
Lightness ($L^*$) for roselle-mango blends stored in glass and plastic bottles ranged from 13.3 to 18.6 (28°C) and 13.5–18.6 (4°C) for 6 months of storage as shown on Table 4. The $L^*$ value which is an indicator of lightness of color had shown to decrease with increased storage time. The decrease might be due to nonenzymatic browning reactions occurred to mango juice during storage. Falade et al. (2004) reported 47.4% and 36.8% decrease of $L^*$ values in sweetened Julie and Ogbomoso mango juice stored at 25°C. Marti et al. (2002) also reported a significant decrease in $L$ value during storage of pomegranate juice for 150 days at 25°C.

Redness ($a^*$) for roselle-mango blends stored in glass and plastic bottles ranged from 14.6 to 20.6 (28°C) and 15.2–20.6 (4°C) for 6 months of storage. Yellowness ($b^*$) for roselle-mango blends stored in glass bottles ranged from 2.7 to 8.2 (28°C) and 2.8–8.2 (4°C) after 6 months of storage. The yellowness in roselle-mango juice blends is due to the presence of carotenoids in mango juice. However, these carotenoids are highly susceptible to degradation by heat, low pH, and light exposure (Hewavitharana et al. 2013). The effect of packaging material, storage time, storage temperature, and their interactions, significantly ($P < 0.001$) affected the yellowness $b^*$ for roselle-mango juice blends (Table 5).

TMA of the roselle-mango juice blends (40%R) stored in glass bottles at 4°C were higher than those stored at 28°C (Fig. 1). The decrease was significant ($P < 0.05$) during storage, irrespective of storage temperature and packaging material (Table 5). Waskar and Gaikwad (2004) observed similar trends on pomegranate kokum mango-based blends stored for 150 days. The amount of anthocyanin remained after 6 months was (127.7–144.1 mg/100 g) at 4°C and 100–107 mg/100 g at 28°C in all roselle-mango blends (40%) stored in glass and plastic bottles, these amounts were sufficient to provide the amount of anthocyanins per day recommended by the United States of America and Finland (82 and 12.5 mg per day) by Wu et al. (2006).

Vitamin C content of the blends decreased significantly ($P < 0.05$) with increased storage period because vitamin C can easily be oxidized in the presence of oxygen by both enzymatic and nonenzymatic catalyst (Jawaheer et al. 2003). Vitamin C losses was lower in roselle-mango juice blends stored in glass bottles (Fig. 2). Similar results

### Table 4. Initial and final color parameters of roselle-mango juice blends stored in glass and plastic bottles at 28°C and 4°C.

| Source of variation | TSS | pH | TA | RS | TMA | Vit. C | TPC | FRAP | $L^*$ | $a^*$ | $b^*$ |
|--------------------|-----|----|----|----|-----|--------|-----|-----|------|------|------|
| Packaging (A)      | ns  | ns | ns | ns | <0.001| <0.001 | <0.001| <0.001| <0.001| <0.001| <0.001|
| Temperature (B)    | <0.001| <0.001| 0.037| <0.001| <0.001| <0.001| 0.006| <0.001| ns  | ns  | <0.001|
| Time (C)           | <0.001| <0.001| ns  | <0.001| <0.001| <0.001| <0.001| <0.001| ns  | ns  | <0.001|
| $A \times B$       | <0.001| <0.001| <0.001| <0.001| <0.001| <0.001| <0.001| <0.001| <0.001| <0.001| <0.001|
| $A \times C$       | <0.001| <0.001| ns  | <0.001| <0.001| <0.001| <0.001| <0.001| <0.001| <0.001| <0.001|
| $B \times C$       | ns  | ns | ns | <0.001| <0.001| <0.001| <0.001| <0.001| <0.001| <0.001| <0.001|

This table shows the effect of packaging, storage temperature, and time on the color parameters of roselle-mango juice blends. The results are significant at $P < 0.001$. The decrease in lightness ($L^*$) and increase in redness ($a^*$) and yellowness ($b^*$) were observed with increased storage time and temperature.

### Table 5. Effects of packaging, storage time and temperature on the quality of roselle-mango juice blends (40% roselle).

| Source of variation | TSS | pH | TA | RS | TMA | Vit. C | TPC | FRAP | $L^*$ | $a^*$ | $b^*$ |
|--------------------|-----|----|----|----|-----|--------|-----|-----|------|------|------|
| Packaging (A)      | ns  | ns | ns | ns | <0.001| <0.001 | <0.001| <0.001| <0.001| <0.001| <0.001|
| Temperature (B)    | <0.001| <0.001| 0.037| <0.001| <0.001| <0.001| 0.006| <0.001| ns  | ns  | <0.001|
| Time (C)           | <0.001| <0.001| ns  | <0.001| <0.001| <0.001| <0.001| <0.001| ns  | ns  | <0.001|
| $A \times B$       | <0.001| <0.001| <0.001| <0.001| <0.001| <0.001| <0.001| <0.001| <0.001| <0.001| <0.001|
| $A \times C$       | <0.001| <0.001| ns  | <0.001| <0.001| <0.001| <0.001| <0.001| <0.001| <0.001| <0.001|
| $B \times C$       | ns  | ns | ns | <0.001| <0.001| <0.001| <0.001| <0.001| <0.001| <0.001| <0.001|

This table shows the effect of packaging, storage time, and temperature on the quality of roselle-mango juice blends. The results are significant at $P < 0.001$. The decrease in lightness ($L^*$) and increase in redness ($a^*$) and yellowness ($b^*$) were observed with increased storage time and temperature.
were observed by Alaka et al. 2003 when mango juices were packaged in polyethylene films, polyethylene terephthalate (PET or plastic) bottles and transparent glass bottles, and stored at 6°C, 26°C, and 34°C. Despite the fact that the vitamin C losses in roselle-mango blends (40% roselle) stored at 4°C for 2 months was more than 45 mg per 100 mL, i.e., only 100 mL of the blends will contain sufficient vitamin C to provide the recommended daily allowance (RDA) for adults, which is 45 mg (FAO/WHO 2001).

Polyphenols are the most abundant antioxidants in the diet and are widespread constituents of fruits and vegetables (Fang et al. 2006). However, they are susceptible to degradation during storage, which was demonstrated by the value of 30.9 mg GAE/100 g initially for roselle-mango blends (40% roselle) stored at 4°C after 6 months of storage (Fig. 3). All variables (time, temperature, and packaging and interaction term, time × temperature, time × packaging, and temperature × packaging) significantly contributed to the loss of total phenol content (TPC) see Table 5.

FRAP for roselle-mango blends stored in glass and plastic bottles ranged from 1.04 to 1.86 mmol/L (28°C) and 1.19–1.86 mmol/L (4°C) after 6 months of storage (Fig. 4). Despite marked losses of TMA in all the roselle-mango blends, FRAP value losses were less than 30% during storage, suggesting that polymeric compounds formed during storage might have compensated the loss of antioxidant activity due to degradation of monomeric anthocyanins (Tsai and Huang 2004).

In the case of seasonal variation and storage time and temperature, a bi-plot of observations and variables is shown in Figure 5. Most of the variation (85%) was explained by the first two principle components (PC) with the first component (PC1) accounting for 68% and associated with parameters (TSS, Vitamin C, and TPC) and the second components account for 17% of the total variation associated with parameters (color L*, a*, b* RS, FRAP, and TMA). The PC1 explained roselle-mango juice blends stored in plastic bottles at refrigerated temperature with more blends from season 2012 while PC2 explained blends stored at ambient temperature with more blends from season 2011.

The roselle-mango juice blends stored at refrigerated temperature for zero and 2 months were on the positive side of PC2 while those blends stored for 4 and 6 months were on the negative side of PC2. Those blends stored at
ambient temperature for 0 months were on the positive side of PC1 while those blends stored at ambient temperature for 2, 4, and 6 months were on the negative side of the PC1.

The results showed that most of 2012 blends had high levels of TPC, TSS, and Vitamin C (Fig. 5 and Table 1). Regardless of season or storage temperature, results on the bi-plots showed the effects of storage time on the blends as storage was progressing with storage at 4 and 6 months being on the negative side of the PCs. The Bi plots also showed the TPC, TMA, and Vitamin C were parameters mostly affected by the storage time regardless of storage temperature.

Conclusions

The roselle-mango blends presented some chemical changes during 6 months storage. The most affected components were TMA, total phenols, and vitamin C. The blends stored at 28°C showed remarkable losses of TMA, TPC, and vitamin C as compared to 4°C, hence storage at 28°C should be avoided if good long-term preservation of the roselle-mango juice blends is desired due to retention of more TMA, TPC, and vitamin C. Packaging in glass bottles and storage at 4°C should be encouraged as it retains more vita-

![Figure 3. Total phenolic content (TPC) for roselle-mango juice blend (40% roselle) stored in glass and plastic bottles at ambient (A) and refrigerated (R).](image)

![Figure 4. Antioxidant activity (FRAP) for roselle-mango juice blend (40% roselle) stored in glass and plastic bottles at ambient (A) and refrigerated (R).](image)

![Figure 5. Bi-plot (scores and loadings) for roselle-mango juice blend stored at ambient and refrigerated temperature for 6 months (season 2011 and 2012).](image)
min C and TMA essential in antioxidant capacity of fruits and fruit products. Seasonality and packaging material, storage time, and temperature have shown to affect total monomeric anthocyanin contents, total phenol, and vitamin C content of the roselle-mango juice blends. The quantity of total monomeric anthocyanin and vitamin C remaining after 6 months of storage of the roselle-mango juice blends (40%R) was sufficient to provide recommended amount for daily intake of anthocyanins and vitamin C for adults.

**Conflict of Interest**

None declared.

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