Utilization of dried roselle calyces extract in fruit juice processing

Utnyttelse av roselleekstrakt i fruktjuice

Philosophiae Doctor (PhD) Thesis

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DEDICATION

I dedicate this work to my dear son Timothy

And

My two lovely daughters; Upendo and Agnes

ABBREVIATIONS

Carrez I Solution contains zinc acetate dehydrate and acetic acid

Carrez II Solution contains potassium hexacyanoferrate (II) $K_2 [Fe (CN)_6].3H_2O$

FRAP Ferric reducing antioxidant power

HPLC High performance liquid chromatography

TMA Total monomeric anthocyanin

TPC Total phenol content

TPTZ 2, 4, 6-Tripyridyl-s-Triazine

PCA Principal Component analysis

FCR Folin–Ciocalteu reagent

SUMMARY

There has been an increased consumption of fruit juice as consumers have become aware of the nutritional and health benefits of fruit juices. This increased consumption goes together with the growth of varieties of fruit juices and beverages offered for sale. Among these products is roselle (*Hibiscus sabdariffa* L.) juice, or drink, which is a good source of anthocyanins, vitamins (thiamine, riboflavin, niacin, ascorbic acid) and minerals (Ca, P, Fe). In addition, roselle is also used as flavouring for sauces, jellies, marmalades, soft drinks and as colorant for foods.

Roselle extract has low sugar content, and is acidic, so addition of sweetening products like sugar or blending with tropical fruit juices can help to reduce the sourness and improve the sweetness of the juice. The use of roselle extract mixed with tropical fruit juices is also a way of reducing post-harvest losses of fruits which occurs due to lack of good knowledge in processing, preservation, packaging and proper transport systems in most developing countries like Tanzania.

Blending of roselle extract with different proportion of tropical fruit juices (mango, papaya and guava) produces roselle-fruit juice blends with improved levels of anthocyanins, vitamin C, total phenolics/phenols, minerals (Ca, Mg, P, Fe and Zn). The amount of vitamin C and Fe were enough to provide recommended daily requirements for adults according to WHO/FAO recommendation while amount of anthocyanins provide enough to meet recommendation of 82 mg and 12.5 mg per day per person in Finland and United States.

The roselle-fruit juices were found to be high in glucose and fructose. However, as the concentration of roselle extract in the blends increased, the amount of sugars also decreased. Organic acids (malic acid, succinic acid, tartaric acid) increased also with increased concentration of roselle extract in the blends, while the amount of citric acidic increased with increased concentration of tropical fruit juices.

The sensory evaluation of the roselle fruit juice blends resulted in the highest acceptance of the roselle-fruit juices with 20% roselle followed by blends with 40% roselle. This was probably due to the reduced acidic taste by adding these low concentrations of roselle to the tropical fruit juices. Colour play a very important role in the acceptability of foods as it is one of the

principal characteristics perceived by the senses. The strong colour is due to the presence of anthocyanins in the roselle extract, which imparted the red colour in the blends. The colour of the roselle-fruit juice blends with 80% roselle scored highest among all the sensory parameter evaluated.

Storage temperature of the roselle-fruit juice blends showed to have high effect on the anthocyanin content, total phenolic content, vitamin C with significantly losses at higher temperatures. Lower temperature is therefore recommended for long-term storage.

Packaging material did not have significant influence on the physicochemical properties of the roselle-mango juice blends. Losses in total monomeric anthocyanins, total phenols and vitamin C were higher in blends stored in plastic bottles compared to glass bottles in higher temperatures. Storage in glass bottles at lower temperatures is therefore recommended for storage of roselle-fruit juice blends.

SAMMENDRAG

Inntaket av fruktjuicer har økt etterhvert som kunnskapen om helsefordelene ved inntak av slike produkter har blitt bedre kjent, noe som har ført til en økning i juiceutvalget. Blant disse drikkeproduktene finner en også varianter som inneholder roselle (*Hibiscus sabdariffa* L.). Roselle er en god anthocyaninkilde, og inneholder i tillegg vitaminer (tiamin, riboflavin, niacin og askorbinsyre) og mineraler (Ca, P og Fe). Roselle kan brukes som smakstilsetning i sauser, syltetøy, marmelade og drikkeprodukter og kan også forbedre farge på produktet.

Roselle inneholder lite sukker og har høyt syreinnhold. Ved tilsetning av søtningsstoff eller i blanding med tropiske frukter kan en bedre smaksopplevelsen. Bruk av tropiske frukter i blanding med roselle kan også bidra til bedre utnyttelse av disse, men det trengs kunnskap om prosessering, konservering, forpakning og transportmuligheter i de fleste utviklingsland, inkludert Tanzania.

Ved å blande roselle i ulike blandingsforhold med tropiske frukter som mango, papaya og guava vil dette gi drikkeprodukter med høyere innhold av anthocyaniner, vitamin C, fenolforbindelser og mineraler (Ca, Mg, P, Fe og Zn). Innholdet av vitamin C og Fe i juiceblandingene tilfredsstiller behovet for disse komponentene for voksne, i henhold til WHO/FAOs anbefalte daglige inntak, mens innholdet av anthocyaniner tilfredsstiller behovet på 82 mg og 12,5 mg pr person anbefalt i Finland og USA.

Innholdet av glukose og fruktose i roselle-juiceblandingene var relativt høyt. Ved å tilsette mer roselle i blandingene ble sukkerinnholdet derimot lavere. Innholdet av organiske syrer som eple-, rav- og vinsyre økte med økende konsentrasjon av roselle, mens sitronsyre økte med høyere konsentrasjon av fruktjuice.

Sensorisk vurdering viste at roselle-juiceblandingen med 20 % roselle var best likt, deretter 40 % roselle. Dette kan henge sammen med at en ved laveste tilsetting av roselle får lavere syreinnhold og høyere sukkerinnhold i blandingene. Roselle bidrar med en attraktiv rød farge på grunn av innholdet av anthocyaniner, noe som virker tiltalende på konsumenten. Dette var

en viktig parameter ved de sensoriske vurderingene, hvor blandinger med 80 % roselle fikk høyest poengsum for utseende.

Lagringstemperaturen for juiceblandingene med roselle hadde innvirkning på anthocyaniner, fenoler og vitamin C. Høyere lagringstemperatur viste nedgang i konsentrasjonen av disse komponentene. Av den grunn bør det ferdige produktet lagres ved relativt lav temperatur hvis det skal oppbevares lenge.

Valg av pakkemateriale hadde også innvirkning på innholdet av antioksidantegenskapene. Dette ble bekreftet i forsøket med roselle-mangoblandinger. En fant større tap av anthocyaniner, fenoler og vitamin C ved lagring i plastflasker sammenliknet med glassflasker. Lagring av produktet ved 4 ºC og i glassflasker vil være å foretrekke.

LIST OF PAPERS

Paper I

B. Mgaya-Kilima, S.F. Remberg, B.E. Chove, T. Wicklund. (Accepted) Physiochemical, mineral composition and antioxidant properties of Roselle (*Hibiscus sabdariffa* L.) extract blended with tropical fruit juices *African Journal of Food, Agriculture, Nutrition and Development (AJFAND).*

Paper II

B Mgaya-Kilima, S.F. Remberg, B.E. Chove, T. Wicklund. Influence of storage temperature and time on physiochemical and bioactive properties of roselle-fruit juice blends in plastic bottles *Journal of Food Science and Nutrition,* **2**(2): 181-191.

Paper III

B. Mgaya-Kilima, S.F. Remberg, B.E. Chove, T. Wicklund. Influence of storage temperature and time physiochemical and antioxidant properties of roselle-fruit juice blends in glass bottles *African Journal of Food, Agriculture, Nutrition and Development (Submitted)*

Paper IV

B. Mgaya-Kilima, S.F. Remberg, B.E. Chove, T. Wicklund. Physiochemical and antioxidant properties of roselle-mango juice blends: effects of packaging materials and storage temperature and time. *Manuscript to Journal of Food Science and Nutrition (submitted)*

Paper V

B. Mgaya-Kilima, S.F. Remberg, B.E. Chove, T. Wicklund. Determination of organic acids and sugars and consumer acceptability of roselle-fruit juice blends (Manuscript).

1.0 INTRODUCTION

Fruits have been a part of the human diet for centuries. They contain high quantity of water, carbohydrates, proteins, vitamins A, B1, B2, C, D and E; and minerals such as Ca, Mg, K, Zn and Fe [1]. They are rich in antioxidants that help in lowering incidence of degenerative diseases such as cancer, arthritis, arteriosclerosis, heart disease, inflammation, brain dysfunction and acceleration of the ageing process [2, 3].

Large quantities and different varieties of fruits are produced annually throughout the world. However, a greater proportion of these fruits especially in developing countries are wasted during the season due to lack of good knowledge in storage, processing, preservation, packaging and proper transport systems. For example, Tanzania has capacity of producing 2 000 000 metric tons of fruits worth at least US $ 900 000, however, 40-60% of the fruits are wasted [4]. Processing of fruits into juice will help reducing post-harvest loss and increase consumption of fruits.

Worldwide, there has been increased fruit juice consumption, as consumers have been more aware of the nutritional and health benefits of fruit juices. This increased consumption is due to the increased variety of fruit juices and beverages offered for sale. Beverages are produced from various types of plants, especially leaves, flowers and fruits [5].

One of such plants whose flowers are used to prepare juices is *Hibiscus sabdiriffa* L., commonly known as roselle. Dried red roselle calyces are usually extracted making a bright red drink with a unique flavour. The drink is consumed hot or cold. The calyces contain organic acids (tartaric, citric, malic, and hibiscic), glucoside compounds, and phenolic compounds, such as anthocyanins [6]. They also contain vitamins (thiamine, riboflavin, niacin and ascorbic acid) and minerals (Ca, Mg, K and Fe). The extract is also used as flavouring for sauces, jellies, marmalades, and soft drinks and as food colorant. Hibiscus extracts are reported as having medical properties such as decreasing serum cholesterol in humans and animals [7, 8], protecting the liver against oxidation stress [9], having antihypertensive and cardio protective effects [10], and attenuating nephropathy in diabetes [11].

Most people do not prefer beverages made from pure roselle as it has an acidic and bitter taste. Blending of the extract with juice from tropical fruits such as mango, papaya and guava can improve the aroma, taste and nutritional and antioxidant properties of the juice blends. The main aim of this study was blending roselle extract with various tropical fruits (guava, papaya and mango) to increase the utilization of roselle extracts, enhance nutritional composition and acceptability of roselle-fruit juice blends.

2.0 BACKGROUND

2.1 Hibiscus sabdiriffa L

The genus *Hibiscus* consists of several hundred species of flowering plants in the family *Malvaceae*. These species are well known for their large and colourful flowers. *Hibiscus sabdiriffa* L., is one of the most known species of genus *Hibiscus* [12]. It is an herbaceous plant cultivated largely in tropical and sub-tropical of both hemispheres [13]. China and Thailand are the largest producers and controller of much of the world supply, while Mexico, Egypt, Senegal, Tanzania, Mali, Sudan and Jamaica are important supplier but the production is mostly used domestically [14]. Roselle (*Hibiscus sabdariffa* L) is known in different countries by various common name including roselle, rozelle, sorrel, red sorrel, Jamaican sorrel, Indian sorrel, guinea sorrel, sour-sour, Queensland jelly plant [15].

2.2 Morphological characteristics of roselle

The roselle plant is an annual shrub with growth more than two meters. The leaves are dark green to red divided into three to five lobes and are arranged on the stem alternatively [12] Stems may be green or red depending on the genetic background [15]. Flowers are usually white to pale yellow while the colour of petal may vary from white to pink, red, orange, purple or yellow. The mature fruits are bright red [16] (Figure 1).

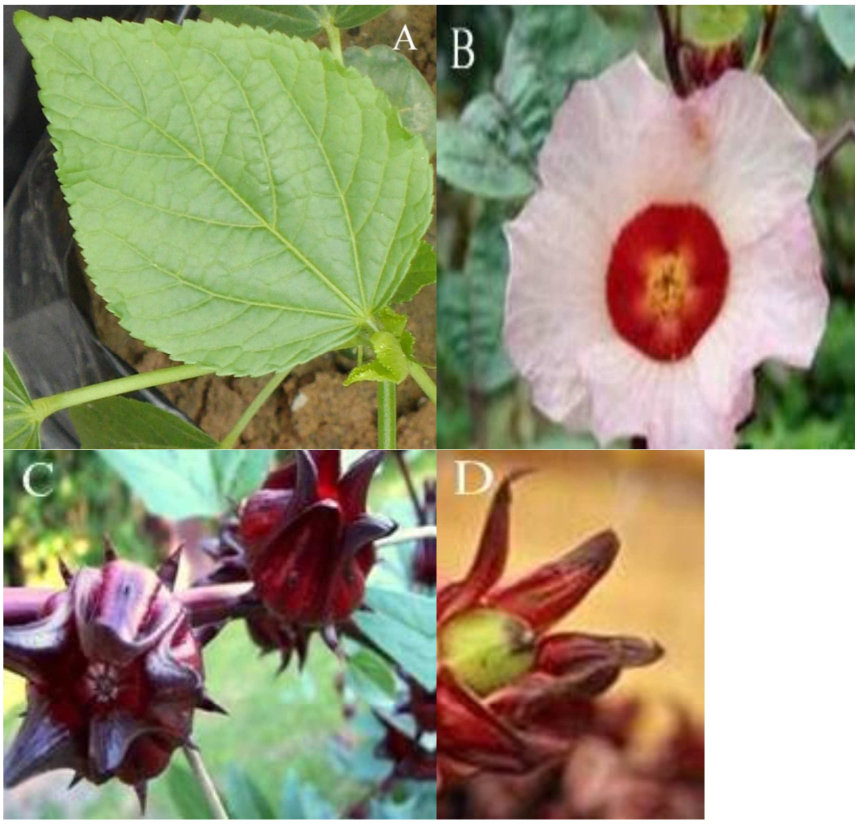


Figure 1 Morphology of roselle plant: leaves (A), flower (B), calyces (C), fruit (D) [17].

2.3 Phytochemicals

Phytochemicals, also known as bioactive compounds, are secondary metabolites synthesized by plants [18] and can be described as chemicals from plants that may affect health, but do not act as essential nutrients [19]. There are several families of phytochemicals, such as glucosinolates, flavonols, isoflavones, phenolic acids and flavones.

2.3.1 Phenolic compounds

Phenolic compounds or polyphenols are widely distributed groups of phytochemicals, with more than 8000 phenolic structures currently known. They provide essential functions in the reproduction and growth of the plants, act as defence mechanisms against pathogens, parasites, predators, and UV irradiation, and also contribute to the colour of plants [20].

Polyphenols are divided into several classes according to the number of phenol rings and the structural elements attaching the phenolic rings. The main groups of polyphenols are: flavonoids, phenolic acids, tannins (hydrolysable and condensed), stilbenes and lignans [21].

Flavonoids are the most abundant polyphenols. The basic flavonoid structure is the flavan nucleus, containing 15 carbon atoms arranged in three rings (C6-C3-C6), which are labelled as A, B and C (Figure 2).

Flavonoids forms six major subclasses based on the substitution patterns of ring C (Figure 2). The position of ring B as well as the oxidation state of the furan ring is important in this classification. Within each class, individual flavonoids are identified and characterized by conjugation patterns of the hydroxyl groups on the A and C rings as well as the hydroxylation and conjugation patterns of the B ring [21].

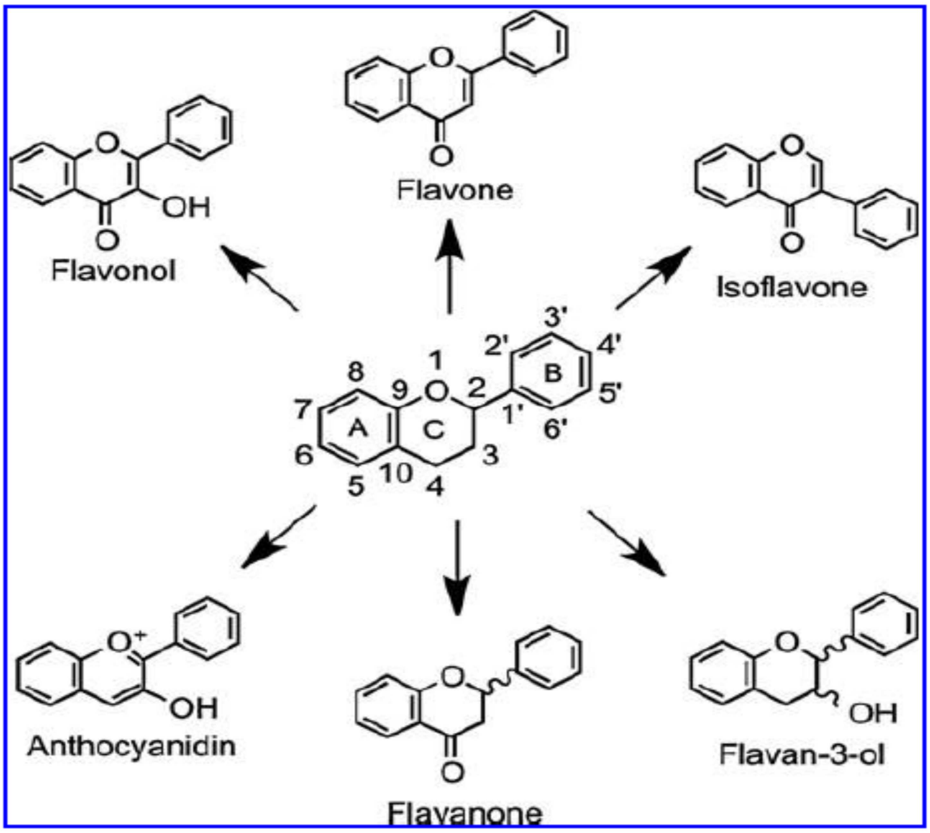


Figure 2 Structure of the flavonoid skeleton [21].

The major flavonoids sub classes are distinguished according to the oxidation state of the central C ring and position of B ring in flavones, flavonols, flavanols, flavanones, isoflavones, and anthocyanins. The structure variation in each subclass is partly due to the degree and pattern of hydroxylation, methoxylation, prenylation, or glycosylation.

2.3.2 Anthocyanins

Anthocyanins are water-soluble plant pigments and are particularly evident in fruit and flower tissue where they are responsible for a diverse range of red, blue, and purple colours.

They have at least three functions in plant physiology. First, they assist in plant propagation due to their bright colour which attracts insects for pollination and animals for seed dispersion [22].

Secondly, they prevent predation by imparting a bitter taste to plants and third, owing to strong absorption of light, they may also be important in protecting plants from UV-induced damage [23]. Chemically, anthocyanidins are polyhydroxy or polymethoxy derivatives of 2 phenylbenzopyrylium: 2 benzoyl rings (A and B) separated by a heterocyclic (C) ring (Figure 3)

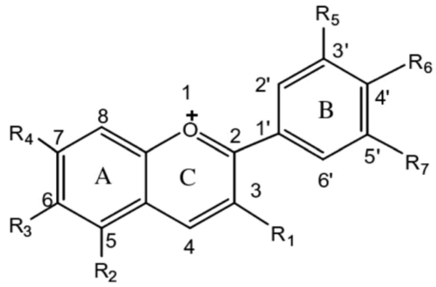
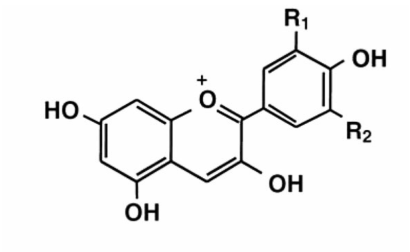


Figure 3 The flavylium cation. R1, R2 = H, OH or OCH3; R3 = OH or glycosyl; R4 = OH or glycosyl **[24]**

Approximately 17 anthocyanidins are found in nature, only six of which are widely distributed: cyanidin (Cy), delphinidin (Dp), petunidin (Pt), peonidin (Peo), pelargonidin (Pl), and malvidin (Mv) [25, 26] (Figure 4).



R_1	R_2	Anthocyanidin
H	H	Pelargonidin
OH	H	Cyanidin
OCH_3	H	Peonidin
OH	OH	Delphinidin
OCH_3	OH	Petunidin
OCH_3	OCH_3	Malvidin

Figure 4 structural classifications of six most common anthocyanidins [27].

The structural variations of anthocyanins are due to differences in the number of hydroxyl groups attached to the molecule, the degree of methylation of these hydroxyl groups, the nature and number of the sugar moiety attached to the phenolic (aglycone) molecule and the position of the attachment, as well as the nature and number of aliphatic or aromatic acids attached to the sugars [24].

Anthocyanins frequently occur as 3-monosides, 3-biosides and 3-triosides as well as 3, 5-diglycosides and more rarely 3, 7-diglycosides associated with the sugars glucose, galactose, rhamnose, arabinose, and xylose. The most widespread anthocyanin is cyanidin 3-glucoside (27, 25). delphinidin 3-sambubioside is the major pigment responsible for the reddish-violet colour. cyanidin 3-sambubioside is also present with lesser amounts of delphinidin and cyanidin 3-glucosides [27].

These pigments have been identified in edible plant materials as diverse as apple, berries (blackcurrant, boysenberry, blueberry, bilberry, strawberry, blackberry, raspberry, cranberry, elderberry, lingonberry, chokeberry etc.), black carrot, cabbage, cherry, grape, radish, red onions, roselle calyx and sweet potato, to mention only a few of the vast array known [28].

2.3.2.1 Anthocyanins in roselle

The roselle calyces contain two main anthocyanins; delphinidin-3-sambubioside, also known as delphinidin- 3-xylosylglucoside or hibiscin, and cyanidin-3-sambubioside, also known as cyanidin-3-xylosylglucoside or gossypicyanin, and two minor anthocyanins, delphinidin-3-glucoside and cyanidin- 3-glucoside [29]. The calyx of *H. sabdariffa* also contains other polyphenolic compounds including protocatechic acid [30, 31].

2.4 Anthocyanins stability

The isolated anthocyanins are highly instable and very susceptible to degradation [32]. Their stability is affected by several factors such as pH, storage temperature, chemical structure, concentration, light, oxygen, solvents, the presence of enzymes, proteins and metallic ions [24, 29, 33, 34].

2.4.1 pH influence

Anthocyanins are unique among flavonoids as their structures reversibly undergo pH-dependent transformation in aqueous solution (Figure 5). Anthocyanins are more stable in acidic media at low pH values than in alkaline solutions with high pH values. Four major anthocyanin forms exist in equilibria: the red flavylium cation, the blue quinonoidal base, the colourless carbinol pseudobase, and the colourless chalcone [35]. At a pH below 2, anthocyanins exist predominantly in the red flavylium cation form. Rapid hydration of the flavylium cation occurs at the C-2 position to generate the colourless carbinol pseudobase at pH values ranging from 3 to 6. As red colour is bleached out in this transformation, the mechanism of reaction has been extensively investigated [23].

The anthocyanidin's stability is influenced by the ring B substituents and the presence of additional hydroxyl or methoxyl groups which decrease the aglycone stability in neutral media; therefore, delphinidin is the most stable anthocyanidin [36]. In contrast with aglycons, monoglycosides, and mostly, diglycosides derivatives are more stable in neutral pH conditions [36]. The effective pH range for most anthocyanin colorants is limited to acidic foods because of the colour changes and instability that occurs above pH 4.

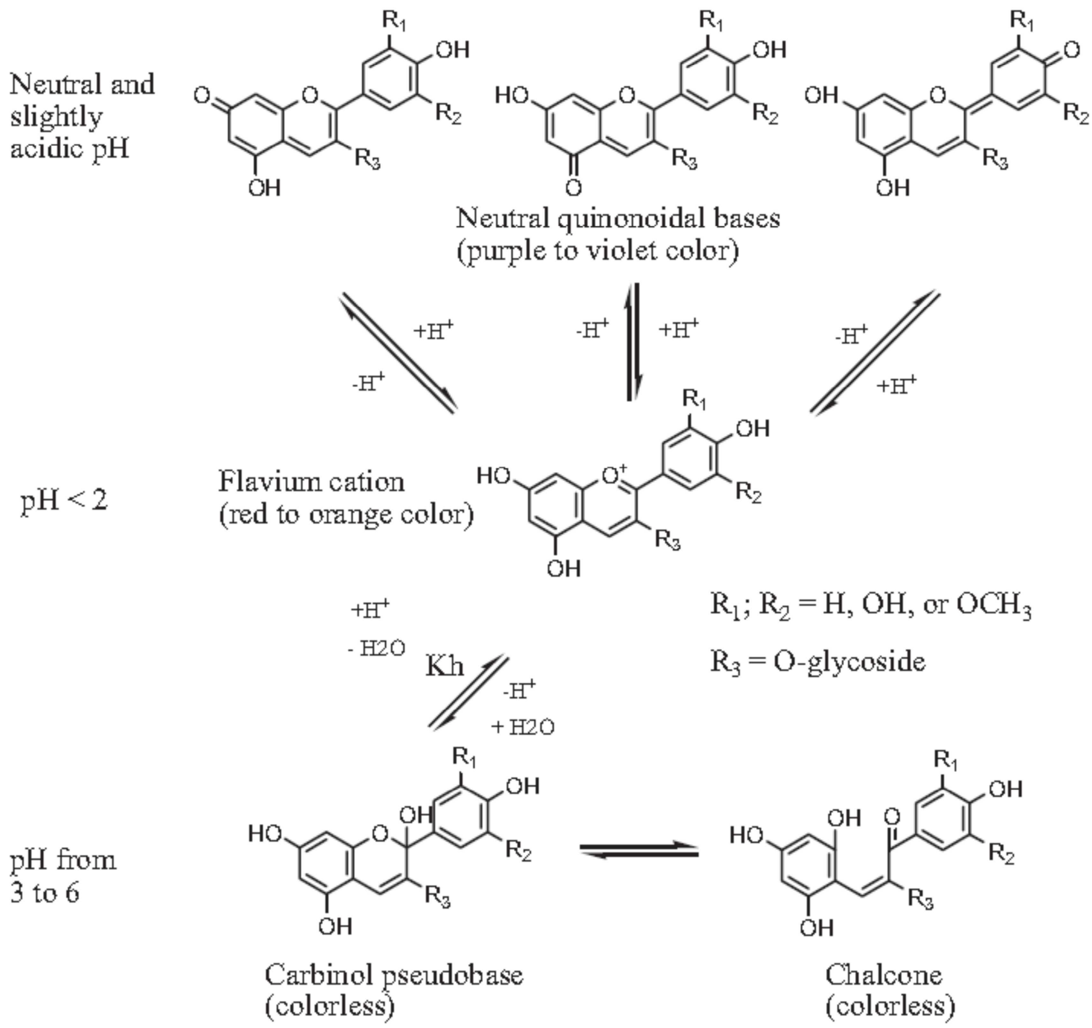


Figure 5 Scheme of the pH-dependent structural interconversion between dominant forms of mono-glycosylated anthocyanins in aqueous phase [37].

2.4.2 Storage effects

Many studies have shown degradation of anthocyanins during storage treatments [38, 39] storage time showing higher losses of total momomeric anthocyanins. The losses of total monomeric anthocyanins were also accompanied by increased polymeric colour values [40]. The large increase in polymeric colour values and corresponding loss of monomeric anthocyanins may be due to several factors, including residual enzyme activity or condensation reactions of anthocyanins with other phenolics [38]. There are also reports on strong influence of storage temperature on the stability of anthocyanins. Higher stability of anthocyanins can be achieved by using lower temperature and short time heating during processing and storage [41].

2.4.3 Structural effects

The glycosyl units and acyl groups attached to the aglycone, and the site of their bonding, have a significant effect on stability and reactivity of the anthocyanin molecule. Also the substitution pattern of the anthocyanidin, the number and placement of the hydroxyl and methoxyl groups in the aglycone, affect the chemical behaviour of the pigment molecule.

The increased hydroxylation of the aglycone stabilizes the anthocyanidin; delphinidin is more stable than cyanidin in acidic methanol [42]. However, there are discrepancies related to the effect of hydroxylation of the aglycone on molecule stability; in a buffered solution at pH 3.1 cyanidin 3-glucoside was more stable than pelargonidin 3-glucoside but delphinidin 3-glucoside was less stable than cyanidin 3-glucoside. Also petunidin 3-glucoside, with two hydroxyl groups in the B-ring, was less stable than peonidin 3-glucoside, which has one hydroxyl group in the same ring [43].

2.4.4 Temperature

The stability of anthocyanins and all pigments found in foods decreases during processing and storage as temperature rises [44]. Eventually thermal degradation leads to brown products, especially in the presence of oxygen [45]. Many authors have studied the influence of temperature in the anthocyanins stability from different sources proving that heating have a detrimental effect on the anthocyanin content [40, 46-49].

2.5 Antioxidant activity

Antioxidants are strong scavengers of free radicals, which are unstable chemical species that react rapidly with other chemical species in a biological system. Reactive species, such as superoxides (O2•- and OOH•), hydroxyl (OH•), and peroxyl (ROO•) radicals, can attack stable molecules in a healthy organism and produce illnesses [50]. Antioxidants, including flavonoids, acids, tocopherols, carotenoids, and vitamin C [51], may neutralize the oxidative effect of free radicals.

There is great number of methods for determination of antioxidant capacity of foods and beverages based on different principles: peroxyl radical scavenging (Oxygen Radical Absorbance capacity, ORAC; Total Radical-trapping Antioxidant Power, TRAP), metal reducing power (Ferric Reducing Antioxidant Power, FRAP; Cupric Reducing Antioxidant Power, CUPRAC), hydroxyl radical scavenging (deoxyribose assay), organic radical scavenging (2, 2-Azino-bis (3-ethylbenz-thiazoline- 6-sulfonic acid, ABTS; 2, 2-Diphenyl-1-

Picrylhydrazyl, DPPH), quantification of products formed during the lipid peroxidation (Thiobarbituric Acid Reactive Substances, TBARS; Low-density Lipoproteins, LDLs oxidation), etc. [52]. Of all these methods, ABTS, FRAP, DPPH and ORAC are some of the most widely used [53]. In this thesis, the antioxidant activity was assessed using Ferric Reducing Antioxidant Power (FRAP) assay.

2.6 Organic acids, fatty acids, oils and aromatic volatiles

The compounds responsible for providing sour taste in foods and beverages are organic acids. Major organic acids in *H.sabdariffa*. L., include citric acid and malic acid in addition to ascorbic acid, tartaric acid and succinic acid [6, 54]. More than 25 volatile hydrocarbons, alcohols and aldehydes have been detected in seed oil of *H.sabdariffa*. L., variety of sterols has also been detected in the seed oil such as cholesterol, campesterol, stigmasterol, -sitosterol, α-spinalsterol and ergoterol [55] while β- sitosterol, campesterol, delta-5-avenasterol, cholesterol and clerosterol were detected by [56].

Different aromatic volatile constituents have been identified in roselle tea and major components in fresh samples were 3-hexenol, 2-hexenol and 1-hexanol and also α-terpineol and eugenol [57]. Fatty acid esters have also been identified in the pressed seed oil of *H. sabdariffa*. The derivatives containing cyclopropene moieties or opoxide functionality; malvalic acid, sterculic acid and epoxy oleic acid [58]. However roselle oil is not removable by hydrogenation hence present problem in the roselle oil processing.

2.7 Nutrients in *H. sabdariffa*

H. sabdariffa is a plant consumed worldwide as a nutritious source of vitamins, minerals, organic acid and minerals. The roselle extract is an excellent source of vitamin C, calcium and phosphorus containing 60%, 80% and 39% of each respective nutrient compared to the content found in orange [59]. A study by Nnam and Onyeke [60] on evaluation of vitamin and mineral composition of the red and yellow calyces showed that roselle was a good source of calcium, iron, phosphorus, zinc, β- carotene, thiamine, riboflavin and vitamin C. The seed contains phosphorus, calcium, zinc, manganese magnesium, copper, riboflavin in addition to 18 amino acids [61].

2.8 Medicinal uses and biological studies

Hibiscus sabdariffa extract has been extensively studied to elucidate and verify the medicinal activities due to its history in traditional medicines. It has shown a wide range of pharmacological properties. Anti-inflammatory activity has been shown in HSE, mediated by inhibition of cyclooxygenase enzymes 1 and 2. The extract showed higher inhibition of cyclooxygenase-1 (COX-1) than cyclooxygenase-2 (COX-2), indicating its potential use as blood thinner as well [62]. Anthocyanins derived from HSE have been screened against certain human cancer cell lines (Chang et al., 2005). The study shown to induce apoptosis in human promyelocytic leukemia cells, thought to be mediated by the p38-FasL and Bid pathway [63]. Aqueous hibiscus extract has also been shown to reduce the levels of LDL and the ratio of LDL to HDL in rats [64]. Antihypertensive activity of the hibiscus extract in pre-hypertensive and mild-hypertensive adults has been confirmed by MacKay et al., [65] whose results suggested the possibility of using hibiscus tea as a dietary supplement to prevent and control hypertension in adults.

2.9 Other roselle uses

Roselle is a multi-use plant, whose young shoots, leaves and calyces are used as a cooked vegetable or cut and used as vegetable sauce [66]. They can also be eaten raw in the salads, the red freshly calyx lobes are chopped and used in fruit salads. The dried red calyces have been used to prepare tea, syrup, jam and jellies [59]. The calyces can also be harvested as fodder for livestock while roselle seed oil is used in soap and cosmetic industries. The seeds can also be pounded into meal, added to cereals or roasted and boiled as a coffee replacement in some parts of Africa [16]. The seeds are also eaten roasted as snacks or ground into meal to make cakes. Roselle is also an attractive garden plant. The cut flowers and also the decorative red stalks with ripe red fruits have been exported to Europe [67]. The bust fibres and sometimes the whole stem can be used in the paper industry in the USA and Asia [17]. Figure 6 is an example of tea bags and dried roselle calyces in sacks used to make tea, which can be drunk hot or cold

Figure 6 Roselle tea bags and dried roselle calyces in sacks [17].

2.10 Exotic fruits

Tropical and subtropical fruits, known as “exotic” fruits, includes a number of tropical fruits that are not yet commonly found in global markets but have the potential to do so in view of their appearance, taste, and textural and nutritional quality parameters [68]. The exotic fruits includes mango, guava, passion fruit, rose apple, papaya, lime, passiflora, pineapple, carambola, sapodilla, mamey, lychee and longan, and are common ingredients which are frequently used in varieties of juices, purees and many fruit based deserts [69].

Countries in the tropics produce a large amount of fruit species which could be interesting for the food industry. Exotic fruits consumed regionally, are gaining popularity in the market due to their nutritional and therapeutic value, but also because of their pleasant flavors and variety of color [70-72]. The nutritional and therapeutic value is mainly due to the presence of bioactive compounds, secondary metabolites, which have potential effects on human health [71]. In this study, mango, papaya and guava were used as exotic fruits.

Mango (*Mangifera indica* L.) is a tropical fruit that originated from Southeast Asia and has been cultivated for at least 4000 years. Worldwide mango production has increased about 9% from 35.5 million metric tons in 2008 to 38.7 million metric tons in 2010, highlighting the economic importance of mango in the international commodity market [73]. The mango fruit are excellent source of fiber, vitamins A, C and the B complex, iron and phosphorus [74].

Carica papaya belongs to the small family Caricaceae and is one of the major fruit crops cultivated in tropical and subtropical zones. Worldwide, in an area of 438 588 Ha, over 11.2 million tons of fruits were produced in 2010 [73]. Papaya fruits are rich in vitamin A, C and iron [75]. Guava (*Psidium guajava* L.) is one the most cultivated fruit crop in many tropical and subtropical countries. It is rich in vitamin C and A, dietary fiber, pectin, sugars, folic acid, potassium, manganese, and copper. Guavas are processed and preserved as jam, pulp, puree, squash, nectar and juices [76].

2.11 Fruit juice

Fruit juice is a drink consisting of 100% pure fruit juice, which typically contains no preservatives or other added ingredients [77] Fruit juices can be classified as: Freshly squeezed, concentrate, juice drinks, nectars, smoothies and fruit juices with added ingredients.

Freshly squeezed juices are produced by 'squeezing' the juice from the fruit of choice, which is then packaged and transported to the retailer usually within 24 hours. These juices are not pasteurization and often have a very short shelf life (2–3 days) [77]. Juices 'from concentrate', are produced by 'squeezing' the juice from the fruit, then concentrated (by evaporating the water naturally present in the juice) [77]. During production, fruit juices from concentrate are typically heat-treated, to ensure that any unwanted spoilage pathogens, including bacteria or moulds, are destroyed [78].

Juice drinks are products which contain anything less than 100% pure fruit juice. The level of fruit juice contained in these drinks can be found in the ingredients panel, usually on the back of the pack. These drinks may include those that are purchased in a ready-to-drink format, or those that are purchased as 'cordial', also known as dilutable drinks [78]. 'Functional' fruit juice products ('functional foods being those that encompass potentially healthful products that may provide a health benefit beyond that provided by the traditional nutrients it contains') with added ingredients [78].

2.12 Materials used for fruit juice packaging

Package design and construction play a significant role in determining the shelf life of a food product. The right selection of packaging materials and technologies maintains product quality and freshness during distribution and storage. Materials that have traditionally been used in food packaging include glass, metals (tinplate, and tin-free steel), paper and paperboards, and plastics.

Plastic materials are the most utilized material used for food preserving/storage. The main plastics used are: polypropylene, polystyrene, polyvinyl chloride, polyethylene terephthalate, high density polyethylene and low density polyethylene. Polyethylene has low permeability to water, excellent electrical insulation, resistance to acids, alkalis and organic solvents [79].

Glass bottles provide excellent protection due to perfect gas and aroma barriers. Insufficient tightness around the metal closure is a potential source of oxygen ingress, but can be minimised by various liner solutions. Visible light and part of the ultraviolet light spectra penetrate through clear glass, and may affect photosensitive compounds, such as certain vitamins. Addition of UV absorbers to the glass will protect the bottle content against ultraviolet rays [80] .

2.13 Chemical preservatives

The chemical preservatives are used to prevent food spoilage due to microbial attack and thus are effectively used in combinations for better preservation [81]. No single preservative is completely effective against all microorganisms [82]. The most commonly used preservatives are benzoic acid, sodium benzoate, potassium metabisulphite, sorbic acid and sulphur dioxide. Acid is an essential universal constituent of juice and the most commonly used acid is citric acid. Sodium benzoate (SB), potassium sorbate and potassium metabisulphite (PMS) are commonly used as preservatives for long term storage of fruit pulp because of their better antimicrobial activity [81, 83]. The chemical preservative used in this study was sodium benzoate.

2.14. Conducting of experiment

This study was conducted in two consecutive years (2011 and 2012). Objectives one, two and five were done in 2012 while objectives three and four were done in 2012. The fruits (mango, papaya and guava) were bought from horticulture garden at Sokoine University of Agriculture and dried roselle calyces were bought from Morogoro Municipality. 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 (ie 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 plastic bottles or glass bottle, loosely capped and pasteurized in a water bath at a temperature of 82.5 ^{o}C for 20 min and cooled rapidly to room temperature by immersing the bottles in water bath (28 ^{o}C). Samples were drawn for initial chemical analyses and thereafter in one (Paper I) and two months (Paper II, III and IV) intervals.

2.14.1 Chemical analysis

The chemical analyses employed were physiochemical properties (pH, total soluble solids, titratable acidity) and mineral analysis (Calcium, magnesium, phosphorus, iron and zinc) using AOAC methods [84]. Reducing sugars were analysed using Luff-Schoorl [85], vitamin C content by the Folin-Ciocalteu reagent [86], total monomeric anthocyanin with the pH differential method [87], total phenolic content by Folin-Ciocalteu reagent (FCR) method [88], antioxidant activity analysed with the FRAP (Ferric Reducing Antioxidant Power) assay [89]. The concentration of some organic acids (e.g. citric, succinic, tartaric and malic) and carbohydrates (e.g. glucose and fructose) in roselle-fruit blends was analyzed by High Performance Liquid Chromatography (HPLC) as described by Castellari *et al* [90]. Sensory analysis was carried out according to a 9-point Hedonic scale where 9 was "like extremely" and 1 was "dislike extremely. Detailed information on these methods are given in the section of papers, however the principal involved in determination of antioxidant properties are explained as follows:

2.14.1.1 Total monomeric anthocyanin

The pH differential method is a simple, rapid, and economical means for determining the amount of anthocyanins in a sample, and this method has been verified by AOAC's strict validation guidelines. It is a good alternative for laboratories that do not have access to a HPLC.

The pH differential method for the determination of total monomeric anthocyanin content is a spectrophotometric method based on the structural change of the anthocyanin chromophore between pH 1.0 and 4.5 (Figure 7). The method is used in research and for quality control of anthocyanin-containing fruit juices, wines, natural colorants, and other beverages.

Monomeric anthocyanins undergo a reversible structural transformation as a function of pH (colored oxonium form at pH 1.0 and colorless hemiketal form at pH 4.5; Figure 7). Thus, the difference in absorbance at the $\lambda_{vis\text{-}max}$ (ca 520 nm) of the pigment is proportional to the concentration of pigment [87].

Absorbance should be measured at the $\lambda_{vis\text{-}max}$ of the pigment solution, and the pigment content should be calculated by using the molecular weight (MW) and molar extinction coefficient of the major anthocyanin in the matrix. For example, the anthocyanin content of wines is customarily calculated as the content of malvidin-3-glucoside (MW = 493.2) by using a molar extinction coefficient of 26900.

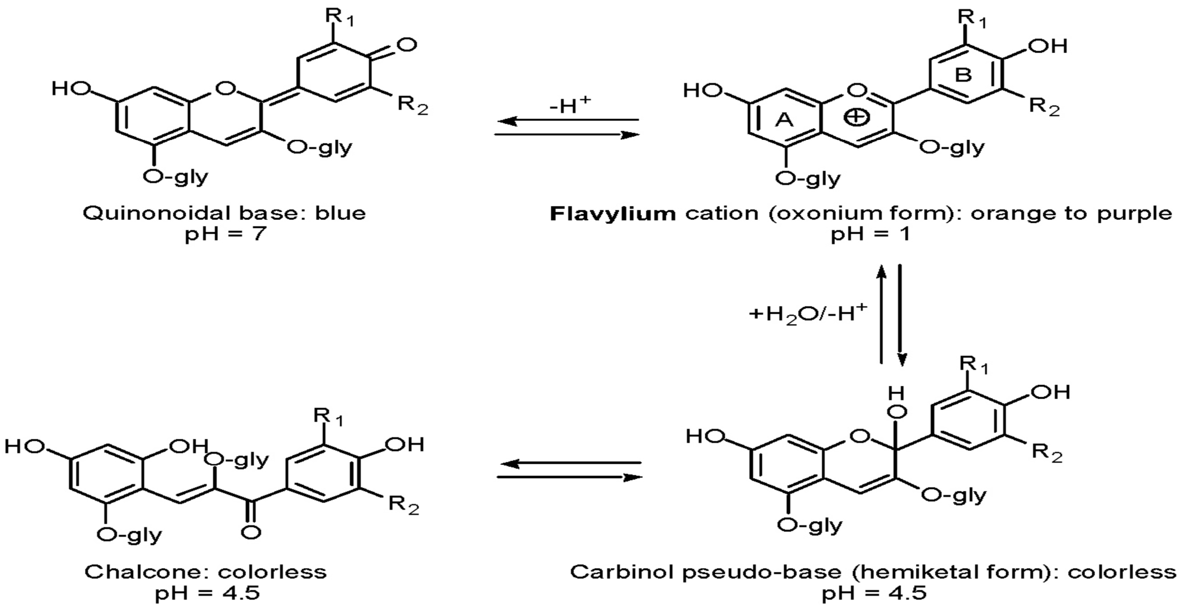


Figure 7 Predominant anthocyanin structural forms present at different pH levels [87].

2.14.1.2 Total Phenolic compounds

Folin-Ciocalteu Reagent **(**FCR) was initially intended for the analysis of proteins taking advantage of the reagent's activity toward protein tyrosine (containing a phenol group) residue [91]. Many years later, Singleton and co-workers extended this assay to the analysis of total phenols in wine; since then the assay has found many applications [88]. The reagent consists of a mixture of sodium molybdate, sodium tungstate, and other reagents. Upon reaction with phenols, it produces a blue color, which absorbs at 765 nm.

The Folin-Ciocalteu reaction has its basis in oxidation/reduction chemistry. First, sample is added to the FCR and the phenolate ions are mixed with oxidizing agents, which change from yellow to blue once reduced. The mixture of oxidizing agents and sample is then added to an alkaline solution. Under alkaline conditions, phenolics ionize completely to their phenolate form and can be readily oxidized by the FCR. The oxidized phenolate changes to the quinoid structure, while the oxidizing agents gain an electron going from a 6+ to a 5+ oxidation state. The color change is monitored with a spectrophotometer and converted into a concentration using a standard.

The sample must be first mixed with the FCR and then the base because FCR is unstable under alkaline condition. The reaction takes about two hours for completion at room temperature. The temperature can be raised to speed up the reaction; however, there is a slight loss in sensitivity [92]. A sample blank without FCR is necessary because of significant background interference [88].

2.14.1.3 Antioxidant activity

The FRAP assay is commonly used in routine analysis for evaluation of antioxidant activity because it is simple, rapid, sensitive and inexpensive. The reducing activity of a compound might serve as a significant indicator of its potential antioxidant activity.From a mechanistic standpoint, FRAP is an electron transfer (ET)-based assay like Folin, ABTS/TEAC, and CUPRAC in the sense that the oxidant probe accepts an electron from the antioxidant analyte to be converted into the reduced probe which is coloured. FRAP assay depends upon the reduction of ferric tripyridyltriazine (Fe (III)–TPTZ) complex to ferrous tripyridyltriazine (Fe(II)– TPTZ) (Figure 8) with an intensive blue colour by a reductant at low pH [89]. Reductants polyphenols and anthocyanins have strong electron-donating capacity, which can induce the formation of a blue coloured Fe (II)–TPTZ from the colourless oxidized Fe (III) form [89]. The increase in absorbance at 593 nm (ΔA) due to Fe (II)–TPTZ complex formation is proportional to the combined (total) ferric-reducing antioxidant power (FRAP) of the antioxidants in the sample [93].

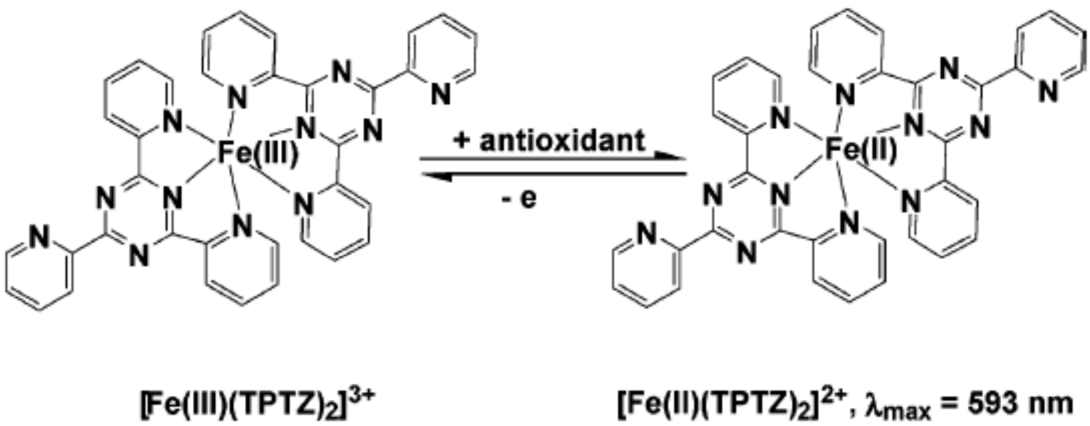


Figure 8 Electronic transfer reaction in FRAP assay [94].

2.14.2 Statistical analysis

Analysis of variance (ANOVA) was performed using Minitab Statistical Software (Version 16.0, 2008, Minitab Statistical Software, Minitab Inc., Enterprise Drive State College, PA, USA). The main factor in the model were type of blends (Paper I and V), storage time and temperature (Paper II and III) and packaging material, storage time and temperature (Paper IV) with three parallels and two replicates.

Physicochemical composition and antioxidant properties for roselle fruit juice blends (Paper II-IV) were analysed by general linear model (GLM) using Minitab Statistical Software (Version 16.0, 2008, Minitab Statistical Software, Minitab Inc., Enterprise Drive State College, PA, USA). The treatments were separated using Tukeys test. From the results obtained from different parameters measured, all the roselle-fruit juice blends (20%, 40%, 60%, and 80% roselle) showed similar trends. Therefore 40% roselle blend was chosen to represent the blends in statistical analysis using ANOVA and PCA.

Principle component analysis (PCA) is a method used to study a data set with a large number of interrelated variables [95]. The main idea of PCA is to reduce a dimensionality of data set (matrix) by transforming it into new set of variables, the principle components (PCs). The first PC (PC1) explains the main variation of the data, while the second PC (PC2) shows the second largest variation. PCA was therefore used in Paper II-IV in order to study the effects of storage time and temperature on the roselle-fruit juices blends (Paper II and III). Effects of packaging material, storage time and temperature on roselle-mango juice blends and effect of seasonality (Paper IV)

3.0 OBJECTIVES OF THE STUDY

3.1 Main objective

The main objective of this study was to utilize the dried Roselle calyces extract to increase the nutritional value and antioxidant properties of guava, mango and papaya juice.

3.2 Specific objectives

- Formulation of different combinations of Roselle extract and fruit juices and determine physiochemical, antioxidant properties and mineral composition (Paper 1).
- To evaluate effects of storage time and temperature on physiochemical and antioxidant properties of roselle–fruit juice blends packed in plastic bottles (Paper 11) and glass bottles (Paper 111)
- To evaluate effects of packaging materials, storage time and temperature on physiochemical and antioxidant properties roselle-mango juice blends (Paper IV)
- Organoleptic assessment of the formulated Roselle blends drinks so as to determine the most acceptable blend (V).
- To determine organic acid and sugar content of roselle blends (V).

4.0 MAIN RESULTS AND GENERAL DISCUSSION

4.1 Physiochemical, antioxidant properties and mineral composition

4.1.1 Physiochemical properties

4.1.1.1 pH and titratable acidity

The pH increased significantly ($p<0.05$) with the decreased in concentration of roselle extract in the blends (Paper I)**.** The addition of fruit juices in roselle extract increased pH is due to high level of pH of the blends. The titratable acidity of the blends ranged from 1.28 to 1.92. The acidity in roselle-fruit juice blends decreased with the decreased concentration of roselle extract in the blends as roselle extract is known to be acidic.

4.1.1.2 Total soluble solids and reducing sugars

Total soluble solids and reducing sugars increase with increased amount of fruit juices in the blends. Tropical fruit juices are good source of sugars and roselle extract has low sugar content [6]. The increase in TSS might be due to increase in total sugars by inversion in the presence of organic acids from polysaccharides like starch and cellulose into simple sugars in course of time [96].

Total soluble solids and reducing sugars in the roselle-fruit juice blends ranges from 5.6-10.6°brix and 2.95-5.55 mg/100g respectively. Both total soluble solids and reducing sugars increased significantly ($p<0.05$) with decreased concentration of roselle extract in the blends (Paper I). Roselle extract is low in sugar [6] while the fruit juices are known to be high in sugar content so the increased amount of sugar in the blends increased the sugar content in the blends.

4.1.1.3 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. The lightness (L*) ranges from 19.6 to 14.7, the redness (a*) ranged from 20.0 to 15.4 while yellowness (b*) ranges from 8.5 to 4.5 (Paper I). The red colour in the roselle-fruit juice blends was due to the anthocyanin content of the roselle extract. The higher redness (a*) values for the blends with high levels of roselle extract were due to the lower pH (Paper I), which favoured the coloured flavylium form of anthocyanins [24]. The pink guava used in roselle-guava blends contributes to red colour due to carotenoid pigment lycopene [97]. Tropical fruit juices such as mango, papaya and guava are good source of carotenoids [71] which is responsible for yellowness in these fruits. The increase in yellowness (b*) with increased concentration of fruit juice is due to presence of carotenoids in these fruits.

4.1.2 Mineral composition

Mineral composition (calcium, magnesium, phosphorus, iron, sodium, zinc) increased significantly ($p<0.05$) with increased concentration of roselle extracts in all the roselle blends (with exception of sodium). According to the dietary reference intakes (DRI), the daily adequate intake of magnesium (mg) for an adult is 320 mg for female and 420 mg for male [98, 99]. Therefore, 100 g of roselle fruit juice blends in this study would supply 3.6-68% (female) and 2.7-51% (male) of the mg requirement for the average adult.

The DRI for iron (Fe) is 320 mg for female and 400–420 mg for male adult [98, 99]. Therefore, roselle-fruit juice blends (100 g) would supply (7.5-164%) for female and (17-369%) for male while the DRI for calcium (Ca) is 1000mg for adults per day, roselle fruit-juice blends would supply (2.3-55%) for adults.

4.1.3 Antioxidant properties

4.1.3.1 Vitamin C and total monomeric anthocyanins

Roselle-fruit juices blends (20% roselle to 80% roselle) were between 40.0-61.2 mg/100g. Vitamin C content in all roselle fruit blends increase significantly ($p<0.05$) with increased concentration (Paper I). Tropical fruit juices such as mango, papaya and guava are good source of vitamin C [71]. The increased amount of vitamin C content in the blends might be due to high content of vitamin C in fruit juices. Total monomeric anthocyanin (TMA) in roselle fruit juices were between 493.5-118.2 mg/100g. TMA increased significantly ($p<0.05$) with increased concentration of roselle extract in the all the roselle-fruit juice blends, this is due to contains high amounts of anthocyanins in roselle extract [6, 100].

4.1.3.2 Total Phenol content and antioxidant activity

Antioxidant activity measured by Ferric Reducing Antioxidant Power (FRAP) and total phenolic content (TPC) ranged between 1.80-1.37 mmol/L and 53.7-10.8 GAE mg/100g respectively The FRAP value and TPC increased significant with increased concentration of roselle extract in the all the roselle-fruit juice blends**.** Phenolic antioxidants in foods include flavonoids, catechins, chalcones, hydroxybenzoic and hydroxycinnamic acids and many of which are present in fruit juices. The tropical fruit juices used in this study contained phenolic compounds, vitamin C and carotenoids which might have contributed to the antioxidant activity of the blends. However, the blends containing higher concentration of roselle extract have greatest antioxidant activity, this shows that roselle extract containing high anthocyanin content have greater contribution to the antioxidant activity of the roselle-fruit juice blends.

4.2 Effect of storage time and temperature

4.2.1 Physiochemical properties

Retention or minimum increase in total soluble solids content of juice during storage is desirable for the preservation of good juice quality. Total soluble solids (TSS) and reducing sugar increased with increase storage period regardless of the storage temperature (Paper II and III). The increase in TSS might be due to increase in total sugars by inversion in the presence of organic acids from polysaccharides like starch and cellulose into simple sugars in course of time [96]. pH increase as the storage progressed while titratable acidity decreased significantly with increased storage period. Acidity in juice is important because it determines proper acid: sugar ratio of blend juices. Therefore, maintenance of juice acidity is significance during storage Decreased acidity might be due to acidic hydrolysis of polysaccharides were acid is utilized for converting non reducing sugars into reducing sugars [101].

Colour is one of the most important parameters to which consumer are sensitive when selecting foods. In this study Lightness values (L***),** redness (a*) and yellowness (b*) values of the roselle-fruit blends decreased significantly ($P < 0.05$) with increased storage time (Paper II and III) with lower values measured at higher temperatures**.** These decrease of a* and L* values can be attributed to the degradation or polymerization of anthocyanins [102]. Martí *et al* [103] also reported a significant decrease in L value during storage period of 150 days at 25 °C, resulting in darker colour during the storage period of pomegranate juice.

4.2.2 Antioxidant properties

There was a significant ($p<0.05$) decrease in vitamin C, total monomeric anthocyanin, total phenolic content and antioxidant activity regardless of the storage temperature (Paper II and III). Vitamin C content in roselle fruit juice blends (40% roselle) decreased by 55-58%

(28°C) and 41-43% (4°C) (Paper II) and 60-62% (28°C) and 30-34% (4°C) (Paper III) roselle-fruit juice blends respectively after 6 months of storage. The decrease in the vitamin C content was probably due to the fact that ascorbic acid being sensitive to oxygen, light and heat can easily be oxidized in presence of oxygen by both enzymatic and non-enzymatic catalyst [104, 105]. Also presence of anthocyanins in the blends might have caused mutual degradation of both compounds through oxidation as well as to the direct condensation reaction of the ascorbic acid (AA) on carbon 4 of the anthocyanin [32, 38, 106, 107].

The interaction between fruit juice, time and temperature of storage on total monomeric anthocyanins during the storage of roselle-fruit juice blends (40% roselle) were significant ($P < 0.05$). Total anthocyanins decreased by 86-65% (28°C) and 75-53% (4°C) (Paper II) and 71-74% (28°C) and 41-44% (4°C) (Paper III) roselle-fruit juice blends respectively after 6 months of storage and losses were higher at 4°C. The decrease in anthocyanin concentration in roselle-fruit juice blends may be the result of anthocyanin polymerization [40], non-enzymatic activity or condensation reactions of anthocyanins with ascorbic acid or other phenolics [38, 40]. Oxygen can either directly react with anthocyanins or oxidize other compounds that eventually react with anthocyanins to give colorless or brown products [108, 109]

Total phenolic content decreased by 66-58% (28°C) and 51-22 (% (4°C) (Paper II) and 55-51% (28°C) and 28-25% (4°C) (Paper III) roselle-fruit juice blends respectively after 6 months of storage.

The decrease in TPC and antioxidant capacity content during storage was principally attributed to condensation with ascorbic acid, to hydrolysis reactions and to non-enzymatic browning [110, 111]. Anthocyanins stability was studied by several authors, who observed that monomeric anthocyanins diminished considerably during storage. However, condensation compounds or the resulting polymers contributed to the overall phenolic content and antioxidant capacity, as a consequence of which, less losses of these parameters during storage [110].

4.3 Effect of packaging, storage time and temperature

4.3.1 Physiochemical properties

The pH, total soluble solids, reducing sugar in the roselle-fruit juice blends increased during storage regardless of the packaging material used and storage temperature, however the increase was not significant**.** The pH of fruit juice plays an important role in the preparation of beverages. The pH of juice increased during storage of juice. The increase in pH of juice could be attributed to decrease in acidity of juice during storage. The decrease in titratable acidity in juice might be due to the chemical reaction between organic constituents, which increased upon prolonged storage and temperature. It is a measure of the acidity, which not only influences the flavor or palatability of a product but also the shelf life. Changes in TSS and RS content were natural phenomenon that occurs during storage and it is correlated with hydrolytic changes in carbohydrates during storage [101].

There was reduction in the values of the L* parameter (darkening), a* parameter (loss of red colour) and the b* parameter (loss of yellow colour) for the roselle-mango juice during storage. The colour changes were more pronounces in the sample stored at the high temperature and those stored in plastic bottles. It was possible to see colour changes with human eye, the blends stored in glass and plastic bottles at 28 °C (Appendix 1 b & d) showed clear changes on the colour of the blends however, the blends stored in plastic bottles showed remarkable colour changes (Appendix 1 c & d) at 28 °C and 4 °C.

4.3.2 Antioxidant properties

Vitamin C and anthocyanin content was found to decrease with increase in storage time, regardless of packaging material and storage temperature. (Paper IV). These results indicate that ascorbic acid and anthocyanin loss is greater in roselle-mango juice blends stored in plastic bottles than those in glass bottles and also losses were higher at 28 °C compared to 4 °C storage temperature. Anthocyanins and vitamin C are reported to be heat-labile compounds and are unstable at high temperature during processing or storage [112]. Despite the vitamin C losses in roselle-fruit juice blends (40% roselle), its content at the end of the storage ranged from 26.2-31.3 mg per 100 mL, i.e. only 100 mL of the blend provide 58-70% the recommended daily allowance (RDA) of vitamin C for adults, which is 45 mg (FAO/WHO, 2001). Also at the end of storage the loss in anthocyanin ranges between 100-127 mg/100g while recommended daily intake of anthocyanin is estimated to be 82 mg and 12.5 mg per day per person in Finland and United States [113].

4.2.3 Seasonality effects

Effect of seasonal variation, storage time and temperature was well described by a bi-plot of observations and variables (Appendix 2) 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 (colour L*, a*, b* RS, FRAP and TMA) and the second components account for 17% of the total variation associated with parameters (TSS, Vitamin C and TPC). The PC1 explained roselle-mango juice blends stored in plastic bottles at ambient temperature with more blends from season 2011 while PC2 explained blends stored at refrigerated temperature with more blends from season 2012.

The results shows that most of blends 2012 blends had high levels of TPC, TSS and vitamin C (Appendix 2). Regardless of season or storage temperature , results on the bi-plot showed the effects of storage time on the blends as storage was progressing with storage at four and six months being on the negative side of the PCs. The Bi plots also showed the TPC, TMA and vitamin C being parameters mostly affected by the storage time regardless of storage temperature.

4.4 Organic acid, sugar content and sensory evaluation

4.4.1 Organic acid

The quantity of organic acid in roselle-fruit juice blends (100-0% roselle) were succinic acid (21.7-0.7) $\times 10^2$mg/kg, citric acid (1.9-4.0)$\times 10^2$mg/kg, tartaric acid (0.19-0) $\times 10^2$mg/kg, malic acid (0.76-0.14) $\times 10^2$mg/kg (Paper V). The results showed that as the concentration of roselle extract decreased in the blends, the quantity of organic acids (with exception for citric acid) decreased significantly ($P < 0.05$).The major organic acids in fruits are malic and citric acid and their organic acid composition varies and depends on the fruit type, ripening, environmental conditions, and cultural practices.

4.4.2 Sugar content

The quantity of sugar in roselle-fruit juice blends (100-0% roselle) were glucose (8.15-1.88) g/kg and fructose (7.04-2.04) g/kg (Paper V). The results showed that as the concentration of roselle extract decreased in the blends, the quantity of sugar increased significantly ($P<0.05$). Roselle extract is low in sugar content [6, 59] while tropical fruit juices have high sugar content.

4.4.3 Sensory evaluation

Sensory characteristics of any food product contribute significantly to its consumer acceptance or rejection. Appearance, flavour and colour are the most important attributes determining consumer's choices of food products. The sensory attributes of the roselle-fruit blends are showed that all roselle-fruit blends (20% roselle) scored almost highest in all the organoleptic properties (colour, flavor, taste, appearance, odour consistency (mouthfeel) and acceptability). The increase in levels of roselle extract in all the roselle-fruit blends (60%

and 80% roselle) resulted in decreased sensory score which might be due to increased acidity in the blends.

Colour was the attribute that panelists rated higher in all roselle fruit juice blends, however roselle fruit juice blends with 80% roselle were rated highest. The decreased concentration of roselle extracts in the blends lowered colour scores in all the roselle fruit blends. This is an indication that the red colour of roselle extract was very attractive to panelists. Roselle extract is known to be a good source of anthocyanins [6, 54, 100] which imparts the red colour to the blends. Colour is known to play a major role in the acceptability of zobo (roselle) beverage by consumers [59].

5.0 CONCLUDING REMARKS AND FUTURE PERSPECTIVES

5.1 Conclusions

Roselle extract can be mixed with other tropical fruit juices and still produce a roselle-like juice. The percentage of roselle extract in the blend and the type of blended juice greatly affect the final product.

Results indicated that low temperature storage (4 °C) is ideal for storage of roselle-fruit juice blends as loss of vitamin C and anthocyanin is significantly lower compared to higher temperature (28 °C).

Total phenols and total antioxidant activity of roselle-fruit juice blends were relatively lower at higher temperatures. Thus, the effects of storage conditions on the antioxidant properties of roselle-fruit juice blends should be considered prior to selection of storage conditions

Packaging in glass bottles and storage at refrigerated temperature should be encouraged if good long-term preservation of anthocyanins and vitamin C is desired.

The addition of tropical fruit juice with high sugar content in roselle extract can reduce the sourness of the blend. However, the choice of fruit to add to the blend should depend on the availability of fruits.

5.2 Future Perspectives

1. Investigation of stability of the volatile profile and sensory characteristics (aroma and flavour) of roselle-fruit juice blends during storage
2. Determination of browning index and polymeric colour of roselle-fruit juice blends during storage.
3. Investigation of microbiological and sensory qualities of roselle-fruit juice blends during different storage periods.

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7.0 APPENDICES

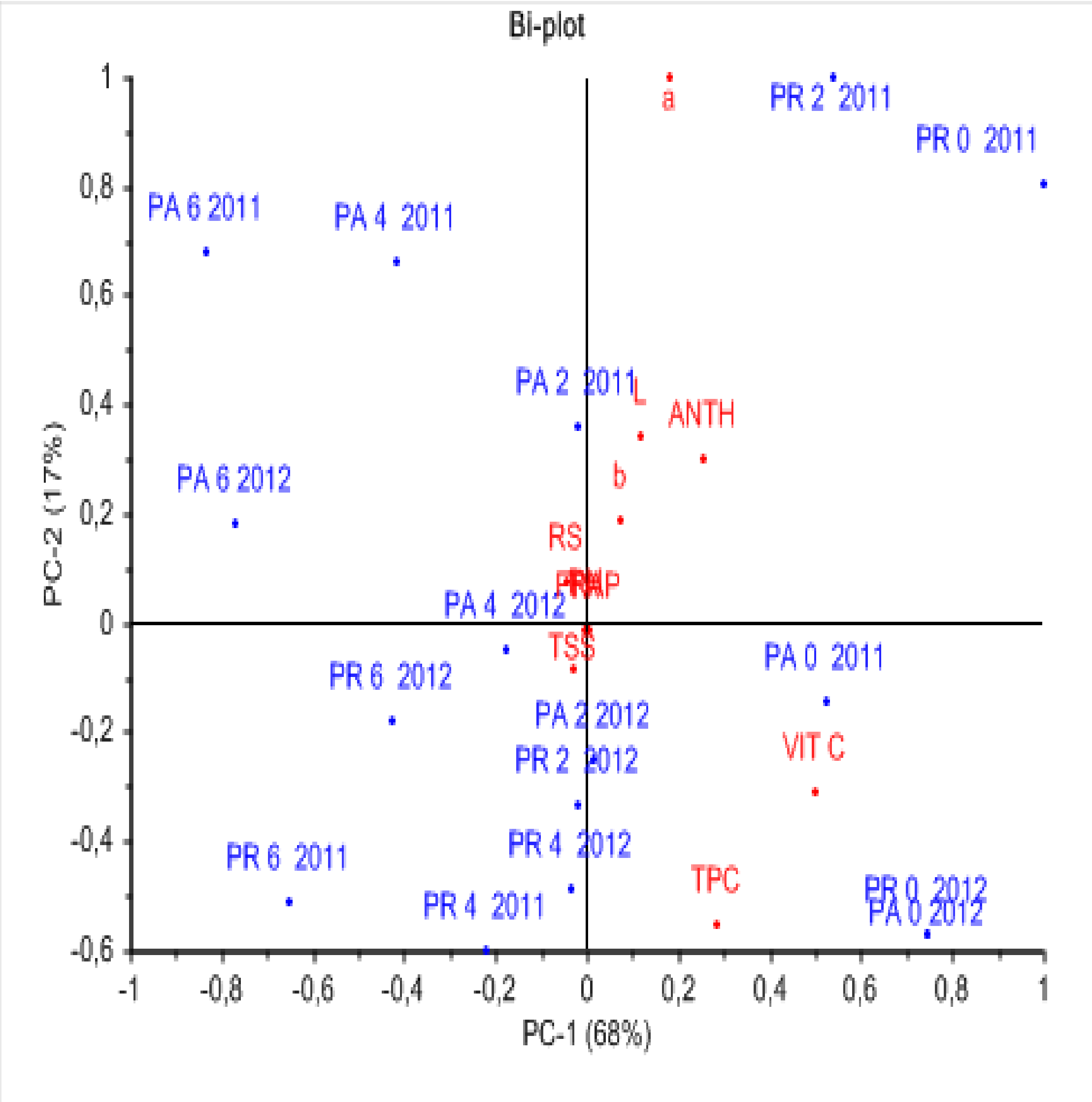
Appendix 1: Colour change for roselle-mango juice blends stored in glass bottle at 28°C (a) and 4°C (b)

(a) (b)

Appendix 2: Colour change for roselle-mango juice blends stored in plastic bottle at 28°C (c) and 4°C (d)

(c) (d)

Appendix 2 Bi-plot of scores and loadings of the PC on roselle-mango juice blends (40% roselle) stored at ambient and refrigerated temperature for six months (season 2011 and 2012)



PA Plastic ambient temperature, PR Plastic refrigerated temperature, 2011,2012 seasons, 0,2,4,6 storage time (months), TSS Total soluble solids, TA Titratable acidity, RS Reducing sugars, L Lightness, a Redness, b Yellowness, VIT C , Vitamin C, TMA Total monomeric anthocyanins, TPC Total phenolic content, FRAP Ferric reducing ability power.

8.0 ENCLOSED PAPERS I-V

Paper I

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

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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

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 a 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^{o}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^{o}C for 20 min and cooled rapidly to room temperature by immersing the bottles in water bath (28^{o}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 ºBrix.

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 2º 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.

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 H_2O: 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).

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).

DISCUSSIONS

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 acts 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 ^{o}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.

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.

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 ($p<0.05$).

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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Paper II

Food Science & Nutrition

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ORIGINAL RESEARCH

Influence of storage temperature and time on the physicochemical and bioactive properties of roselle-fruit juice blends in plastic bottle

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Abstract

Roselle-fruit juice blends were made from roselle extract and mango, papaya, and guava juices at the ratio of 80:20, 60:40, 40:60, and 20:80, % roselle: fruit juice, respectively. The blends were pasteurized at 82.5°C for 20 min and stored in 100 mL plastic bottles at 28 and 4°C for 6 months. The effects of storage time and temperature on physicochemical and bioactive properties were evaluated. Total soluble solids, pH, and reducing sugars increased significantly ($P < 0.05$) in some blends while titratable acidity decrease with increasing storage time. Vitamin C, total monomeric anthocyanins (TMA), total phenols (TPC), and antioxidant activity (ferric reducing ability of plasma, FRAP) in all roselle-fruit blends (40% roselle) decreased significantly ($P < 0.05$) at 28 and 4°C as storage progressed. Vitamin C in all roselle-fruit blends (40% roselle) decreased from 58–55% to 43–42% when stored at 28 and 4°C, respectively. TMA losses were 86–65% at 28°C and 75–53% at 4°C while TPC losses were 66–58% at 28°C and 51–22% at 4°C. Loss of antioxidant capacity (FRAP) was 18–46% at 28°C and 17–35% at 4°C. A principal component analysis (PCA) differentiated roselle- juice fruit blends into two clusters with two principle components PC1 and PC2, which explained 97 and 3% (blends stored at ambient temperature) and 96 and 4% (blends stored at refrigerated temperature) of the variation, respectively. PC1 differentiated roselle-guava juice blends which were characterized by vitamin C, TPC, FRAP, and pH, while PC2 from another cluster of roselle-mango and roselle-papaya juice blends and was characterized by TSS, RS, and color parameters (L^* a^* b^*). However, TMA was the main variable with the highest effect on all roselle-fruit juice blends regardless of the storage time and temperature.

Introduction

Hibiscus sabdariffa L. (family *Malvaceae*), commonly known as roselle, red sorrel, or karkadè, is widely grown in Africa, South-East Asia, and some tropical countries of America (Abou-Arab et al. 2001; Sagayo-Ayerdi et al. 2007; Amor and Allaf 2009; Cisse et al. 2011). Roselle produces red edible calyces with a unique flavor and brilliant red color. The calyces are commonly used to make jelly, juice, jam, wine, syrup, pudding, cake, ice cream, and flavour (Tsai et al. 2002; Tsai and Huang 2004; Duangmal et al. 2008). The beverages produced by roselle calyces are called hibiscus tea, bissap, roselle, red sorrel, agua de Jamaica, Lo-Shen, Sudan tea, or karkadè (McKay et al. 2010).

The calyx contains two main anthocyanins, delphinidin-3-sambubioside, also known as delphinidin-3-xylosylglucoside or hibiscin, and cyanidin-3-sambubioside, also known as cyanidin-3-xylosylglucoside or gossypicyanin, and two minor anthocyanins, delphinidin-3-glucoside and cyanidin-3-glucoside (Wong et al. 2002; Amor and Allaf 2009; Cisse et al. 2011). Roselle anthocyanins can contribute to health benefits as a good source of antioxidants as well as a natural food color (Tsai et al. 2002; Duangmal et al. 2008). They are derivatives of the basic flavylium cation structure with an electron-deficient nucleus, which makes them highly reactive and their reaction involves discolorization of the anthocyanin pigments (Chumsri et al. 2008). Factors like light, pH, temperature, oxygen, ascorbic acid, and sugar are contributing factors in degradation or stability of anthocyanins (Fennema 1996; Tsai and Huang 2004; Chumsri et al. 2008; Cisse et al. 2011).

Most people do not prefer beverages made from roselle extract as it has an acidic and bitter taste. Blending roselle extract with tropical juice from fruits such as mango, guava and papaya could improve the aroma, taste, nutritional, and antioxidant properties of the roselle-fruit blends. The fruits chosen in this study were due to the availability of these fruits during the season. Papaya and guava are also among the underutilized fruits in fruit juice production.

Guava (*Psidium guajava* L.) belongs to the family *Myrtaceae*, commonly known as the apple of the tropics. It grows well in tropical and subtropical regions. The fruits are rich in vitamin C and are almost fivefold higher when compared with oranges (Jawaheer et al. 2003; Ashaye et al. 2005; Thaipong et al. 2006) Most of the guava produced around the world is consumed fresh (Jawaheer et al. 2003).

Papaya (*Carica papaya* L.) is grown in every tropical and subtropical country. A tree-like herbaceous crop, it is a member of the Caricaceae family. It is one of the largest in size of the tropical fruits; it has a pulpy flesh yellow or orange colored with shades of yellow and red, depending on the fruit variety. It has the flavour of a cantaloupe; sweet and juicy with some muskiness (Parker et al. 2010). The fruits are very nutritious due to high contents of vitamin A, C, and iron (Chowdhury et al. 2008).

Mango (*Mangifera indica* L) is one of the most important and widely cultivated fruits of the tropical and subtropical world (Akhter et al. 2012). It is also known as the king of the tropical fruits (Gerbaud 2008). It is an excellent source of fiber, vitamins A, C, and B complex, iron, and phosphorus (Akhter et al. 2012).

Many studies have been conducted on physicochemical and antioxidant properties of roselle extract (Tsai and Huang 2004; Chumsri et al. 2008; Cisse et al. 2011). However, few studies have been conducted on roselle-fruit juice blends, and practically none on the effects of storage time and temperature on roselle-fruit juices. The aim of the present study was to investigate the influence of storage time and temperature on the physicochemical and antioxidant properties of roselle-fruit blends stored in plastic bottles.

Materials and Methods

Plant material

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

Preparation of roselle extract

Dried roselle calyces (10% moisture content) were ground for 1 min using a blender (Kenwood BL 440, Kenwood, Boulogne, France). Roselle calyces were ground at a ratio of 1:10 (roselle:water) and extracted using a water bath at 50°C for 30 min as described previously (Chumsri et al. 2008), and filtered through a cheese cloth.

Fruit juice preparation

Fully matured and high-quality fruits of mango, papaya, and guava were used. Fresh fruits were thoroughly washed, peeled, cut into small pieces (guava were not peeled), and put in a juice extractor (Kenwood JE 810, Edinburgh, U.K.).

Preparation of roselle-fruit juice blends

Three beverage product categories of roselle-mango, roselle-papaya and roselle-guava were formulated in the ratio of 80:20, 60:40, 40:60, and 20:80 roselle extract: fruit juice, 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 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 a cold water bath. Samples were drawn for initial chemical analyses and thereafter analyses were carried every month for 6 months.

pH, titratable acidity and total soluble solids

pH, titratable acid (TA) and total soluble solids (TSS) of roselle-fruit blends were determined according to AOAC (1995). pH was measured using a Hanna portable pH meter (HANNA HI9125, Cluj-Napoca, Romania). TA was determined using 0.1 N sodium hydroxide and phenolphthalein as an indicator and was expressed as % malic acid, while TSS was measured with a hand refractometer (Mettler Toledo, Schwerzenbach, Switzerland) and expressed as Brix.

Reducing sugars

Reducing sugars (RS) were determined by the Luff-Schoorl method as described by Egan et al. (1981). Two grams of sample was weighed in a 100-mL measuring flask and 90 mL hot distilled water, 5 mL Carrez I and 5 mL Carrez II solution were added. The solution was mixed and filtered using a Whatman filter (no. 542), and 10 mL of filtrate was transferred into a 250-mL Erlenmeyer flask, followed by adding 10 mL of copper reagent and swirled. The solution was then boiled in a direct flame for 3 min, cooled in a water bath followed by the addition of 1 g potassium iodide and 10 mL 6 N HCL. The mixture was then titrated with 0.1 N $Na_2S_2O_3$ until a yellow color appeared, 1 mL of starch solution was added and the mixture was titrated continuously until a blue color appeared. RS was determined by interpolation in a table (Egan et al. 1981) after subtracting the blank assay to the volume of sodium thiosulfate of the titration. The results are expressed as mg/100 g fresh weight (FW).

Vitamin C assay

Vitamin C content for the roselle fruit juices was determined according to the method of Dashman et al. 1996 with some modifications using Folin-Ciocalteu reagent (FCR). Twenty milliliters of sample was pipetted into a 100-mL volumetric flask followed by 2 mL of 10% tetrachloroacetic acid solution and diluted to 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 with a Whatman filter (no. 542). One milliliter of sample or standard solution (3 mg ascorbic acid in 1 mL distilled water) was pipetted into a test tube followed by the addition of 3 mL distilled water and 0.4 mL of FCR and incubated at room temperature for 10 min. The absorbance was read at 760 nm using a Jenway 6405 UV–VIS spectrophotometer (Essex, U.K.). The results were expressed as mg/100 g FW.

Determination of antioxidant activity

The antioxidant activity for the roselle fruit 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 HCl and 20 mmol/L $FeCl_3{\cdot}6H_2O$) and 100 μL of sample or standard was incubated at 37°C for 4 min and the absorbance was measured at 593 nm using a spectrophotometer. An intense blue color is formed when the ferric-tripyridyl-s-triazine (Fe^{3+}-TPTZ) complex is reduced to the ferrous (Fe^{2+}) form. A range of iron sulfate concentrations from 0.25 to 2.0 mmol/L was used to prepare a calibration curve. The results are expressed as millimoles of Fe^{2+} per liter of FW (mmol Fe^{2+}/L FW).

Total phenolic assay

Total phenolic content (TPC) for the roselle fruit blends was determined according to the Folin-Ciocalteu method (Singleton et al. 1999) with modifications. An aliquot of 300 μL sample solution was mixed with 1.5 mL of Folin-Ciocalteu 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 in using a spectrophotometer. Gallic acid (0–500 mg/100 g) 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).

Total monomeric anthocyanin content

Total monomeric anthocyanin (TMA) content for roselle-fruit juice blends was determined using the pH differential method (Lee et al. 2005). The 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:

$$A = (A_{520} - A_{700})_{\mathrm{pH1.0}} - (A_{520} - A_{700})_{\mathrm{pH4.5}}$$

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

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

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). The results are expressed as mg cyanidin-3-glucoside equivalent/100 g extract (mg/100 g) FW.

Statistical analyses

Analysis of variance (ANOVA) was applied using a factorial design with two factors including storage temperature (28 and 4°C) and storage time (0, 1, 2, 3, 4, 5, and 6 months). The effect of each factor on the response variable (TSS, pH, TA, RS, vitamin C, FRAP, TMA, TPC) as well as the effects of interactions between the different factors were tested. Significance was accepted at $P < 0.05$ using Minitab Statistical Software (Version 16.0, 2008; Minitab Statistical Software, Minitab Inc., Enterprise Drive State College, PA). ANOVA was only performed on all roselle-fruit juice blends with 40% roselle (40R) as all blends showed a similar trend. Principal component analysis (PCA) was applied to analyze the relationship between roselle-fruit blends (80, 60, 40, 20% roselle) and storage time (0, 1, 2, 3, 4, 5, 6 months) and temperature (ambient and refrigerated) using Unscrambler X 10.2 (Camo Process AS, Oslo, Norway).

Results and Discussions

Total soluble solids

A slight increase in the TSS of the roselle-fruit blends during 6 months of storage at both storage temperatures was

Table 1. Physicochemical and antioxidant properties of roselle-fruit blends stored 0–6 months at 28°C and 4°C.

Parameters	Blends	Mango			Papaya			Guava		
			28°C	4°C		28°C	4°C		28°C	4°C
		0	6	6	0	6	6	0	6	6
TSS	20R	10.6	11.0	12.0	6.9	6.0	8.8	5.6	5.8	6.4
	40R	9.9	11.2	11.2	7.6	6.3	8.3	5.9	6.2	6.8
	60R	7.5	7.4	10.2	7.8	7.7	8.3	6.3	6.9	7.7
	80R	6.9	7.6	7.5	6.7	8.4	7.3	6.7	7.5	6.6
pH	20R	2.76	2.70	3.03	3.32	4.37	3.38	3.13	3.17	3.25
	40R	2.65	2.57	2.77	2.94	2.93	2.92	2.53	2.58	2.64
	60R	2.40	2.43	2.56	2.69	2.65	2.64	2.83	2.60	2.59
	80R	2.34	2.42	1.90	2.54	2.50	2.54	2.41	2.47	2.40
TA	20R	2.92	1.44	1.44	1.36	2.40	1.36	1.92	1.24	1.92
	40R	3.12	1.44	1.32	2.92	1.28	1.28	1.36	2.40	1.36
	60R	2.34	2.92	2.40	1.60	1.44	1.60	2.40	1.96	1.96
	80R	1.92	2.40	1.40	2.00	1.68	1.92	1.68	2.88	1.68
RS	20R	3.48	6.36	7.35	2.95	2.95	7.00	3.32	6.97	7.01
	40R	4.51	5.22	8.46	3.45	7.70	7.71	3.88	8.16	7.92
	60R	5.06	7.87	8.93	4.87	8.19	8.87	4.10	7.96	8.58
	80R	5.55	9.92	9.32	5.18	9.24	9.24	4.35	9.19	8.98

80R, 80% roselle; 60R, 60% roselle; 40R, 40% roselle; 20R, 20% roselle; TSS, total soluble solids; TA, titratable acidity; RS, reducing sugars.

Table 2. Color parameters (lightness [*L**], redness [*a**] and yellowness [*b**] of roselle-fruit blends stored 0–6 months at 28°C and 4°C.

Parameters	*L**			*a**			*b**		
Storage temperature		28°C	4°C		28°C	4°C		28°C	4°C
Storage time	0	6	6	0	6	6	0	6	6
Mango									
20R	18.6	14.7	16.6	18.3	16.2	17.2	19.6	18.1	19.0
40R	17.6	15.2	16.1	17.8	15.8	17.1	16.3	14.7	15.6
60R	16.1	14.8	15.4	16.6	14.8	15.6	15.8	13.8	14.8
80R	14.7	13.8	14.5	15.8	13.8	15.1	15.1	14.2	14.2
Papaya									
20R	16.4	14.8	14.8	15.4	13.0	14.6	16.5	14.7	14.7
40R	17.6	16.1	14.3	16.2	13.5	13.5	17.2	16.2	16.2
60R	19.1	14.7	14.7	18.8	17.3	17.3	17.6	15.8	15.8
80R	20.0	17.9	17.4	19.1	17.5	19.1	19.5	17.4	17.4
Guava									
20R	8.5	6.8	7.4	7.3	5.4	6.7	7.3-	5.4	6.9
40R	7.7	5.8	5.3	6.4	5.4	4.9	6.4	5.4	5.4
60R	5.6	4.3	4.3	6.0	4.0	4.9	5.3	3.2	4.5
80R	4.6	3.9	3.9	4.9	2.7	4.3	4.5	2.7	3.7

80R, 80% roselle; 60R, 60% roselle; 40R, 40% roselle; 20R, 20% roselle.

observed. TSS for roselle-fruit blends ranged from 5.6 to 11.2 brix (28°C) and 5.6–12.0 brix (4°C) during the 6 months of storage (Table 1). Retention or minimum increase in TSS content of juice during storage is desirable for preservation of good juice quality (Bhardwaj and Pandey 2011).

pH

The roselle-fruit juice blends ranged from 2.34 to 4.37 (28°C) and 2.34–3.38 (4°C) during the 6 months of storage (Table 1). An increase in pH was observed at 28°C and 4°C in some roselle-fruit blends. The increased pH was due to the decrease in acidity of the juices. Fruit juices have a low pH because they are comparatively rich in organic acids (Tasnim et al. 2010). Kumar et al. 2012 also observed a significant increase in pH over a period of 120 days of storage at ambient temperature of guava blended with aloe vera and roselle juice nectars.

Titratable acidity

TA for roselle-fruit juice blends ranged from 3.12 to 1.28 (28°C) and 3.12–1.24 (4°C) during the 6 months of storage (Table 1). The TA for some of the roselle-fruit juice blends was found to decrease significantly ($P < 0.05$) at 28°C as well as at 4°C (Tables 3 and 4). Decreased acidity might be due to acidic hydrolysis of polysaccharides where acid is utilized for converting non-RS into RS (Bhardwaj and Pandey 2011).

Reducing sugars

Sugars are one of the most important constituents of fruit products, essential for and also act as a natural food preservative (Bhardwaj and Pandey 2011). The RS value for roselle-fruit ranged from 2.95 to 9.92 mg/100 g (28°C) and 2.95–9.32 mg/100 g (4°C) during the 6 months of storage. The results show a significant increase ($P < 0.05$) in RS with increasing storage period. The sugar content of fruit juices usually increases with increased storage period. The increase is probably due to the hydrolysis of polysaccharides like starch, cellulose, pectin, etc. and conversion into simple sugars (glucose, fructose). Kausar et al. (2012) reported increased RS with increased storage time of a cucumber–melon functional drink and 70% increased RS during the 6 months of storage of bottled gourd–basil leave juice (Majumdar et al. 2011).

Effects of storage temperature and time on color

Visually, no color change was observed in all of the roselle-fruit blends during the 6 months of storage at 4°C. However, minimal loss in visual color was observed in all roselle-fruit blends stored for 4–6 months at 28°C. The results are similar to the findings of Saeed and Ahmed (1977), who did not observe any visual color change in carbonated beverages prepared from roselle calyces during 3 months of storage at ambient temperature.

Lightness values (L*) of the roselle-fruit blends ranged from 19.6 to 13.8 (28°C) and 19.6–14.2 (4°C) for 6 months of storage while redness values (a*) of the roselle-fruit blends ranged from 20.0 to 13.0 (28°C) and 20.0–13.5 (4°C) respectively after 6 months of storage (Table 2). Anthocyanins are responsible for the red color in roselle-mango juice blends and color of anthocyanin is pH dependent (the red flavylium is stable at low

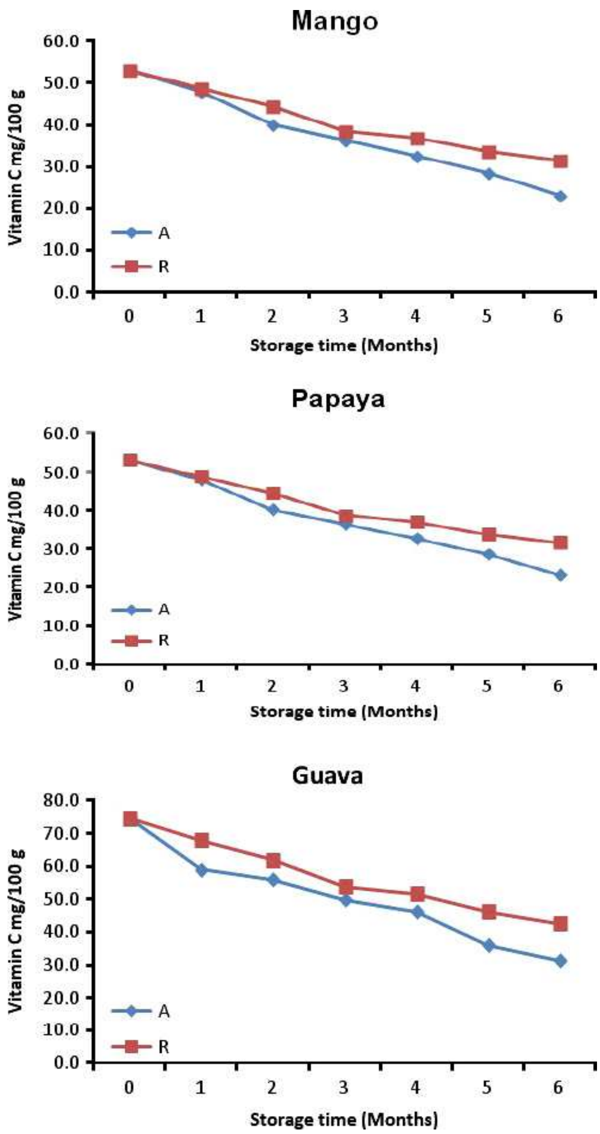

Figure 1. Vitamin C content for roselle-fruit blends (40R) stored for six months at ambient (A) and refrigerated (R).

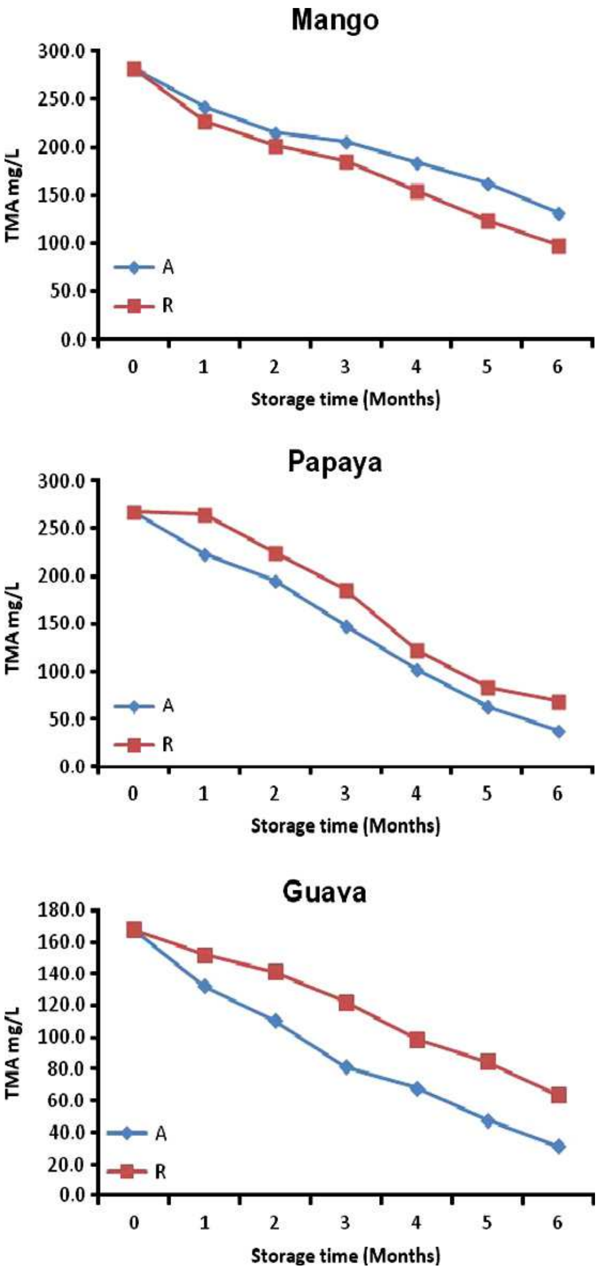

Figure 2. Total monomeric anthocyanin content for roselle-fruit blends (40R) stored for six months at ambient (A) and refrigerated (R).

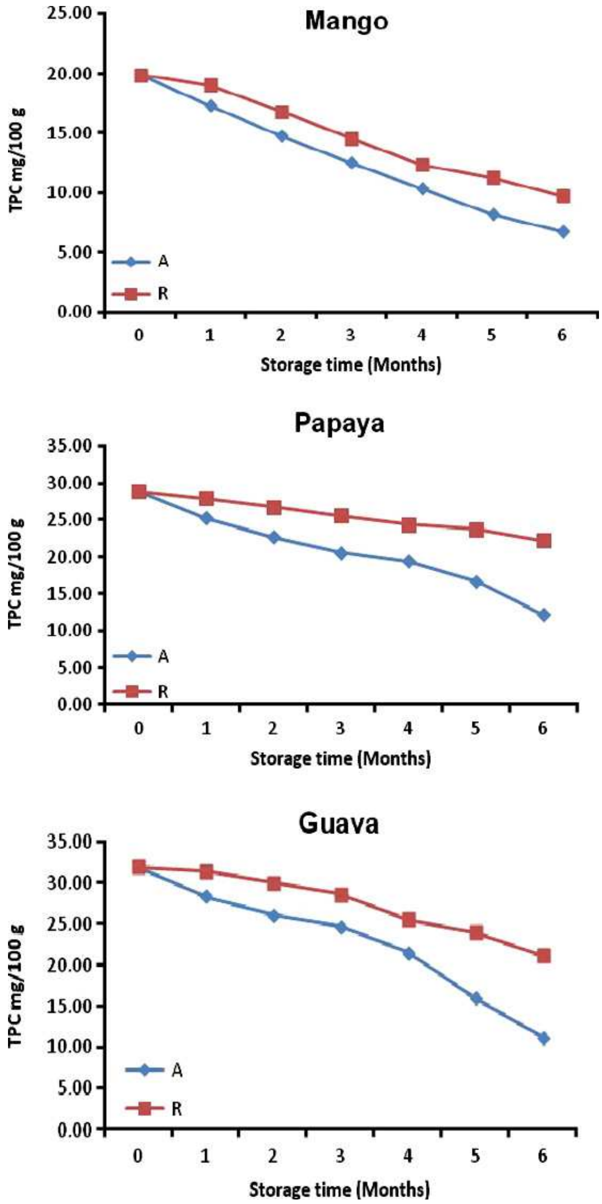

Figure 3. Phenolic content for roselle-fruit blends (40R) stored for six months at ambient (A) and refrigerated (R).

pH) as the pH changes were substantial hence the color changes of the roselle-fruit blends. Yellowness (b*) values of the roselle-fruit blends ranged from 8.5 to 2.7 (28°C) and 8.5–3.7 (refrigerated) during the 6 months of storage.

Effect of time and temperature of storage on vitamin C content

The vitamin C contents for roselle-mango, roselle-papaya, roselle-guava juice (40R) blends were 54.4, 53.0, 74.7 mg/100 g FW initially and changed to 24.5, 23.2, 31.3 mg/100 g FW (28°C) and 31.5, 31.5 42.6 mg/100 g FW (4°C) during the 6 months of storage (Fig. 1). Vitamin C content of all roselle-fruit blends decreased during storage with the advancement of storage period, which was probably due to the fact that vitamin C being sensitive to oxygen, light and heat are easily oxidized in the presence of oxygen by both enzymatic and non-enzymatic catalysts (Ziena 2000). A decrease in vitamin C was observed in guava blended with aloe vera and roselle during 120 days of storage at ambient temperature (Kumar et al. 2012).

Effect of time and temperature of storage on TMA

The TMA for roselle-fruit juice blends (40R) is shown in Figure 2. TMA for roselle-mango, roselle-papaya, and roselle-guava juice (40R) blends was 282.6, 268.6, and 167.8 mg/100 g FW initially and changed to 97.8, 37.4, and 31.3 mg/100 g FW (28°C) and 131.4, 68.0, and 63.7 mg/100 g FW (4°C) after 6 months of storage (Fig. 3). The losses in TMA for roselle-fruit juices were higher at 28°C than 4°C. The TMA for roselle-fruit juice blends was found to be decreased during storage but this decrease was statistically significant ($P < 0.05$) at 28°C as well as at 4°C (Tables 3 and 4). The presence of ascorbic acid and higher pH of the prepared roselle-fruit juice blends could have accelerated anthocyanin degradation. It is also known that interaction of ascorbic acid with anthocyanins may result in the degradation of both compounds through a condensation reaction (Choi et al. 2002; González-Molina et al. 2009). From the results roselle-guava blends with higher vitamin C content had greater loss of anthocyanin than roselle-mango and roselle-papaya blends.

Effect of time and temperature of storage on the total phenolic content

TPC for roselle-mango, roselle-papaya, roselle-guava juice (40R) blends were 19.8, 28.8, and 32.0 GAE mg/100 g FW initially and changed to 6.7, 12.2, 11.1 GAE mg/100 g FW (28°C) and 9.71, 22.3, 21.2 GAE mg/100 g FW (4°C) at 6 months of storage (Fig. 3). The data reveal that the TPC decreased during storage and significantly ($P < 0.05$)

Table 3. Influence of treatment effects on physicochemical and bioactive properties of roselle-fruit juice blends (40R).

Parameters	TSS	pH	TA	RS	Vit C	FRAP	TMA	TPC	*L**	*a**	*b**
Fruit	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Temp	ns	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Time	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Fruit × Temp	<0.001	<0.001	ns	<0.001	<0.001	<0.001	<0.001	<0.001	0.031	<0.001	<0.001
Fruit × Time	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.008	<0.001	<0.001
Temp × Time	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.022	0.004

TSS, total soluble solids; TA, titratable acidity; RS, reducing sugars; Vit C, vitamin C; FRAP, ferric reducing ability of plasma; TMA, total monomeric anthocyanins; TPC, total phenolic content; ns, not significant.

Table 4. Main effects of fruit juice, storage temperature and time on the physicochemical and bioactive properties of roselle-fruit juice blends during storage.

Parameters	TSS	pH	TA	RS	Vit C	FRAP	TMA	TPC	*L**	*a**	*b**
Fruit juice											
Mango	9.3a	2.6a	1.7a	6.4a	39.6b	1.2b	105.0b	25.2a	17.0a	16.9a	6.8a
Papaya	9.1b	2.6a	1.4b	6.0b	39.3c	1.2b	175.8a	19.2b	16.5b	15.2c	5.8c
Guava	6.2c	2.5b	1.4b	5.6c	53.7b	2.5a	177.8a	17.8c	15.7c	16.7b	6.2b
Storage temperature (°C)											
28	8.2a	2.5b	1.5b	5.6b	41.7b	1.6b	137.7b	16.8b	16.2b	16.1b	6.2b
4	8.2b	2.6a	1.6a	6.3a	46.7a	1.7a	168.1a	24.7a	16.6a	16.4a	6.4a
Storage time (months)											
0	7.7d	2.7a	1.5b	4.5e	60.7a	1.8a	236.5a	26.9a	17.2a	17.2a	6.8a
1	7.7d	2.7a	1.5b	4.6e	53.4b	1.8a	207.2b	24.9b	16.8b	16.9a	6.7a
2	8.2c	2.4c	1.4bc	4.6e	48.0c	1.8a	181.4c	22.9c	16.4c	16.6b	6.7a
3	8.2c	2.3d	1.3c	5.3d	42.9d	1.7b	154.6d	21.1d	16.2c	16.2bc	6.5b
4	8.4b	2.5b	1.4bc	6.5c	39.5e	1.5c	121.7c	18.9e	16.2c	16.1c	6.1c
5	8.5b	2.4c	1.6b	7.1b	34.6f	1.4d	94.1f	16.6f	16.1c	15.5d	5.7d
6	8.7a	2.7a	1.9a	8.5a	30.4g	1.3e	71.5 g	13.9 g	15.7d	15.2d	5.3c

TSS, total soluble solids; TA, titratable acidity; RS, reducing sugars; Vit C, vitamin C; FRAP, ferric reducing ability of plasma; TMA, total monomeric anthocyanins; TPC, total phenolic content; *L**, lightness; *a**, redness; *b**, yellowness. Means separated in columns by main effects of Tukey's test. Numbers followed by the same letter are not significantly different ($P < 0.05$).

more decrease was found at 28°C than at 4°C, irrespective of storage intervals (Tables 3 and 4).

During storage, some monomeric anthocyanins might have been transformed into polymeric compounds (Iversen 1999; Ochoa et al. 1999). This might be the reason for less reduction of TPC and high losses in TMA in the blends.

Antioxidant activity

FRAP values for roselle-mango blends ranged from 1.8 to 0.76 mmol/100 g (28°C and 4°C) during the 6 months of storage (Fig. 4). The antioxidant capacity of fruits and vegetables, which benefits human health, is highly correlated with their anthocyanin and TPC (Fang et al. 2006). The results showed that antioxidant activity levels did not decrease substantially.

Despite marked losses of monomeric anthocyanins in the roselle-fruit juice blends, FRAP values were not higher during storage, suggesting the possibility of formation of polymeric compounds from monomeric anthocyanins during storage which were able to compensate the loss of antioxidant capacity due to decreased monomeric anthocyanins (Brownmiller et al. 2008).

PCA of roselle-fruit blends

A PCA was applied to characterize the different roselle-fruit blends by their storage time and storage temperature (Fig. 5A and B). The two principal components were able to explain all total variation. The principal component 1 (PC1) and PC2 divided the roselle-fruit juice blends into two clusters depending on the type of fruit mixed with roselle extract. PC2 was used to explain roselle-guava blends and was characterized by high levels of vitamin C, TPC, FRAP, and pH, while PC1 explained roselle-mango and roselle-papaya juice blends with high level of TMA, total soluble solids, RS, lightness (L^*), redness (a^*), and yellowness (b^*).

The roselle-guava juice blends stored at ambient temperature formed a cluster with blends stored at 0 and 1 months on the positive side of PC2 and blends stored from 2 to 6 months on the negative side of PC. The roselle-papaya and roselle-mango juice blends form the second cluster with blends stored at 0–3 months (roselle-papaya) and 0–4 months (roselle-mango) on the positive side of PC1 and blends stored at 4–6 months (roselle-papaya) and 5–6 months (roselle-mango) on the negative side of the PC (Fig. 5A).

The roselle-guava juice blends stored at refrigerated temperature formed a cluster with blends stored at 0–2 months on the positive side of PC2 and blends stored from 3 to 6 months on the negative side of PC. The roselle-papaya and roselle-mango juice blends form the second cluster with blends stored at 0–3 months (roselle-papaya) and 0–2 (roselle-mango) on the positive side of PC1 and blends stored at 4–6 months (roselle-papaya) and 3–6 months (roselle-mango) on the negative side of the PC (Fig. 5B). Regardless of the storage time, TMA, TPC, and vitamin C were mostly affected during storage of roselle-fruit juice blends stored for 6 months. This shows that the storage temperature had a clear effect on the loss of TMA.

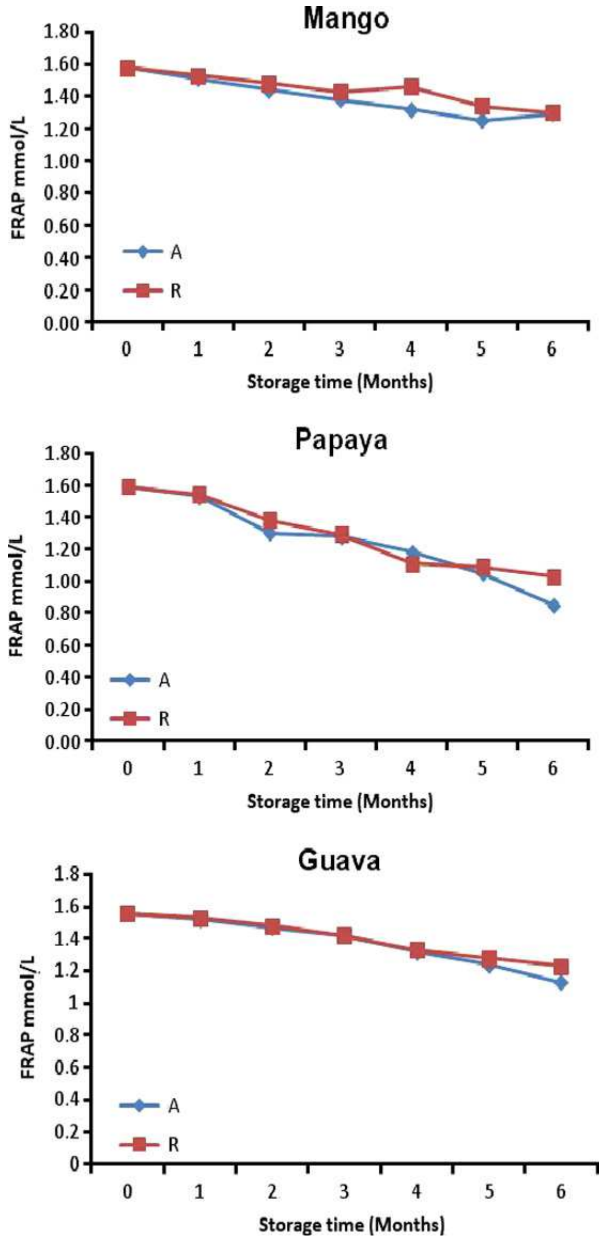

Figure 4. Antioxidant activity (FRAP) for roselle-fruit blends (40R) stored for six months at ambient (A) and refrigerated (R).

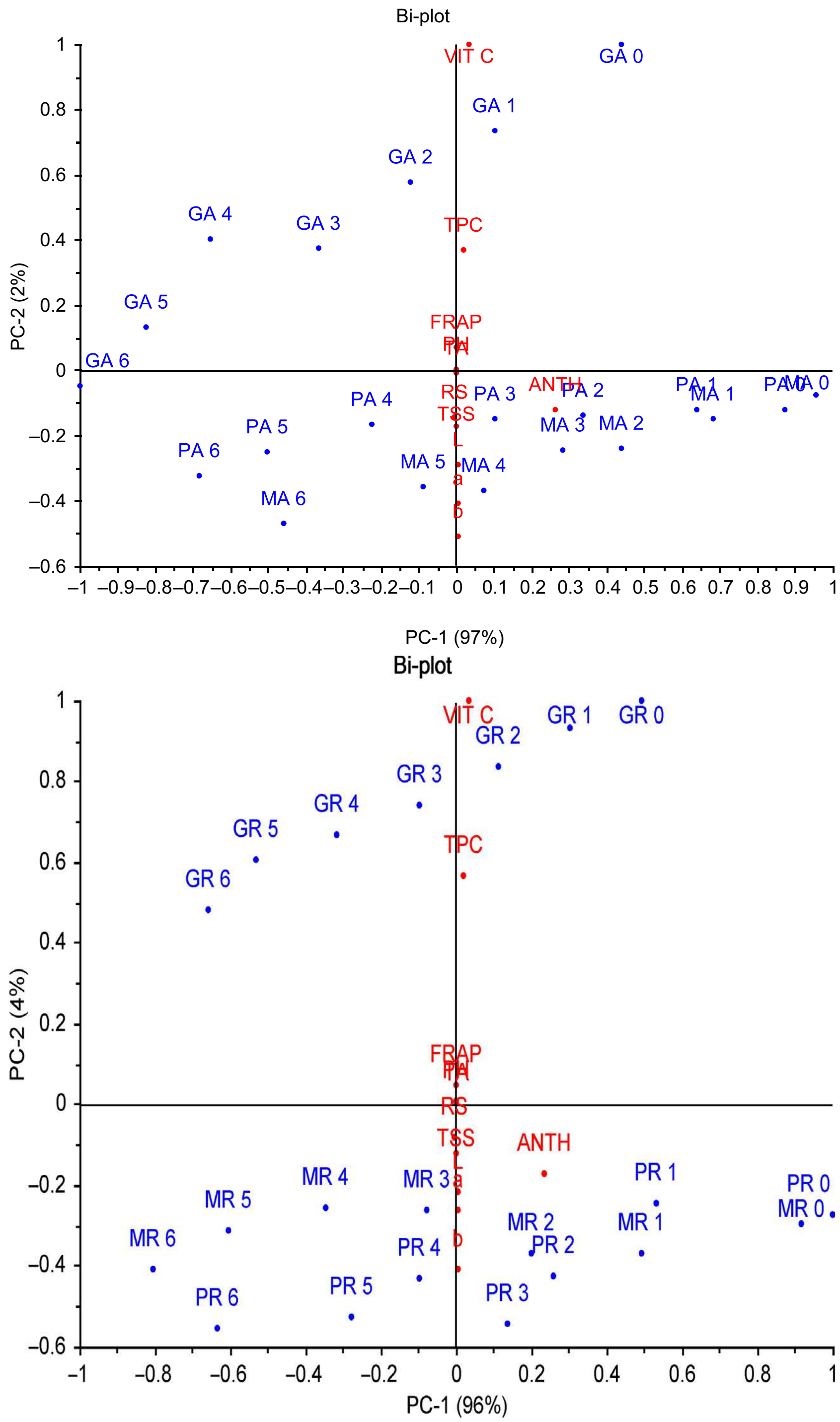

Figure 5. (A, B) Bi-plot (PCA) showing the effects of storage time and temperature on the roselle-fruit blends at ambient and refrigerated temperature.

Conclusions

The roselle-fruit blends have high content of vitamin C, anthocyanin, and total phenol. However, these compounds were lost during 6 months of storage at 28°C and 4°C. The loss of vitamin C and anthocyanin was more pronounced at 28°C; therefore, storage at 4°C should be encouraged when the products need to be stored for long time.

Acknowledgments

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Conflict of Interest

None declared.

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Paper III

INFLUENCE OF STORAGE TEMPERATURE AND TIME PHYSIOCHEMICAL AND ANTIOXIDANT PROPERTIES OF ROSELLE-FRUIT JUICE BLENDS IN GLASS BOTTLES

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ABSTRACT

Roselle extract as it has an acidic and bitter taste. Blending roselle extract with tropical juice from fruits such as mango, guava and papaya could improve the aroma, taste, nutritional and antioxidant properties of the roselle-fruit blends. Roselle extract have large quantities of anthocyanin which are known to be unstable during processing and preservation. Therefore the aim of this study was to assess the effect of storage temperature and time on physiochemical and antioxidant properties in roselle-fruit juice blends. Different blends with roselle extract and fruits (mango, papaya and guava) were prepared (80:20, 60:40, 40:60, 20:80, % roselle: fruit juice, respectively). The juice blends were preserved by pasteurization (82.5° C for 20 min) and by addition of 1g (Sodium benzoate and citric acid). Blends were stored in 100mL glass bottles at ambient (28°C) and refrigerated (4°C) temperatures for up to six months and analyzed at two month intervals for physiochemical properties, vitamin C, total monomeric anthocyanin (TMA), total phenolic content (TPC) and antioxidant activity (FRAP assay). The changes in pH, titratable acidity, total soluble solids and reducing sugars were not significant with increased storage period. Vitamin C in all roselle-fruit blends decreased with ranged from (60-62) % and (30-34) % when stored at 28°C and 4°C, respectively. TMA losses were (71-74) % at (28°C) and (41-44) % (4°C) while TPC losses were (51-55) % (28°C) and (25-28) % (4°C). Loss of antioxidant capacity (FRAP) loss were less than 56% at 28°C and less than 30% at 4°C. From the results the storage at 4°C is desirable if long term storage of roselle-fruit juices is required. A principal component analysis (PCA) differentiated roselle- juice fruit blends into two clusters with two principle components PC1 and PC2, which explained all of the variation. PC1 differentiated roselle-guava juice blends which were characterized by vitamin C, TPC, FRAP and pH, while PC2 form another cluster of roselle-mango and roselle-papaya juice blends and were characterized by TSS, RS and colour parameters (L*, a*, b*). However, TMA was the main variable affected in all roselle-fruit juice blend regardless of the storage temperature.

Key words: Roselle fruit juices, anthocyanins, storage

INTRODUCTION

The increased consumption of fruit juices goes together with increased variety of fruit juices and beverages offered for sale. Among these juices and beverages is roselle juice and/or drink which tastes good and contains large amounts of anthocyanins and ascorbic acid. Roselle produces red edible calyces with a unique flavor and brilliant red colour. The calyces are commonly used to make jelly, juice, jam, wine, syrup, pudding, cake, ice cream and flavour [1, 2]. The beverages produced by roselle calyc*es* are called hibiscus tea, bissap, roselle, red sorrel, agua de Jamaica, Lo-Shen, Sudan tea, or karkade [3]. The fleshy flowers provide a soft drink consumed as a cold or hot beverage [1, 2]

Hibiscus sabdariffa L. (family *Malvaceae*), commonly known as roselle, red sorrel, or karkadè, is widely grown in Africa, South East Asia, and some tropical countries of America [4, 5, 6]

The calyces are rich in anthocyanins, ascorbic acid and hibiscus acid [7]. There are four main types of anthocyanins in roselle; dephinidin 3-sambubioside, cyanidin 3-sambubioside, delphinidin 3-glucoside and cyanidin 3-glucoside [5, 7]. Roselle anthocyanins can contribute to health benefits as a good source of antioxidants as well as a natural food colourant [1]. They are derivatives of the basic flavylium cation structure with electron deficient nucleus which make them highly reactive and their reaction involve discolorization of the anthocyanin pigments [8].

Factors like light, pH, temperature, oxygen, ascorbic acid and sugar are contributing factors in degradation or stability of anthocyanins [2, 5, 8]. Most people do not prefer beverages made from roselle extract as it has an acidic and bitter taste. Blending roselle extract with tropical juice from fruits such as mango, guava and papaya could improve the aroma, taste, nutritional and antioxidant properties of the roselle-fruit blends. The fruits choicen in this study were due to the availability of these fruits during the season. Papaya and guava are also among underutilized fruits in fruit juice production.

Guava (*Psidium guajava* L.) belongs to the family *Myrtaceae*, commonly known as Apple of Tropics. It grows well in tropical and subtropical regions. The fruits are rich in vitamin C and contain almost five times as in oranges [9].

Papaya (*Carica papaya* L.) is grown in every tropical and subtropical country. A tree-like herbaceous crop, it is a member of the *Caricaceae* family [10]. It is one of the largest in size of the tropical fruits; it has a pulpy flesh yellow or orange coloured with shades of yellow and red, depending on the fruit variety. It has a flavour of a cantaloupe; sweet and juicy with some muskiness [11]. The fruits are very nutritious due to high contents of vitamin A, C and iron [12].

Mango (*Mangifera indica* L) is one of the most important and widely cultivated fruit of the tropical and subtropical world [13]. It is also known as the king of the tropical fruit [14]. They are an excellent source of fiber, vitamins A, C and the B complex, iron and phosphorus [13].

Many studies have been conducted on physiochemical and antioxidant properties of roselle extract [2, 5, 8]. On the other hand, only few studies have been conducted on roselle-fruit juice blends, and practically none on the effects of storage time and temperature on roselle-fruit juices have been studied. The aim of the present study was to investigate the influence of storage time and temperature on physiochemical and antioxidant properties of roselle-fruit blends stored in glass bottles.

MATERIALS AND METHODS

Plant material

Dark red dried roselle calyces were purchased from the Municipality market in Morogoro. Guava (pink), papaya (Solo) and mango (Dodo) were purchased from the horticulture garden at Sokoine University of Agriculture, Tanzania.

Roselle extract preparation

Dried roselle calyxes (10% moisture content) were grounded for 1 minute using a blender (Kenwood BL 440, France). Grounded roselle calyces at a ratio of 1:10 (roselle: water) were extracted using water bath at 50^{o}C for 30 minutes as previously described [8] and filtered through a cheese cloth.

Fruit juice preparation

Fully matured and high quality fruits of mango, papaya and guava were used. Fresh fruits were thoroughly washed, peeled and cut into small pieces (guava were not peeled) and put in a juice extractor (Kenwood JE 810, UK).

Preparation of roselle-fruit juice blends

Three beverage product categories of 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, 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 glass bottles, loosely capped and pasteurized in a water bath at a temperature of 82.5^{o}C for 20 min and cooled rapidly to room temperature by immersing the bottles in cold water bath (10^{o}C). Samples were at drawn at 0, 2, 4 and 6 months for chemical analyses.

pH, titratable acidity (TA) and total soluble solids (TSS)

pH, TA and TSS of roselle-fruit blends were determined according to AOAC [15].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, while TSS was measured with a hand refractometer (Mettler Toledo, Switzerland) and expressed as ^{o}Brix.

Reducing sugars

Reducing sugars (RS) were determined by Luff-Schoorl method as described by Egan *et al* [16]. Two grams of sample were weighed into a 100mL measuring flask and 90mL distilled water; 5mL Carrez I and 5mL Carrez II solution were added. The solution was mixed and filtered using a Whatman filter (no. 542), and 10mL of filtrate was transferred into a 250mL Erlenmeyer flask, followed by adding 10mL of copper reagent and swirled. The solution was then boiled in a direct flame for 3 minutes, cooled in a water bath followed by adding of 1g potassium iodide and 10mL 6N HCL.

The mixture was then titrated with 0.1N $Na_2S_2O_3$ until a yellow colour appeared, 1mL of starch solution was added and the mixture was titrated continuously until a blue colour appeared. RS was determined by interpolation in a table (Egan et al., 1981) after subtracting the blank assay to the volume of sodium thiosulfate of the titration. The results are expressed were expressed as mg/100g fresh weight (FW).

Vitamin C assay

Vitamin C content for the roselle fruit juices was determined according to the Folin-Ciocalteu reagent (FCR) method [17]. 20mL of sample was pipetted into 100mL volumetric flask followed by 2mL of 10% Tetrachloroacetic acid (TCA) solution and diluted to 100mL with distilled water. The sample was poured into a conical flask, swirled gently for one minute and left to stand for one minute and filtered with a whatman filter (no. 542). [Equal amounts (1mL) of both sample and standard solution (3 mg ascorbic acid in 1mL distilled water)] was pipetted into a test tube followed by adding 3mL distilled water and 0.4 mL of FCR and incubated at room temperature for 10 min. The absorbance was read at 760 nm using a spectrophotometer (Jenway 6405 UV/VIS Spectrophotometer, UK). The results were expressed as mg per 100g fresh weight (FW).

Total phenol content

The total phenolic content (TPC) in the extracts was determined spectrophotometrically, using the Folin–Ciocalteu method [18]. The added reagent volumes were proportionally reduced so that the final reaction volume amounted to 2 mL and could be prepared in disposable plastic cuvettes. Gallic acid was used as a standard and the results were expressed as milligram of gallic acid equivalents per 100 g of fresh weight (mg GAE/100 g FW).

Total anthocyanin content

Anthocyanin quantification was performed by the pH-differential method [19]. Calculation of the anthocyanins concentration was based on a cyanidin-3-glucoside molar extinction coefficient 26,900 and a molecular mass of 449.2 g/mol. Results were expressed as miligrams (mg) of cyanidin-3-glucoside equivalents (CGE) per 100 g of fresh weight (FW).

Antioxidant activity

Ferric reducing antioxidant power (FRAP assay)

The FRAP assay was used to estimate the reducing capacity of tested extracts, according to the original method by Benzie and Strain [20]. A calibration curve was prepared, using an aqueous solution of ferrous sulphate $FeSO_4 \cdot 7H_2O$ and the results, obtained from three replicate extractions, were expressed as mmol $FeSO_4 \cdot 7H_2O$ per 100 g of fresh weight (mmol Fe^{2+}/100 g FW).

Statistical analyses

Analysis of variance (ANOVA) was applied using a factorial design with two factors including storage temperature (28^0C and 4^0C) and storage time (0, 2, 4, 6 months) for only roselle-fruit blends (40% roselle) since other blends (80, 60, 20 % roselle) also showed similar trend. The effect of each factor on the response variable (TSS, pH, TA, RS, vitamin C, FRAP, TMA, TPC) as well as the effects of interactions between the different factors were tested. Significance was accepted at $p<0.05$ using Minitab Statistical Software (Version 16.0, 2008, Minitab Statistical Software, Minitab Inc., Enterprise Drive State College, PA, USA) for Microsoft Windows. Principal component analysis was applied to analyse the relationship between roselle-fruit blends (40 % roselle) and storage time (0, 2, 4, 6 months) and temperature (Ambient and refrigerated) using Unscrambler X 10.2 (Camo Process AS, Oslo, Norway).

RESULTS

Effect of storage time and temperature on physiochemical and antioxidant properties

Total soluble solids and reducing sugar

A slightly increase in the total soluble solids (TSS) of the roselle-fruit blends during six months of storage at both storage temperatures was observed. TSS for roselle-fruit blends ranged from 13.8-7.2 ^{o}brix (28^{o}C) and 13.8-4 ^{o}brix (4^{o}C) during the 6 months of storage (Table 1). Data reveals that the TSS increased during storage and statistically ($p<0.05$) more increase was found under ambient conditions than refrigerated conditions (Table 3).

Reducing sugar (RS) content for roselle-fruit juice blends ranged from 2.5-5.7mg/100g (28^{o}C) and 2.5-5.1 mg/100g (4^{o}C) during the 6 months of storage (Table 1). The results show significant increase ($p<0.05$) in RS with increase storage period (Table 3).

pH and titratable acidity.

The roselle-fruit juice blends ranged from 4.19-2.2 at 28^{o}C and 4^{o}C during the 6 months of storage (Table 1). An increase in pH was observed at (28^{o}C) and (4^{o}C) in roselle-fruit blends. The increased pH was due to corresponding decrease in acidity of the blends. Titratable acidity (TA) for roselle-fruit juice blends ranged from 1.99-0.58 (28^{o}C) and 1.84-0.58 (4^{o}C) during six months of storage (table 1). The titratable acidity was found to be decreased during storage but this decrease was statistically was significant ($p<0.05$) for storage time and interaction time*temperature (Table 3).

Colour

One of the most important parameters to which consumer are sensitive when selecting food is colour. Table 2 shows the changes in colour parameters for roselle-fruit juice blends during time and temperature storage. Lightness values (L**)** of the roselle-fruit blends ranged from 19.6-13.6 (28ºC) and 19.6-14.4 (4ºC) for six months of storage while redness (a*) roselle-fruit blends ranged from 20.7-14.7 (28ºC) and 20.7-14.5(4ºC) respectively after six months of storage (table 2). The reduction in the redness (a*) color in roselle fruit juices blends could be related to a decrease in TMA, which is responsible for this red color. Yellowness (b*) values roselle-fruit juice blends ranged from 8.5-3.1 (28ºC) and 8.5-3.9 (4ºC) for the six months of storage. Statistical analysis showed that interaction time and temperature factor had significant effect ($p<0.05$). The decrease of yellowness (b*) with increase storage time was due to loss of carotenoids

Vitamin C content

The Vitamin C content for roselle-mango, roselle-papaya, roselle-guava juice (40R) blends were 55.1, 58.9, 76.2 mg/100g FW initially and changed to 22.2, 22.4, 28.6 mg/100g FW (28°C) and 36.3, 40.5, 53.5 mg/100g FW (4°C) at 6 months of storage (Fig 1). All variables (time, temperature, and interaction term interaction term, time × temperature) were significant contributors ($P < 0.05$) to the loss of vitamin C (table 3). The reduction was higher with longer

Total monomeric anthocyanin

The total monomeric anthocyanin (TMA) for roselle-fruit juice blends (40R) is shown in fig TMA for roselle-mango, roselle-papaya, roselle-guava juice (40R) blends were 230.4, 236.3, 166.4 mg/L FW initially and changed to 62.0, 69.7, 42.5 mg/L FW (28°C) and 135.8, 147.4, 93.1 mg/L FW (4°C) after 6 months of storage (Fig 2). Temperature affected significantly the decrease in anthocyanins and the decrease was always fastest at room temperature (28°C) followed by storing at 4°C, respectively ($p < 0.05$).

Total phenolic content

Total phenolic content (TPC) for roselle-mango, roselle-papaya, roselle-guava juice (40R) blends were 30.9, 27.6, 34.3 GAE mg/100g FW initially and changed to 13.8, 12.5, 16.7 GAE mg/100g FW (28°C) and 23.1, 20.4, 24.6 GAE mg/100g FW (4°C) after 6 months of storage (Fig 3). As it was shown in Table 3, time and temperature of storage significantly affected the total polyphenol content as determined by Folin–Ciocalteu assay. All variables (time, temperature, and interaction term interaction term, time × temperature) were significant contributors ($P < 0.05$) to the loss of TPC.

Antioxidant activity

Antioxidant activity (FRAP) for roselle-mango, roselle-papaya, roselle-guava juice (40R) blends were 1.58, 1.66, 1.33 mmol/L FW initially and changed to 1.17, 0.78, 0.59 mmol/L FW (28°C) and 1.32, 1.42, 0.94 mmol/L FW (4°C) after 6 months of storage (Fig 4)**.**

Principal component analysis (PCA) of roselle-fruit blends

A Principal component analysis (PCA) was applied to characterize the different roselle-fruit juice blends by their storage time and storage temperature (Fig 5a & b). The two principal components were able to explain all total variation. The principal component 1 (PC 1) and principal component 2 (PC 2) divided the roselle-fruit juice blends into two clusters depending on the type of fruit mixed with roselle extract. PC2 was used to explain roselle-guava blends and characterized by high levels of vitamin C, total phenolic content, FRAP and pH while PC1 explained roselle-mango and roselle-papaya juice blends with high level of total monomeric anthocyanins, total soluble solids ,reducing sugars, lightness (L*), redness (a*) and yellowness (b*).

The roselle-guava juice blends stored at ambient temperature formed a cluster with blends stored at zero months on the positive side of the PC 2 and blends stored from 2, 4,6 months on the negative side of PC. The roselle-papaya and roselle-mango juices blends forms the second cluster with blends stored at 0 and 2 months on the positive side of the PC1 and blends stores at 4 and 6 months on the negative side of the PC 1 as shown in score plot (Fig 5a)

The roselle-guava juice blends stored at refrigerated temperature formed a cluster with blends stored at 0-6 months on the negative side of the PC 2 The roselle-papaya and roselle-mango juices blends forms the second cluster with blends stored at zero months on the positive side of the PC2 while blends stores at 2-6 months on the PC1 as shown in score plot (Fig 5c). Regardless of the storage time, TMA was mostly affected during storage of roselle-fruit juice blends stored for six months. This shows that the storage temperature had a clear effect on the loss of TMA (Fig 5 b&c).

DISCUSSIONS

The increase in TSS might be due to increase in total sugars by inversion in the presence of organic acids from polysaccharides like starch and cellulose into simple sugars in course of time [21]. Increased TSS was also observed by Kumar *et al* [21] in guava blended with aloe vera and roselle and stored for 120 days at ambient temperature. Boghani *et al* [10] reported increased TSS blended papaya-aloe vera juices stored glass bottles at refrigeration temperature for 4 months. The increased RS was also reported by Kumar *et al* [21] for guava RTS and nectar blended with aloe vera and roselle and stored for 120 days at ambient temperature.

The significant increased pH was also reported in guava blended with aloe and roselle juice nectars [21] Decreased acidity might be due to acidic hydrolysis of poly saccharides were acid is utilized for converting non reducing sugars into reducing sugars [22] Decrease in TA was also observed in guava RTS and nectar blended with aloe vera and roselle and stored for 120 days at ambient temperature [21].

It is well known that anthocyanin properties, including colour expression, are highly influenced by anthocyanin structure and pH [23].The losses in redness might be due to loss of anthocyanin which impart red colour to the blends. Carotenoids are known to be responsible to yellowness of the blends, however carotenoids are highly susceptible to degradation by external agents such as heat, low pH and light exposure [24].Therefore losses in yellowness might be due to degradation of carotenoids during storage.

According to the literature data, the content of vitamin C in different juices decreases during storage, depending on storage conditions, such as temperature, oxygen and light access [9].The storing temperature has been previously shown to affect the stability of anthocyanins in different juices [25, 26]. Several factors can influence the stability of anthocyanins in juices, e.g. pH, ascorbic acid and anthocyanin degrading enzymes [25]. Marti *et al* [27] found that anthocyanin losses in pomegranate juice stored for two months were about 60% at 5°C and 85% at 25°C. The retention in antioxidant capacity during storage conflicts with the marked losses observed in total anthocyanins, and may be explained by the formation of anthocyanin polymers [1, 2], which compensated for the loss of monomeric anthocyanins.

CONCLUSION

The results of this study showed that total monomeric anthocyanin, vitamin C and total phenol content of roselle-fruit juice blends decreased significantly with storage time. The most affected parameter was total monomeric anthocyanin. The interaction time-temperature had a significant effect on the TMA, TPC and vitamin C content of roselle-fruit juice blends with higher losses observed at ambient temperature. Storage of roselle-fruit juice blends at room temperature should be avoided if good long-term preservation of anthocyanin, phenols and vitamin C is desired.

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Table 1: Physiochemical and antioxidant properties of roselle-fruit blends stored (0-6) months at 28ºC and 4ºC.

		Mango			Papaya			Guava		
Parameters	Blends		28ºC	4ºC		28ºC	4ºC		28ºC	4ºC
		0	6	6	0	6	6	0	6	6
TSS	20R	13.8±0.08	13.7±0.11	14.1±0.08	13.8±0.08	13.7±0.11	13.8±0.08	7.8±0.08	8.3±0.14	7.9±0.07
	40R	11.2±0.14	13.0±0.17	11.5±0.15	11.2±0.14	13.0±0.17	11.5±0.15	7.5±0.09	7.4±0.14	7.3±0.08
	60R	10.0±0.15	12.2±0.20	12.2±0.42	10.0±0.15	12.0±0.20	10.0±0.19	7.2±0.09	7.3±0.08	7.3±0.05
	80R	8.0±0.10	9.6±0.12	8.5±0.09	8.0±0.12	9.6±0.12	8.5±0.09	6.1±0.08	6.3±0.09	7.2±0.10
pH	20R	2.9±0.01	3.1±0.01	3.2±0.01	4.2±0.1	3.4±0.01	4.0±0.01	3.4±0.01	3.2±0.02	3.3±0.01
	40R	2.5±0.01	2.8±0.01	3.0±0.01	3.1±0.01	3.0±0.01	3.1±0.01	3.1±0.01	2.9±0.01	2.9±0.01
	60R	2.4±0.01	2.6±0.01	3.0±0.03	3.0±0.01	2.7±0.01	2.7±0.02	2.9±0.01	2.7±0.09	2.9±0.02
	80R	2.2±0.01	2.5 ±0.01	3.0±0.02	2.8±0.01	2.8±0.01	2.6±0.06	2.8±0.01	2.5±0.06	2.4±0.01
TA	20R	0.8±0.02	1.3±0.03	0.8±0.02	0.8±0.02	1.2±0.02	0.9±0.02	0.8±0.02	1.6±0.02	0.8±0.01
	40R	1.5±0.03	1.3±0.02	1.4±0.02	1.3±0.03	1.3±0.02	1.4±0.02	1.2±0.03	1.8±0.03	1.6±0.01
	60R	1.5±0.03	1.9±0.02	1.5±0.02	1.5±0.03	1.8±0.02	1.6±0.15	1.5±0.01	1.9±0.01	1.7±0.02
	80R	1.7±0.02	2.1±0.02	1.6±0.09	1.6±0.02	2.0±0.02	1.8±0.09	1.7±0.01	2.0±0.01	1.8±0.02
RS	20R	4.4±0.01	5.7±0.01	5.1±0.01	4.0±0.01	5.4±0.01	5.1±0.01	3.5±0.02	4.7±0.01	4.1±0.01
	40R	3.6±0.01	4.9±0.00	4.2±0.01	3.7±0.00	4.8±0.01	4.2±0.01	3.2±0.03	4.3±0.01	3.9±0.01
	60R	2.9±0.01	4.0±0.01	4.0±0.01	3.1±0.01	4.2±0.18	4.0±0.01	2.9±0.02	4.1±0.05	3.5±0.01
	80R	2.5±0.00	3.7±0.01	3.2±0.01	2.7±0.01	3.9±0.01	3.1±0.01	2.4±0.01	3.4±0.01	3.1±0.01

80R=80% Roselle; 60R=60% Roselle; 40R=100% Roselle; 20R=20% Roselle;

TSS Total soluble solids; TA Titratable acidity; RS Reducing sugars

Table 2: Colour parameters (Lightness (L*), redness (a*) and yellowness (b*) of roselle-fruit blends stored (0-6) months at 28ºC and 4ºC.

Parameters		L*				a*		b*	
Storage temp		28°C	4°C		28°C	4°C		28°C	4°C
Storage time	0	6	6	0	6	6	0	6	6
Mango									
20R	14.9±0.03	14.4±0.41	14.5±0.04	20.6±0.03	19.5±0.03	19.8±0.05	4.7±0.09	3.3±0.02	4.3±0.05
40R	16.3±0.40	15.4±0.03	15.7±0.02	19.8±0.03	18.3±0.04	18.7±0.03	5.7±0.06	4.4±0.040	4.6±0.04
60R	17.9±0.04	16.4±0.02	16.5±0.06	18.3±0.53	17.2±0.06	17.4±0.04	7.8±0.07	6.6±0.07	7.1±0.02
80R	18.6±0.03	17.3±0.04	17.6±0.05	16.7±0.05	14.6±0.06	15.4±0.03	8.5±0.07	7.3±0.05	7.5±0.05
Papaya									
20R	18.3±0.13	16.5±0.18	17.3±0.08	15.4±0.34	13.3±0.21	14.7±0.24	7.4±0.14	5.8±0.06	7.0±0.15
40R	17.8±0.38	16.2±0.89	17.2±0.12	16.2±0.38	13.3±0.23	15.6±0.07	6.4±0.11	4.9±0.11	5.6±0.07
60R	16.6±0.03	15.2±0.05	15.8±0.08	18.8±0.09	17.8±0.07	18.2±0.15	6.0±0.03	4.4±0.11	5.0±0.19
80R	15.8±0.02	14.2±0.43	15.2±0.06	19.9±0.07	17.9±0.10	19.3±0.07	4.9±0.07	3.7±0.04	4.4±0.04
Guava									
20R	19.6±0.41	18.4±0.64	19.1±0.47	16.5±0.24	15.0±0.19	15.7±0.67	7.3±0.46	5.7±0.28	7.0±0.50
40R	16.3±0.03	15.0±0.64	15.7±0.07	17.2±0.38	16.4±0.07	16.5±0.09	6.4±0.04	5.8±0.04	6.0±0.54
60R	15.8±0.56	14.2±0.10	15.0±0.10	17.6±0.04	16.1±0.14	16.6±0.41	5.3±0.02	3.8±0.34	4.6±0.06
80R	15.1±0.02	13.6±0.12	14.4±0.25	19.5±0.10	17.8±0.06	18.8±0.09	4.5±0.18	3.1±0.53	3.9±0.17

L* Lightness; a* redness; b* yellowness; 80R=80% roselle; 60R=60% roselle; 40R=100% roselle; 20R=20% roselle

Table 3: Main effects of fruit, temperature, blends and time on the physicochemical and antioxidant properties of roselle-fruit juice blends during storage

Parameters	TSS	pH	TA	RS	VITC	TMA	TPC	FRAP	L*	a*	b*
Fruit juice											
Mango	11.7a	2.8c	1.5c	4.1a	41.0b	179.7b	24.2c	1.4a	17.2a	17.9a	7.4a
Papaya	11.7a	3.2a	1.3c	4.0b	39.0c	203.4a	25.6b	1.4a	17.2a	17.0b	6.0c
Guava	7.3b	3.0b	1.4b	3.6c	59.6a	104.8c	27.0a	1.0b	15.9c	16.8c	6.3b
Storage temperature (°C)											
28°C	10.4a	3.0a	1.4a	4.1a	45.6b	159.6b	24.6b	1.3a	16.9a	16.4b	6.4b
4°C	10.1b	3.0a	1.4a	3.7b	47.4a	165.7a	26.5a	1.3a	16.6b	18.0a	6.7a
Storage time (Months)											
0	10.6a	2.9b	1.2d	3.5d	62.1a	213.0a	32.0a	1.5a	17.4a	17.8a	6.9a
2	10.2b	3.1a	1.3c	3.6c	48.8b	165.8b	26.4b	1.4b	17.0b	17.6b	6.7b
4	10.2b	3.1a	1.5b	4.0b	41.8c	159.9b	23.6c	1.3c	16.4c	17.0c	6.6c
6	10.0c	2.9b	1.6a	4.4a	33.4d	111.8c	20.4d	1.1d	16.1d	16.6b	6.0d

TSS, total soluble solids; TA, titratable acidity; RS, reducing sugars; Vit C, vitamin C; FRAP, ferric reducing antioxidant power; TMA, total monomeric anthocyanins; TPC, total phenolic content; L*, lightness; a*, redness; b*yellowness. Means separated in columns by main effects of Tukeys test. Numbers followed by the same letter are not significantly different ($P<0.05$).

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Figure legends

Figure 1: Vitamin C content for roselle-fruit blends stored for six months at 28ºC and 4ºC

Figure 2: Total monomeric anthocyanin content for roselle-fruit blends stored for six months at 28ºC and 4ºC

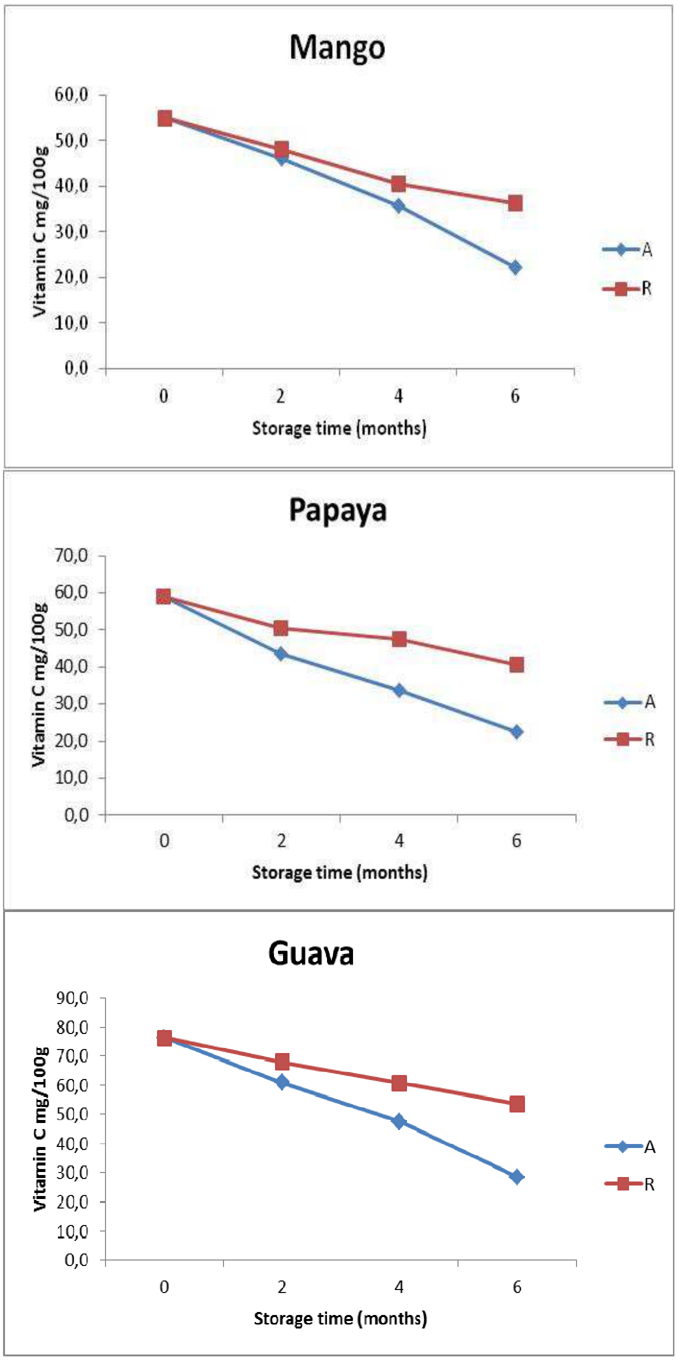
Figure 3: Total phenol content for roselle-fruit blends stored for six months at 28ºC and4ºC.

Figure 4: Antioxidant activity (FRAP) for roselle-fruit blends stored for six months at 28ºC and 4ºC

Figure 5: a & b Score and loading plots showing the effects of storage time the roselle-fruit juice blends at stored at 28ºC

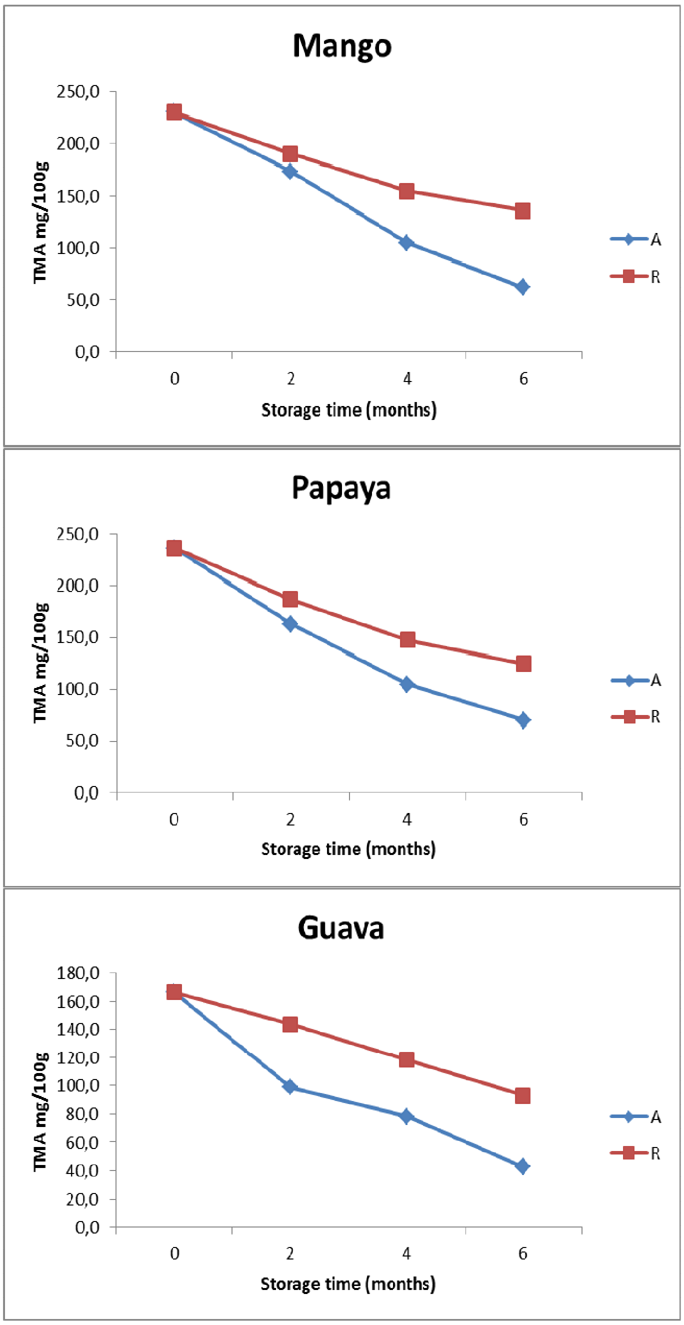
Figure 5: c & d Score and loading plots showing the effects of storage time the roselle-fruit juice blends at stored at 4ºC.

Figure 1



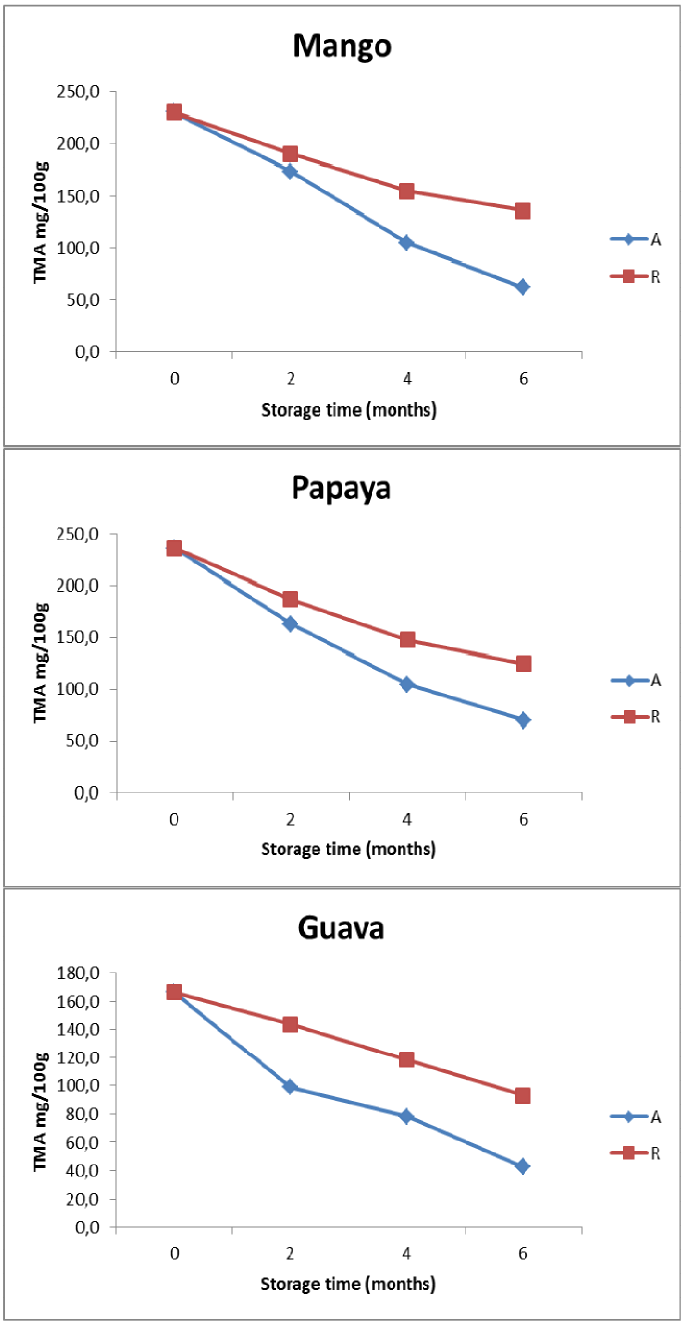
A Ambient temperature (28°C); R Refrigerated temperature (4°C)

Figure 2



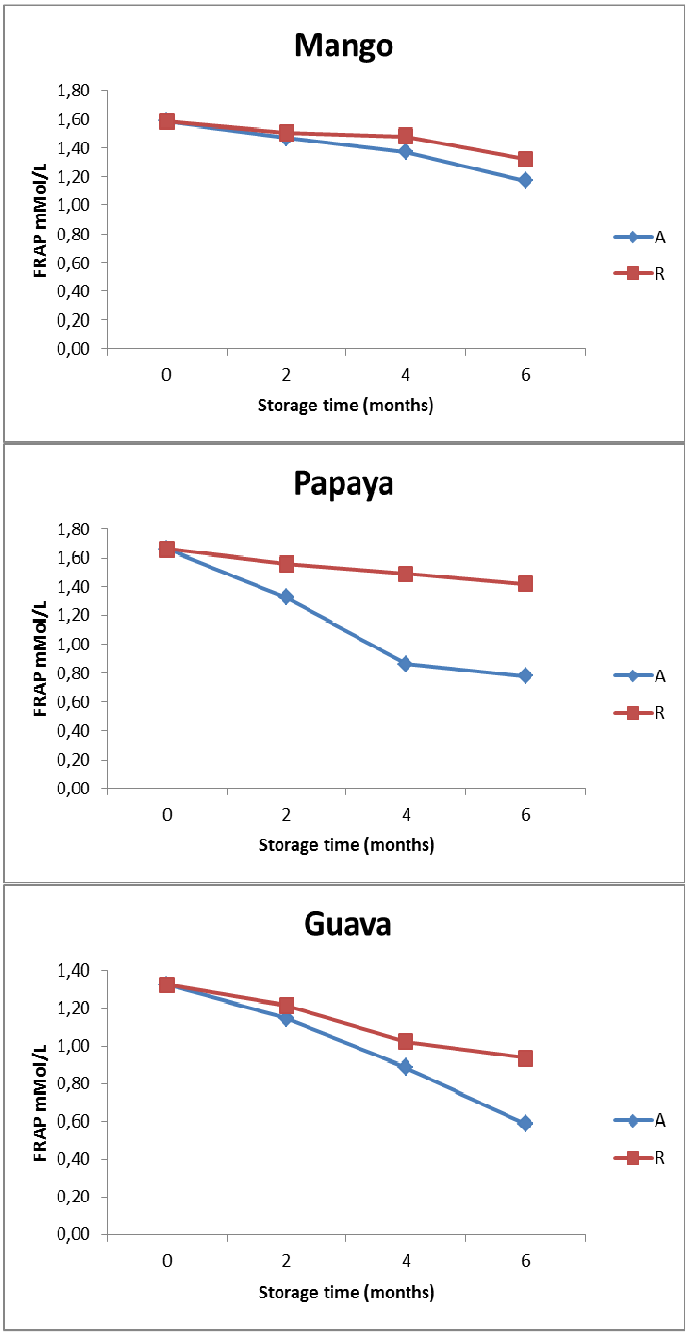
A Ambient temperature (28°C); R Refrigerated temperature (4°C)

Figure 3



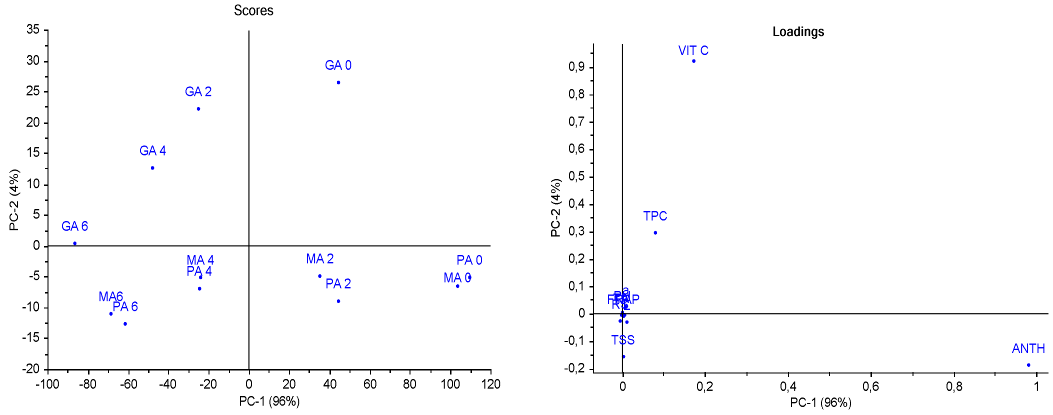
A Ambient temperature (28°C); R Refrigerated temperature (4°C)

Figure 4



A Ambient temperature (28°C); R Refrigerated temperature (4°C)

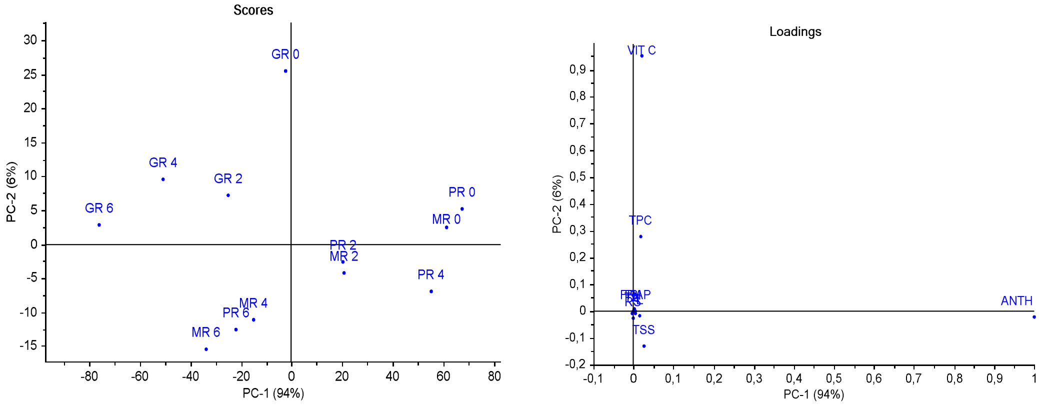
Figure 5 a & b



(a) (b)

M Mango; G guava; P papaya; R refrigerated temperature; A ambient temperature; 0, 2, 4, 6 storage time (months);

Figure 5 c & d



(c) (d)

M Mango; G guava; P papaya; R refrigerated temperature; A ambient temperature; 0, 2, 4, 6 storage time (months);

Paper IV

Physiochemical 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 and 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, colour, 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 bottles. The acidity, colour, 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.

Keywords: roselle; mango; juice blend; physiochemical properties; storage conditions

Introduction

Hibiscus sabdariffa L. (family *Malvaceae*), commonly known as roselle, red sorrel, or Karkadè, is widely grown in Africa, South East Asia, and some tropical countries of America (Abou-Ara *et al*., 2011, Amor and Allaf., 2009, Cisse *et al*., 2011., Sayago-Ayerd *et al*., 2007*)* Roselle produces red edible calyces with unique brilliant red colour and flavour. The calyces are commonly used in the production of jelly, juice, jam, wine, syrup, gelatine, pudding, cake, ice cream and flavouring (Duangmal *et al*., 2008, Hussein *et al*., 2010, Tsai and Huang 2004 and Tsai *et al*., 2002). 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*., 2009).

Two anthocyanins are dominant in roselle calyxes, 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 (Amor and Allaf., 2009, Cisse *et al*., 2011, Wong *et al*., 2002). Roselle anthocyanins render health benefits as a good source of antioxidants as well as a natural food colorant (Duangmal *et al*., 2008, Tsai *et al*., 2002). Anthocyanins possess antioxidative, antitumor, and anticarcinogenic activity (Fasoyiro *et al*., 2005 and Gonzalez *et al*., 2009). They are derivatives of the basic flavylium cation structure with electron deficient nucleus which make them highly reactive and their reaction involve discoloration of the anthocyanin pigments. Factors like light, pH, temperature, oxygen, ascorbic acid and sugar are contributing factors in degradation or stability of anthocyanins (Cisse *et al*., 2011, Chumsri *et al*., 2008, Tsai and Huang 2004).

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 overproduction, 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 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 at ambient and refrigerated temperatures. The aim of this study was to determine effects of packaging materials, storage temperature and time on physiochemical changes and antioxidant properties of roselle-mango juice blends.

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 horticulture unit at Sokoine University of Agriculture, Tanzania.

Dried roselle calyxes (10% moisture content) were grounded for 1 minute using a blender (Kenwood BL 440, Boulogne, France). Grounded calyces were mixed with water (1:10 w/v) and extracted using a water bath at 50^{o}C for 30 minutes (Chumry *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^{o}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^{o}C and 28^{o}C for six 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 (^{o}Brix) was measured with a hand refractometer (Mettler Toledo, Schwerzenbach, Switzerland) and expressed as %.

Colour measurements

The colour for roselle-mango 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 2º 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* for lightness, a* for redness and b*for yellowness.

Reducing sugars

Reducing sugars were determined by Luff-Schoorl method as described by Egan *et al*. (1981). Two grams of sample were weighed into a 100 mL measuring flask and 90 mL distilled water, where 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 minutes, cooled with tap water, and 1g potassium iodide and 10 mL 6N HCl were added. This mixture was titrated with 0.1N $Na_2S_2O_3$ until a yellow colour appeared followed by adding a few drops of starch solution, titrated continuously until a blue colour disappeared. Sugar content was then determined by interpolation in a table (Egan *et al*., 1981) after subtracting the blank assay to the volume of sodium thiosulfate of the titration. The results are expressed in mg/100g 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 20mL of sample was pipetted into 100 mL volumetric flask followed by 2 mL of 10% TCA solution and diluted to the 100 mL with distilled water. The sample was poured into a conical flask, swirled gently for one minute and left to stand for one minute and filtered (Whatman filter no 542). One mL of the sample or 1 mL of standard solution 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 760 nm using a Jenway 6405 UV/VIS Spectrophotometer (JENWAY, Essex, UK). The results are expressed in mg/100g FW.

Determination of antioxidant activity

Antioxidant activity for the roselle-mango blends was determined by the ferric reducing ability of plasma (FRAP) assay (Benzie & Strain, 1996) with some modifications. 3 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°C for 4 minutes, absorbance was measured at 593 nm using a spectrophotometer. An intense blue colour was formed when the ferric-tripyridyltriazine (Fe^{3+}-TPTZ) complex is reduced to the ferrous (Fe^{2+}) form. A range of iron sulphate concentrations from 0.25 to 2.0-mmol/L was used to prepare the calibration curve. The results are expressed as millimoles of (Fe^{2+}) per litre of fresh weight (mmol (Fe^{2+})/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 (0–500 mg/100g) was used for calibration of a standard curve. The results are expressed as milligrams of gallic acid equivalents per 100 gram of fresh weight (mg GAE/100 g FW).

The total monomeric anthocyanin content (TMA)

The total monomeric anthocyanin 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 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/mol-cm); 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 are expressed as mg cyanidin-3-glucoside equivalent/L extract (mg cyn-3-glu/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, USA). Multifactorial analysis of variance (General Linear Model (GML) was applied using a factorial design with three factors including packaging bottles (plastic, glass), storage temperature (ambient A, refrigerated R) and storage time (0, 2, 4, 6 months). Principal component analysis (PCA) was used to evaluate seasonal variation of blends stored in plastic bottles (2011 and 2012 seasons) using Unscrambler X 10.2 (CAMO Software AS, Norway).

Results and discussion

The initial physiochemical properties of roselle-mango juice blends (2011 and 2012) are shown in Table 1. The total soluble solids and reducing sugars were increasing with the increased concentration of mango juice in the blends as the fruit juice is known to contain high sugar content while roselle extract is low in sugar content (Wong *et al*., 2002). The pH of the roselle-mango fruit juices was increasing with decreasing concentration of roselle extract as roselle is low in pH while titratable acidity was increasing with increased concentration of roselle extract. Total monomeric anthocyanins, total phenol and FRAP was also decreasing with decreased concentration of roselle extract in the blends as the roselle extract is known to be a good source of anthocyanins (Wong *et al*., 2002) .

Total soluble solids (TSS) for roselle-mango blends stored in glass and plastic bottles ranged from 8.0-13.7 ºBrix (28°C) and 8.0-14.1 ºBrix (4°C) while reducing sugars (RS) ranged from 5.7-2.5 mg/100g (28°C) and 5.1-2.5 mg/100g (4°C) during six months of storage (Table 2). The results showed TSS and RS of roselle-mango blends increased during storage under all the storage temperature. The increase in TSS and RS was significant ($p<0.001$) with storage temperature and storage time and packaging*storage temperature (Table 5).

The TSS and RS increased gradually throughout storage, this might be due to hydrolysis of polysaccharides into monosaccharides and oligosaccharides (Bhardwaj & Pandey., 2011). Similar trend of increased TSS with increased storage time were observed in mango-sea buckthorn blended juice stored for 90 days (Khan *et al* 2012) and pomegranate kokum mango blends stored for 150 days (Waskar & Gaikwad 2004).

pH for roselle-mango blends stored in glass and plastic bottles ranged from 3.3-2.2 (28°C) and 3.5-2.2 (4°C) while titratable acidity (TA) ranged from 1.61-0.79 % (28°C) and 1.61-0.8 % (4°C) during six months of storage (Table 3). Roselle extract is known to have low pH and addition of mango juice has influence on the increased pH of the blends. The changes of TA were affected significantly by storage temperature ($p<0.05$) and packaging*storage temperature interaction ($p<0.001$) while pH was affected by storage temperature, storage time and their interactions ($p<0.001$).

Lightness (L*) for roselle-mango blends stored in glass and plastic bottles ranged from 18.6-13.3 (28°C) and 18.6-13.5 (4°C) for six months of storage as shown on Table 4. The L* value which is can be an indicator of lightness of colour, decrease with increased storage time. The decrease might be due to non enzymatic 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 juices stored at 25°C. Martí *et al*. (2002) also reported a significant decrease in L value during storage period of 150 days at 25 °C, resulting in darker colour during the storage period of pomegranate juice.

Redness (a*) for roselle-mango blends stored in glass and plastic bottles ranged from 20.6-14.6 (28°C) and 20.6-15.2 (4°C) for six months of storage. Yellowness (b*) for roselle-mango blends stored in glass bottles ranged from 8.2-2.7 (28°C) and 8.2-2.8 (4°C) after six months of storage. The yellowness in roselle-mango juice blends is due to presence of carotenoids in mango juice. However, these carotenoids are highly susceptible to degradation by external agents such as 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).

According to results shown in Figure 1, total monomeric anthocyanin of the roselle-mango juice blends (40%R) stored in glass bottles at 4°C were higher than those stored at 28°C. The decrease was significant ($p<0.05$) during storage, irrespective of storage temperature and packaging material (Table 5 and 6). Waskar & Gaikwad (2004) observed similar trends on pomegranate kokum mango based blends stored for 150 days. The amount of anthocyanin remaining after six months (127.7-144.1 mg/100g) at 4°C and 100-107 mg/100g at 28°C in all roselle-mango blends (40%) stored in glass and plastic bottles , these amounts were sufficient to provide the amount recommended by 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 being sensitive to oxygen, light and heat can be easily oxidized in presence of oxygen by both enzymatic and non-enzymatic catalyst (Jawaheer *et al*., 2003).Vitamin C losses was lower in roselle-mango juice blends stored in glass bottles (Figure 2). Similar results were observed by Alaka et al., 2003 when mango juices were packaged in polyethylene films, polyethylene tetraphthalate (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 during storage, which was demonstrated by the value of 30.9 mg GAE/100g initially for roselle-mango blends (40%R) which decreased to 18.8 (28°C) and 20.1 (4°C) after 6 months of storage (Fig c). All variables (time, temperature, and packaging and interaction term, time*temperature, time*packaging and temperature*packaging) significantly contributed to the loss of TPC (Table 5).

Ferric reducing ability of plasma (FRAP) for roselle-mango blends stored in glass and plastic bottles ranged from 1.86-1.04 mMol/L (28°C) and 1.86-1.19 mMol/L (4°C) after six months of storage. 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 capacity due to degradation of monomeric anthocyanins (Tsai and Huang 2004).

For the case of seasonal variation and storage time and temperature, a bi-plot of observations and variables is shown in Figure 2. 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 (colour L*, a*,b* RS, FRAP and TMA)and the second components account for 17% of the total variation associated with parameters (TSS, Vitamin C and TPC). The PC1 explained roselle-mango juice blends stored in plastic bottles at ambient temperature with more blends from season 2011 while PC2 explained blends stored at refrigerated temperature with more blends from season 2012.

The roselle-mango juice blends stored at refrigerated temperature for zero and two months were on the positive side of PC2 while those blends stored for four and six months were on the negative side of PC2. Those blends stored at ambient temperature for zero and two months were on the positive side of PC1 while those blends stored at ambient temperature for six months were on the negative side of the PC1.

These results showed the effect of seasonality and the storage conditions as PC1 contained most of the blends from season 2011 and those blends stored at ambient temperature while the PC2 contained most blends from season 2012 and those blends stored at refrigerated temperature.

The results shows that most of blends 2012 blends had high levels of TPC, TSS and Vitamin C (Figure 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 four and six months being on the negative side of the PCs. The Bi plots also showed the TPC, TMA and Vitamin C being parameters mostly affected by the storage time regardless of storage temperature.

Conclusions

The roselle-mango blends presented some chemical changes during six months storage. The most affected components were total monomeric anthocyanins, 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 vitamin C and total monomeric anthocyanin essential in antioxidant capacity of fruits and fruit products. Seasonality and packaging material, storage time and temperature have shown to influence 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 six months of storage of the roselle-mango juice blends (40%R) was more than sufficient to provide recommended amount for daily intake of vitamin C for adults.

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 (mg/100g FW)	L*	a*	b*	Vit C (mg/100g FW)	TMA (mg/L)	T (mg/10 GA
2011	0R	14.0 a ±0.50	3.4a ±0.12	0.3 c ±0.43	5.9 a±0.05	42.4 a ±0.7	06.6e ±0.3	43.9a±0.6	62.2 a ±0.00	48.0 f ±0.75	10.9 ±5.2
	20R	10.6 a ±0.49	2.8 b ±0.14	1.4 b ±0.00	5.6 b±0.00	18.6b ±0.4	16.4 d ±0.2	8.5 b ±0.2	58. 5 b ±0.00	134.7 e ±1.50	21.3 ±0.0
	40R	9.9 a ±0.19	2.7b ±0.01	1.9 b ±0.43	5.1 c±0.00	17.6c±0.3	18.1 c ±0.7	7.7c±0.5	53.0 c ±0.00	282.6 d ±1.81	28.8 ±0.0
	60R	7.5 b ±0.63	2.4 c ±0.06	3.1 a ±0.40	4.5 d±0.00	16.1d ±0.2	19.2a ±0.4	5 6d±0.2	44.4 d ±0.00	335.2 c ±1.54	37.9 ±0.0
	80R	6.9 b ±0.20	2.6c ±0.01	1.4 b ±0.00	3.5e ±0.0 0	14.7 e ±0.1	20.0a ±0.5	4.7e ±0.6	40.0 e ±0.00	493.5 b ±5.15	53.7 ±0.0
	100R	5.7 c ±0.10	2.3 d ±0.01	1.9 b ±0.00	2.4 f±0.00	14.3 e ±0.0	20.6a ±0.0	3.9f ±0.0	37.4 f ±0.00	555.3 a ±2.03	54.6 ±0.8
2012	0R	15.5a±0.13	3.1a±0.01	0.3f±0.02	5.2a±0.02	42.4a±0.80	14.6e±0.02	44.5a±0.05	65.3a±0.01	32.9f±0.01	14.5f±0.
	20R	13.8b±0.07	2.9b±0.01	0.8e±0.02	4.4b±0.00	18.6b±0.03	16.7d±0.04	8.5b±0.06	60.5b±0.01	82.4e±0.05	23.4e±0.0
	40R	11.2c±0.12	2.5c±0.01	1.3d±0.03	3.6c±0.01	17.9b±0.03	18.3c±0.49	7.8c±0.07	55.1c±0.02	236.3d±0.38	30.8d±0.0
	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±0.06	47.2d±0.03	280.5c±0.01	38.4c±0.
	80R	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.
	100R	5.9f±0.07	2.1f±0.01	1.8a±0.03	2.0f±0.03	14.5e±0.02	20.9a±0.02	3.7f±0.04	39.3f±0.08	572.3a±0.01	56.3a±0.0

100R=100% roselle, 80R=80% roselle; 60R=60% roselle; 40R=40% roselle; 20R=20% roselle and 0=0% roselle.

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

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

	TSS (%)					RS (mg/100 g FW)				
Packaging materials		Glass		Plastic			Glass		Plastic	
Storage (M)	0	6	6	6	6	0	6	6	6	6
Temp		28°C	4°C	28°C	4°C	0	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.

	pH					TA (%)				
Packaging material		Glass		Plastic			Glass		Plastic	
Storage (M)	0	6	6	6	6	0	6	6	6	6
Temp		28°C	4°C	28°C	4°C	0	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±0.0
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±0.0
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±0.0
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±0.0

TA titratable acidity, M months, 80R=80% roselle; 60R=60% roselle; 40R=100% roselle; 20R=20% roselle

Table 4. Initial and final colour parameters (lightness L*, redness a* and yellowness b*) of roselle-mango juice blends stored in glass and plastic bottles at 28°C and 4°C

		L*				a*		b*	
Storage temp		28°C	4°C		28°C	4°C		28°C	4°C
Storage time	0	6	6	0	6	6	0	6	6
Glass									
80R	14.9±0.03	14.4±0.41	14.5±0.04	20.6±0.03	19.5±0.03	19.8±0.05	4.7±0.09	3.3±0.02	4.3±0.05
60R	16.3±0.40	15.4±0.03	15.7±0.02	19.8±0.03	18.3±0.04	18.7±0.03	5.7±0.06	4.4±0.040	4.6±0.04
40R	17.9±0.04	16.4±0.02	16.5±0.06	18.3±0.53	17.2±0.06	17.4±0.04	7.8±0.07	6.6±0.07	7.1±0.02
20R	18.6±0.03	17.3±0.04	17.6±0.05	16.7±0.05	14.6±0.06	15.4±0.03	8.5±0.07	7.3±0.05	7.5±0.05
Plastic									
80R	14.9±0.03	13.3±0.03	13.5±0.05	20.6±0.03	17.8±0.06	17.9±0.07	4.7±0.09	2.8±0.06	2.7±0.06
60R	16.3±0.40	13.8±0.05	14.2±0.05	19.8±0.03	17.1±0.05	17.2±0.04	5.7±0.06	3.3±0.05	3.7±0.05
40R	17.9±0.04	14.7±0.06	15.1±0.04	18.3±0.53	15.3±0.07	15.2±0.05	7.8±0.07	4.6±0.41	5.9±0.05
20R	18.6±0.03	16.5±0.08	16.8±0.05	16.7±0.05	16.1±0.02	16.7±0.04	8.5±0.07	6.4±0.08	6.4±0.05

80R=80% roselle; 60R=60% roselle; 40R=100% roselle; 20R=20% roselle

Table 5: Probability level of significance (ANOVA) on the quality of roselle-mango juice blends (40% roselle).

Source of variation	TSS (%)	pH	TA (%)	RS (mg/100g FW)	TMA (mg/L FW)	Vit. C (mg/100g FW)	TPC (mg/100g GAE FW)	FRAP (mmol/100g FW)	L*	a*	b*
Packaging (A)	ns	ns	ns	ns	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Temperature of storage(B)	<0.001	<0.001	0.037	<0.001	<0.001	0.006	<0.001	ns	0.05	ns	<0.001
Time of storage (C)	<0.001	<0.001	ns	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
AXB	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	ns	ns	<0.001
AXC	ns	<0.001	ns	ns	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
BXC	ns	ns	ns	ns	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

ns =non significant

TSS, total soluble solids; TA, titratable acidity; RS, reducing sugars; Vit C, vitamin C; FRAP, ferric reducing ability of plasma; TMA, total monomeric anthocyanins; TPC, total phenolic content; L*, lightness; a*, redness; b*yellowness.

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Figure caption

Figure 1: Total monomeric anthocyanin for (TMA) roselle-mango juice blend (40% roselle) stored in glass and plastic bottles for 6 months at 28°C and 4°C.

Figure 2: Vitamin C content for roselle-mango juice blend (40% roselle) stored in glass and plastic bottles for 6 months at 28°C and 4°C

Figure 3: Total phenol content (TPC) for roselle-mango juice blend (40% roselle) stored in glass and plastic bottles for 6 months at 28°C and 4°C

Figure 4: Antioxidant capacity (FRAP) for roselle-mango juice blend (40% roselle) stored in glass and plastic bottles for 6 months at 28°C and 4°C

Figure 5: Bi-plot of scores and loadings of the PC on roselle-mango juice blends (40R) stored at ambient and refrigerated temperature for six months (season 2011 and 2012)

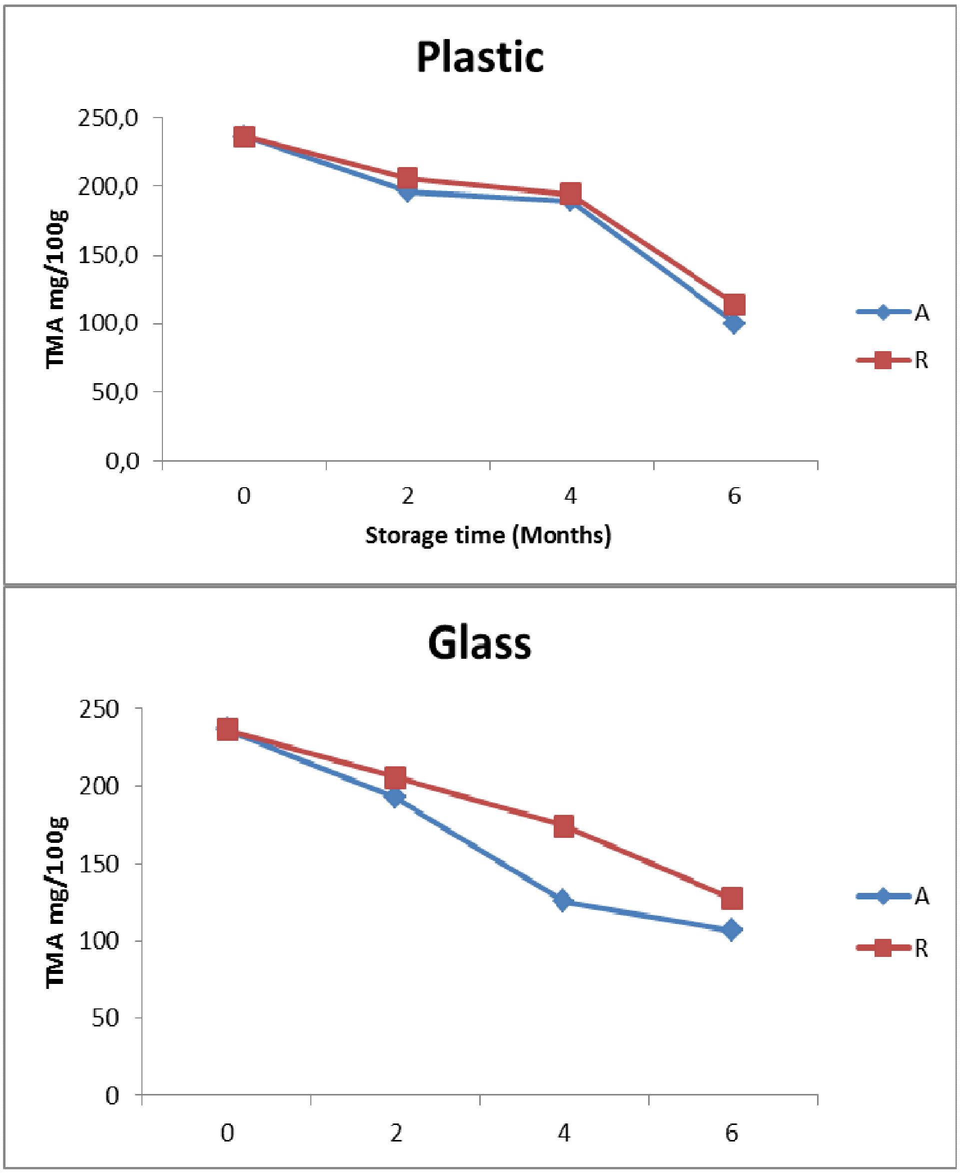

Plastic
TMA mg/100g
250,0
200,0
150,0
100,0
50,0
0,0
0
2
4
6
Storage time (Months)
A
R
Glass
TMA mg/100g
250
200
150
100
50
0
0
2
4
6
A
R

Figure 1

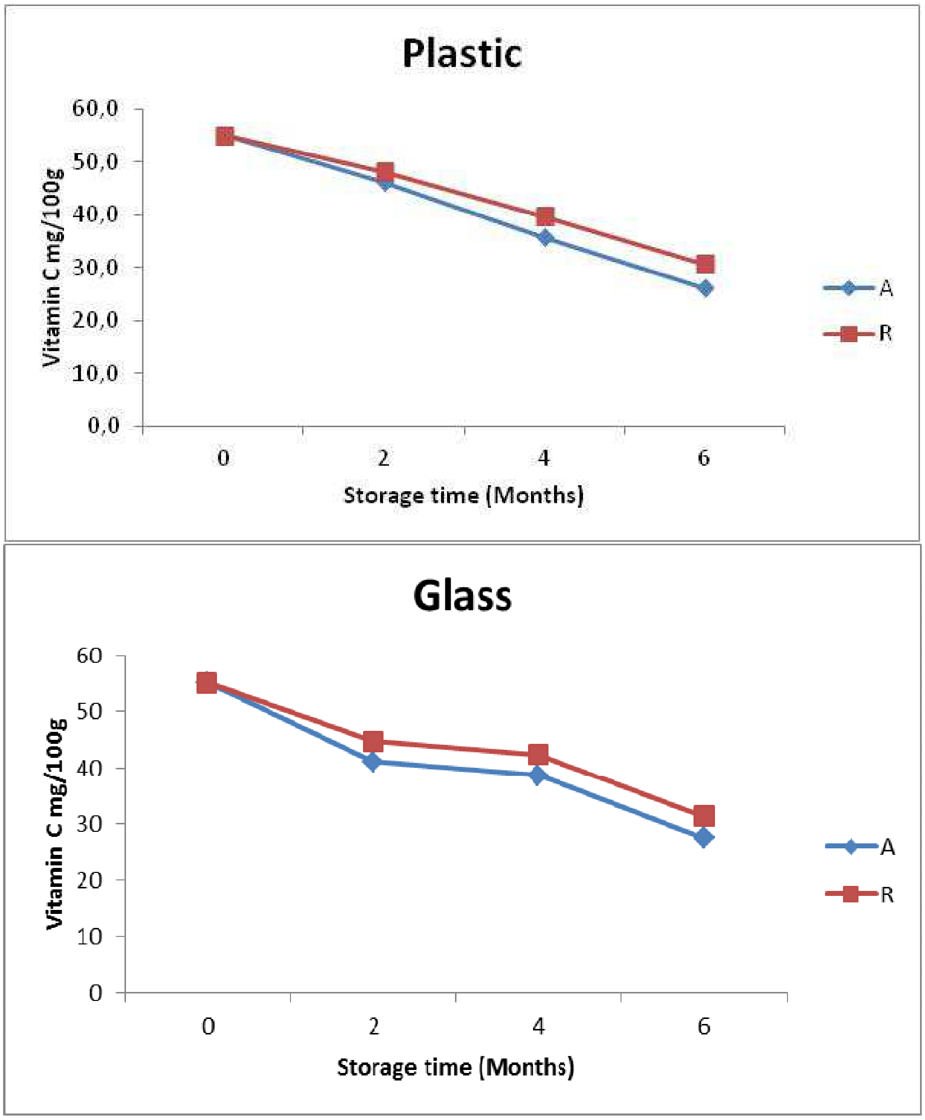

Plastic
Vitamin C mg/100g
60,0
50,0
40,0
30,0
20,0
10,0
0,0
0
2
4
6
Storage time (Months)
A
R
Glass
Vitamin C mg/100g
60
50
40
30
20
10
0
0
2
4
6
Storage time (Months)
A
R

Figure 2

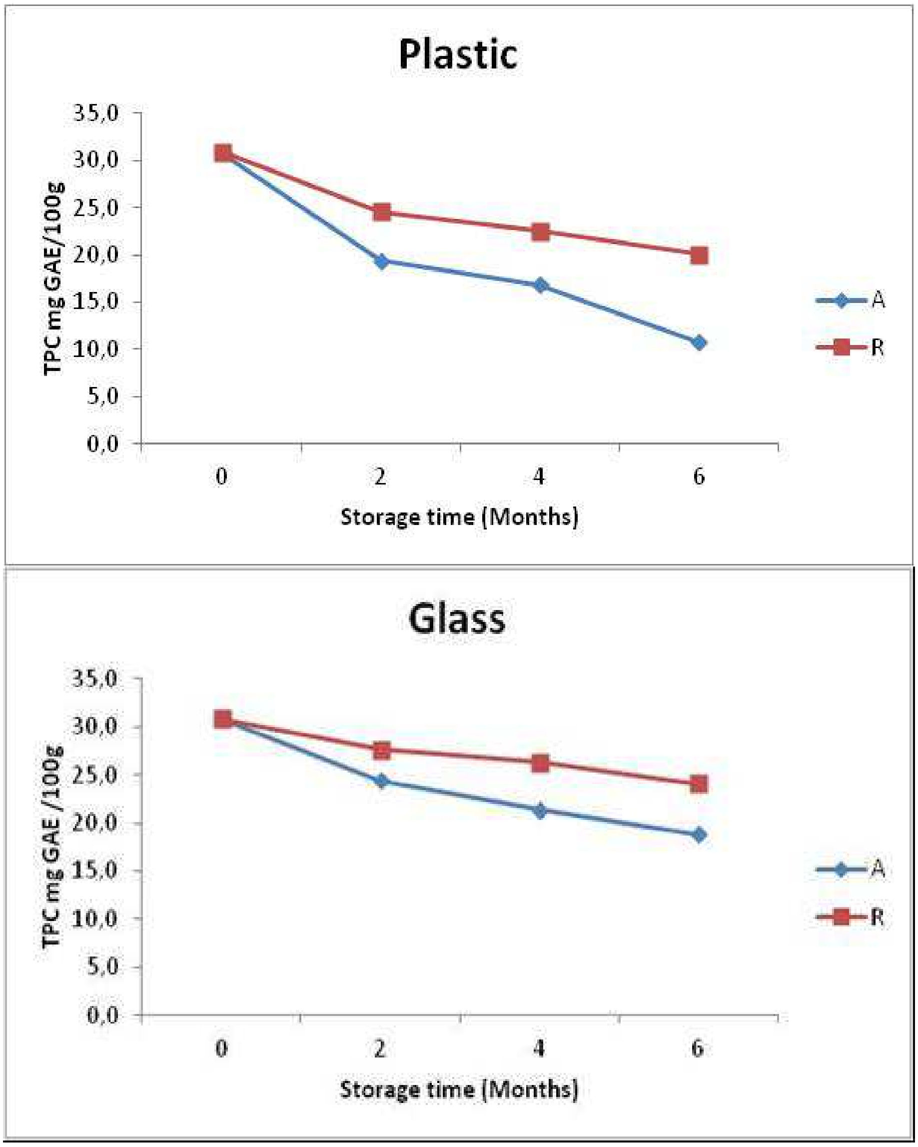
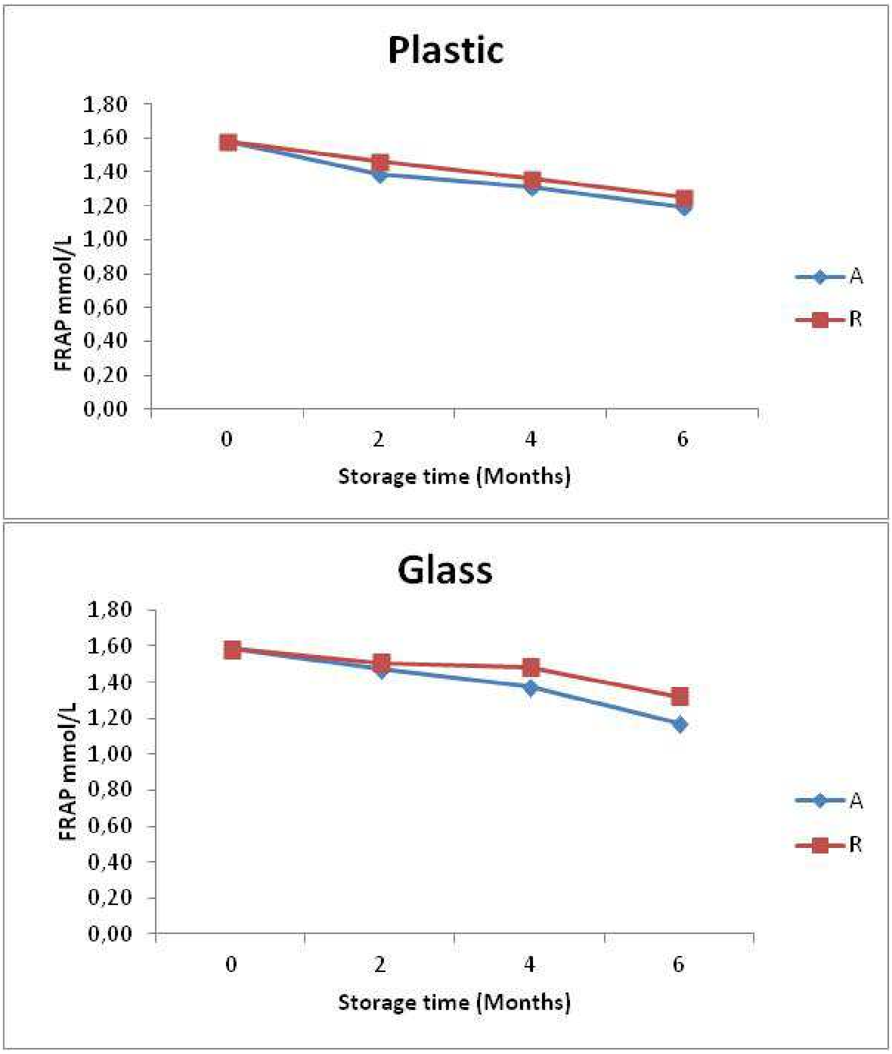

Plastic
TPC mg GAE/100g
35,0
30,0
25,0
20,0
15,0
10,0
5,0
0,0
0
2
4
6
Storage time (Months)
A
R
Glass
TPC mg GAE /100g
35,0
30,0
25,0
20,0
15,0
10,0
5,0
0,0
0
2
4
6
Storage time (Months)
A
R

Figure 3



Plastic
FRAP mmol/L
1,80
1,60
1,40
1,20
1,00
0,80
0,60
0,40
0,20
0,00
0
2
4
6
Storage time (Months)
A
R
Glass
FRAP mmol/L
1,80
1,60
1,40
1,20
1,00
0,80
0,60
0,40
0,20
0,00
0
2
4
6
Storage time (Months)
A
R

Figure 4

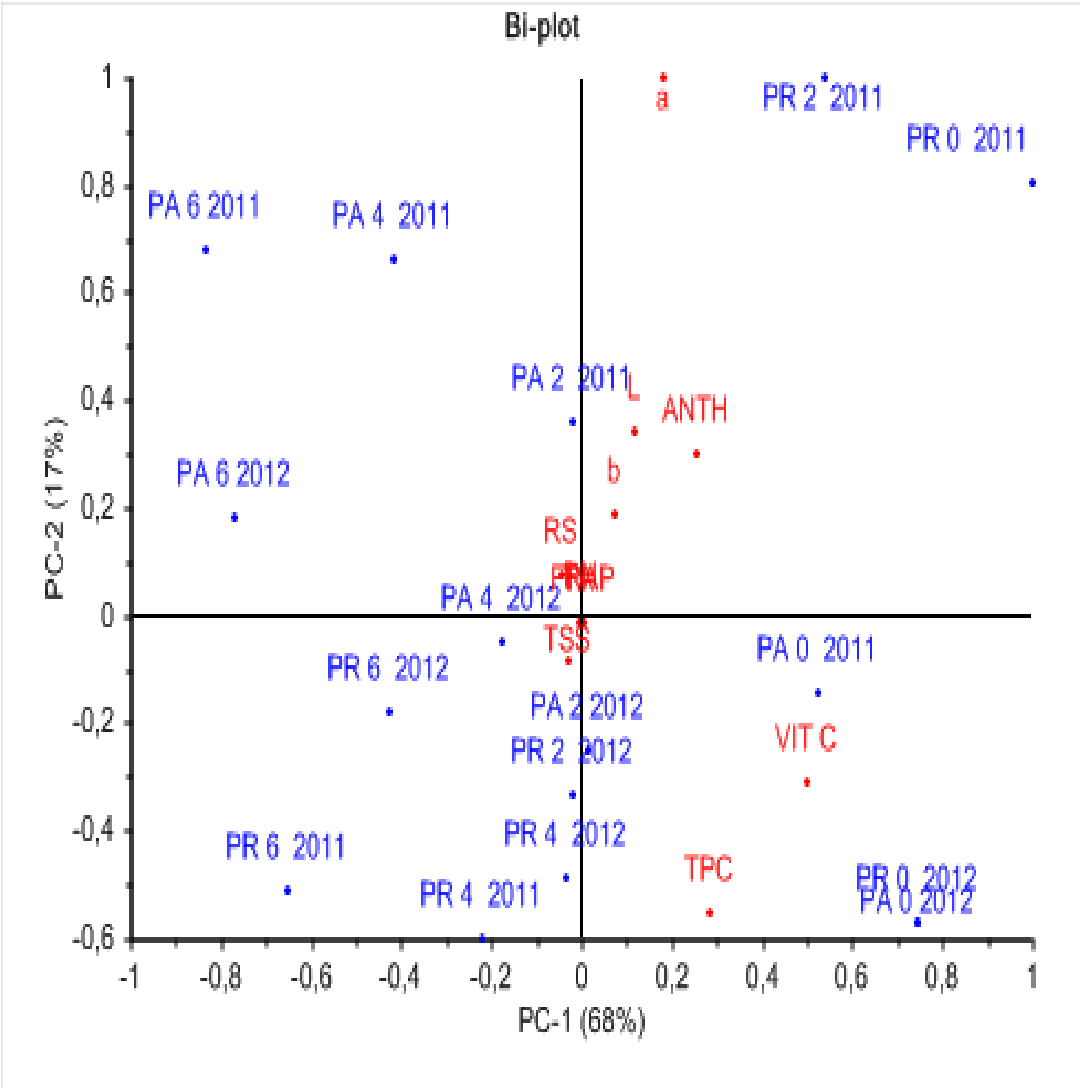

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Figure 5

Paper V

Determination of organic acids and sugars and consumer acceptability of roselle-fruit juice blends

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Abstract

Roselle extract was blended with mango, papaya and guava to produce roselle-fruit blends and organic acids (malic, succinic, citric and tartaric) and sugars (glucose and fructose) were determined using HPLC. In addition, consumer acceptability of roselle-fruit blends was evaluated using un-trained judges. The results showed significant ($p<0.05$) reduction of glucose and fructose with increased amount of roselle extract in the blends .The organic acids (malic, succinic and tartaric were decreased significantly ($p<0.05$) with increased concentration of roselle extract.

All roselle-fruit blend (20R) scored almost highest in all the organoleptic properties (colour, flavor, taste, appearance, odour consistency (mouthfeel) and acceptability) with roselle-mango blends highly acceptable by panelists followed by roselle-papaya and roselle-guava. Addition of fruit juices in the roselle extract had shown to increase sugar content and reducing the acidity of the roselle-fruit juices hence high acceptability of blends with high

sugar contents. The increase in roselle extract in the blends led to high colour score in all the roselle fruit juice blends.

Keywords: roselle extract, fruit juices, organic acids, sugars, sensory attribute

INTRODUCTION

Juicy extracts are produced from various types of plants, mostly their leaves, flowers and fruits. There has been high demand for these juices due to their nutritive values, varying in attractive colours, aroma and flavour [1]. One such plant whose flowers are used to prepare juices is *Hibiscus sabdiriffa*, commonly known as roselle.

Roselle is a tropical plant initially cultivated for the use of its leaves, seed and stem, but is now grown commercially for the use of the calyces. Roselle juice is known to be highly acidic with a mixture of organic acids such as citric, malic and tartaric acids, and low sugar content [2, 3, 4]. Organic acids and sugars have effects on the chemical and sensorial characteristics of the fruit. While organic acids give different perception of acidity, sugars present different sweetness level [5].The whole plant can be used as raw material for beverage, or the dried calyces can be soaked in water to prepare a colourful drink, consumed either cold or hot [6].

The low pH of roselle extract is known to cause sour taste, hence the need for intensive sweetening and flavouring to attain consumer acceptability. Roselle extract can be blended with various fruits to produce fruit-flavoured roselle drinks. Blending will give beverages with improved organoleptic quality and higher nutritive values richer in vitamins and minerals. Organoleptic assessment is influenced greatly by the relative and total amounts of sugars and acids in fruits and fruit juices [7]. The determination of organic acids and sugars in beverages is very important, as their presence and relative ratio have effect on both chemical and sensory characteristics of the product including, pH, total acidity, sweetness and consumer acceptability. It will also provide information on food wholesomeness or how to optimize some selected technological processes

Organic acids are natural compounds in fruits and vegetables [5, 8]. They originate from biochemical processes or from the activity of some microorganisms such as yeasts and bacteria [5]. The nature and concentration of organic acids (malic, citric, tartaric, succinic) and sugars (including glucose, fructose and sucrose) have effect on the organoleptic quality (flavour, colour, and aroma) in fruits and fruit products. The flavour of the flesh fruits dependent on the balance between soluble sugars and non-volatile organic acids. The presence of sugars gives different sweetness levels while the organic acids give different perception of acidity [9] also have influence on the stability and microbiological control of fruits and fruits products [10]. Consequently, the composition of these sugars and organic acids, as well as sugar/acid balance, probably influences the taste of the flesh [5].

In this study, roselle extracts were blended with mango, guava and papaya in different volume ratios. The aim was to determine the organic acid and sugars in these blends as they directly affect flavor and taste and hence general acceptability. In addition, sensory evaluation of the same blends was carried out to assess the acceptability of the blends.

MATERIALS AND METHODS

Fruit material

Dark red dried roselle calyces were purchased from the Municipality market in Morogoro, Tanzania. Guava (pink variety), papaya (‘Solo’) and mango (‘Dodo’) were purchased from the horticulture garden at Sokoine University of Agriculture (SUA), Tanzania, and brought to the Department of Food Science and Technology.

Roselle extract preparation

Roselle extract was prepared according to [11] with modifications. Dried roselle calyxes (10% moisture content) were grounded for 1 minute using a blender (Kenwood BL 440). Dried and grounded roselle calyces were mixed with distilled water (1:10), extracted in a water bath at 50^{o}C for 30 minutes followed by filtration with a cheese cloth. The extract was kept at 4^{o}C before further mixing and analyses.

Fruit juice preparation

Fully matured fruits of premium quality of mango, papaya and guava were used. The fruits were thoroughly washed, peeled and cut into small pieces, except for guava, which were not peeled. The fruit pieces were put into a juice extractor (Kenwood JE 810) to make juice. The different fruit juices were stored at 4^{o}C before mixing with roselle and further analysed.

Preparation of roselle-fruit juice blends

Three different beverage products of roselle-mango, roselle-papaya and roselle-guava were mixed in the ratio of 80:20 (80R), 60:40 (60R), 40:60 (40R) and 20:80 (20R) roselle extract: fruit juice pulp, respectively. In addition, pure roselle extract (100R) and fruit juices (0R) were analysed. Sodium benzoate (1 g/L) and citric acid (1 g/L) were added to all roselle-fruit blends as preservatives.

Juices were bottled in 100 ml sterilized plastic bottles, loosely capped and pasteurized in a water bath at a temperature of 82.5^{o}C for 20 min and cooled rapidly to room temperature by immersing the bottles in a cold water bath.

Sensory evaluation

The panelists were semi-trained and randomly selected from the student, academic and non-academic staff of the Department of Food Science and Technology, at Sokoine University of Agriculture, Morogoro, Tanzania. Participants did not receive any information about the nature, content, nutritional value or potential health benefits of the fruit juices they evaluated. They were only informed that the study concerned tropical fruit juices mixed with a plant extract.

The panellists were asked to read through the questionnaires, and the meaning of each attribute (colour, taste, flavour, odour, consistency (mouthfeel), and overall acceptability) was explained to avoid any misinterpretation. For each roselle-fruit juice sample, participants were asked to score each attribute according to a 9-point Hedonic scale where 9 was “like extremely” and 1 was “dislike extremely”. In total, 90 questionnaires integrating sensory tests of three Roselle tropical fruit blends i.e. Roselle-mango (*n*= 30), Roselle-papaya (*n*=30) and Roselle-guava (*n*=30). The roselle-fruit blends (20R, 40R, 60R and 80R), labeled with a random 3-digit code were served refrigerated in transparent plastic cups (30 mL juice in a 50 mL cup) to the panelists.

Determination of organic acids and sugars

The concentration of some organic acids (e.g. citric, succinic, tartaric and malic) and carbohydrates (e.g. glucose and fructose) in roselle-fruit blends was analyzed by High Performance Liquid Chromatography (HPLC) as described by Castellari et al [12]. Perkin Elmer series 200 HPLC system (Norwark, CT, USA) equipped with a pump system, a refractive index detector (RID-200 SERIES) for sugar analysis, and a UV/Vis detector (SPD-20A) monitored at 210 nm, for the analysis of organic acids. Sugars and organic acids were simultaneously analyzed onto an Aminex HPX-87H column (300×7.8 mm) (Bio-Rad) and kept at 32 °C. The analytical conditions used were as follows: flow 0.4 mLmin−1, eluent 0.05N H_2SO_4 with 6% acetonitrile (v/v) Results are presented as mg of sugar or acid per kg of sample.

Statistical analysis

Data obtained from the study were analyzed using means and standard deviations. Analysis of variance (ANOVA) and Tukey method was used to test significant difference between means. Significance was accepted at P<0.05 using Minitab (Version 16.0, 2008, Minitab Statistical Software, Minitab Inc., Enterprise Drive State College, PA, USA).

Results and discussions

The quantity of organic acid and sugar for roselle-fruit blends (0-100% roselle) are shown in figure 1. The quantity of organic acid and sugar were succinic acid (21.7-0.7) $\times 10^2$mg/kg, citric acid (1.9-4.0)$\times 10^2$mg/kg, tartaric acid (0.19-0) $\times 10^2$mg/kg, malic acid (0.76-0.14) $\times 10^2$mg/kg, glucose (8.15-1.88) g/kg and fructose (7.04-2.04) g/kg.

The major sugars in roselle were found to be glucose followed by sucrose and fructose (Amusa et al., 2012, Wong et al 2002). Succinic and citric acid was found to be higher in the roselle extract while Wong et al 2002 and Babalola et al., 2001 identified oxalic, tartalic, and succinic acids and however succinic acid and oxalic acid were predominant acids. The main acids encountered in fruits are tartaric, malic, citric, succinic, lactic and acetic acids (Tasnim et al., 2010)

The results showed that as the concentration of roselle extract decreased in the blends, the quantity of organic acid, except for citric acid, decreased and the sugar concentration increased (Table 1). Pure roselle extract is known to be low in sugar and highly acidic (Jung et al 2013) while the different fruits used in the blends have high sugar content. Succinic acid was the predominant acid in the roselle-fruit blends with high concentration of roselle (80R) while citric acid was the predominat acid in roselle-fruit blends with low concentration of roselle (20R) (Table 1). The amount of fructose and glucose in blends with high fruit content (20R) was higher compared to blends with high roselle content (80R).

Sensory characteristics of any food product contribute significantly to its consumer acceptance or rejection. Thus, sensory evaluation of food using panelists is routinely carried out to evaluate the acceptability of food product [13]. Appearance, flavour and colour are the most important attributes determining consumer’s choices of food products. The sensory attributes of the roselle-fruit blends are shown in Table 2.

All roselle-fruit blend (20R) scored almost highest in all the organoleptic properties (colour, flavor, taste, appearance, odour consistency (mouthfeel) and acceptability) however the roselle-mango had the highest acceptability by panelists followed by roselle-guava and roselle-papaya blend. The increase in levels of roselle extract in all the roselle-fruit blends (60% and 80% roselle) resulted in decreased sensory score which might be due to increased acidity in the blends.

The maximum scores for all sensory attributes (odour, consistency (mouthfeel) and overall acceptability) in roselle-fruit blends was found for 20% roselle and 40% roselle, and minimum for all roselle blends with 80% and 60% roselle. Colour was the attribute that panelists rated higher in all roselle fruit juice blends, however roselle fruit juice blends with 80% roselle were rated highest. The decreased concentration of roselle extracts in the blends

(Table 1) lowered colour scores in all the roselle fruit blends. This is an indication that the red colour of roselle extract was very attractive to panelists. Roselle extract is known to be a good source of anthocyanins [2, 3, 14, 15], which imparts the red colour to the blends. Colour play a very important role in the acceptability of foods as it is one of the principal characteristics perceived by the senses and is used by consumers for the rapid identification and ultimate acceptance of foods [16]. Colour and taste is known to play a major role in the acceptability of zobo (roselle) beverage by consumers [17].

Conclusion

Addition of tropical fruit juice in roselle extract has shown to reduce the acidity of the blends and also increased sweetness of roselle-fruit blends. All roselle-fruit blends with 20% roselle had higher acceptability

The addition of tropical fruit juice with high sugar content in roselle extract can reduce the sourness of the blend. However, we suggest that the choice of fruit to add to the blend should depend on the availability of fruits.

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Table 1 Organic acid and sugar composition of roselle-fruit blends of 0-100% roselle.

	$\times10^{2}$mg/kg				$\times10^{3}$mg/kg	
Blends	Citric	Succinic	Tartaric	Malic	Fructose	Glucose
Mango						
100R	1.4^{d}	9.7^{a}	0.25^{a}	0.9^{a}	1.9^{c}	2.0^{b}
80R	1.7^{d}	8.3^{b}	0.18^{ab}	0.35^{b}	1.8^{c}	2.2^{b}
60R	1.9^{d}	1.9^{c}	0.18^{ab}	0.31^{bc}	2.3^{bc}	2.4^{b}
40R	2.8^{c}	1.0^{d}	0.14^{bc}	0.26^{bc}	3.0^{b}	2.7^{b}
20R	3.4^{b}	1.0^{d}	0.07^{cd}	0.21^{bc}	3.1^{b}	2.7^{b}
0R	4.0^{a}	0.7^{d}	0.04^{d}	0.20^{c}	8.1^{a}	7.0^{a}
Papaya						
100R	2.9^{c}	22.1^{a}	0.19^{a}	0.76^{a}	1.9^{b}	2.4^{b}
80R	3.1^{c}	21.0^{a}	0.18^{a}	0.32^{b}	1.9^{b}	2.6^{b}
60R	3.6^{bc}	16.6^{b}	0.11^{ab}	0.30^{b}	2.0^{b}	2.7^{b}
40R	4.1^{ab}	14.9^{b}	0.10^{ab}	0.26^{b}	2.6^{b}	3.3^{b}
20R	4.1^{ab}	8.3^{c}	0.08^{b}	0.24^{b}	3.1^{b}	4.0^{b}
0R	4.6^{a}	3.2^{d}	n.d	0.21^{b}	7.4^{a}	6.6^{a}
Guava						
100R	1.7^{c}	23.7^{a}	0.19^{a}	0.38^{a}	1.1^{b}	2.0^{b}
80R	3.1^{b}	11.7^{b}	0.18^{a}	0.26^{ab}	1.9^{ab}	2.0^{b}
60R	3.2^{b}	11.4^{b}	0.17^{a}	0.25^{b}	2.2^{a}	2.4^{b}
40R	3.4^{b}	8.1^{c}	0.15^{a}	0.23^{b}	2.3^{a}	2.8^{b}
20R	3.9^{b}	5.2^{d}	n.d	0.21^{b}	2.3^{a}	2.9^{b}
0R	7.3^{a}	1.0^{e}	n.d	0.14^{b}	2.8^{a}	5.0^{a}

Means in the same columns followed by different letters are significantly different at $p<0.05$. 80R=80% roselle/20% fruit juice, 60R= 60% roselle /40% fruit juice, 40R= 40% roselle /60% fruit juice, 20R= 20% roselle /80% fruit juice.

Table 2 Sensory assessments of roselle-fruit blends (n = 30).

Fruit juice	Blends	Colour	Taste	Flavour	Odour	Consistency	Overall acceptability
Mango	80R	7.0ab	3.9c	4.4c	5.8b	4.9c	4.7b
	60R	7.5a	6.0b	6.1b	6.4ab	6.0b	5.9b
	40R	6.3b	6.7ab	6.6ab	5.6b	6.3b	6.3ab
	20R	7.1ab	7.3a	7.1a	7.2a	7.3a	7.3ab
Papaya	80R	7.4a	4.2c	4.8b	5.4a	5.2a	5.3a
	60R	7.0a	5.1b	5.3ab	5.2a	5.4a	5.6a
	40R	5.8b	6.7a	6.4a	6.0a	5.0a	5.8a
	20R	6.3b	6.1a	5.7ab	5.4a	5.8a	5.9a
Guava	80R	7.0a	3.9d	4.4b	5.8b	4.7c	4.9c
	60R	7.0a	5.2c	5.3b	5.5b	5.4bc	5.7b
	40R	7.1a	6.2b	6.2a	6.2b	5.8b	6.1b
	20R	6.9a	6.9a	6.7a	6.8a	6.7a	7.1a

Means in the same columns followed by different letters are significantly different at $p<0.05$. 80R=80% roselle/20% fruit juice, 60R= 60% roselle /40% fruit juice, 40R= 40% roselle /60% fruit juice, 20R= 20% roselle /80% fruit juice.

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