

CHEMICAL CHARACTERISATION OF *HIBISCUS SABDARIFFA* (ROSELLE) CALYCES AND EVALUATION OF ITS FUNCTIONAL POTENTIAL IN THE FOOD INDUSTRY

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Abstract

Antioxidant capacity, phytochemicals, phyto-nutrients and bioactive compounds, have all become buzzwords in the growing market for natural health food-products and speciality juice drinks. The main objective of this study was to determine the bioactive compounds in the calyces of *Hibiscus sabdariffa* L. and evaluate its functional potential hence provide an incentive to production, processing and consumption of roselle in Kenya where it is not widely cultivated and utilized. Proximate composition was determined using established AOAC methods. Antioxidant activity (AA) color degradation index (CDI) and were carried using 1-1 diphenyl picryl hydrazyl radical (DPPH) and hunters color meter respectively. HPLC, UV-visible spectrophotometer and atomic absorption spectrophotometer (AAS) were used to determine water soluble vitamins (WSV), total polyphenolic content (TPC) and mineral composition respectively. Product formulations were done and their consumer acceptability determined based on a 9-point hedonic. It was found that the properties of roselle extract before and after pasteurization included pH of 3.88 ± 0.00 and 3.42 ± 0.01 , total acidity (as malic acid) of 2.24 ± 0.00 and 2.24 ± 0.03 %, total phenolic contents of 6.06 ± 0.18 and 5.82 ± 0.01 mg/g roselle extract, respectively. The antioxidant activities using DPPH assay with ascorbic acid standard, expressed as EC₅₀ (Efficient Concentration) were 230.01 ± 2.40 and 235.34 ± 0.79 µg/ml, respectively. Iron and calcium were content was 8.59 ± 0.31 and 14.83 ± 0.60 mg/100g respectively. After sensory analysis, the pure roselle drink was generally more acceptable in taste, flavor, aroma, consistency and overall characteristics with an average overall score of 7.6 out of 9 in comparison with Rosela orange drink, rosella apple drink and rosella melon drink. These properties give roselle the potential as a functional ingredient in beverage manufacture and other applications such as utilisation in polymeric natural color development. Roselle can also be applied in the food industry for manufacture of red wine and roselle fruit flavored preserves, thus exhibiting great potential in commercial application in the food and pharmaceutical industry. More studies for in-vivo properties of Roselle extracts are however needed, to further substantiate the health claims of roselle extract in human nutrition.

Key words: Roselle extract, bioactive properties, functional properties, antioxidant activity, food industry

1.0 Introduction

Recently there has been an increased interest in research on food components such as anthocyanins and other phenolic compounds because of their possible linkage to health benefits including reduction in heart disease and cancer, based on their antioxidant activity (Seeram *et al.*, 2002). With the global functional food and beverage market expected to reach \$109 billion by 2010 (Watkins, 2008), diverse sources of phytochemicals are being explored. Kenya cultivates a wide range of fruits and flowers but some fruits/flowers with potential economic, nutritional and functional value remain underexploited.

Roselle is a tropical shrub with red or green inflated calyces. The calyces groups are red, dark red and green types. (Schipper, 2000). The different parts of roselle are seeds, leaves and calyces and this have been used as vegetables, source of oil, refreshing drinks and food preserves. The fleshy calyces roselle have been used in various countries in Africa and the Caribbean as food or a food ingredient such as jellies, syrups, beverages, puddings, cakes, wines and as a colorant. In addition to their use in food, various parts of the roselle plant have been used in traditional medicine for the prevention of disease such as cardiovascular disease and hypertension.

The rationale of studying roselle is to gather information that will provide an incentive for commercial utilisation in Kenya. In this study, the phytochemical composition of roselle was examined, and the functional potential and technological applications were also evaluated. The rate of anthocyanin destruction depends on many factors such as pH, temperature, intermolecular copigmentation, ascorbic acid, and oxygen concentration. The reactions are usually undesirable in juice processing and long-term product storage (Mazza and Miniati, 1993). Therefore, the present study also monitored quality changes and bioactive properties of roselle extract in plastic transparent containers as affected by pasteurization and storage conditions. Anthocyanins are labile compounds that will undergo a number of degradative reactions. Polyphenols in beverages are common because of their beneficial physiological effects on health (Bravo, 1998; Ina *et al.*, 2002).

Colour was objectively measured as well as pigment concentration based on Wrolstad (2005) approach. Colour is one of the first parameters that consumers base their judgment on whether to buy a food product or not. It was important to investigate the pigment degradation since it affects color stability.

The objective therefore of the study was to determine the quality changes and bioactive compounds in the calyces of *Hibiscus sabdariffa* and evaluate their functional potential in food product development.

2.0 Materials and Methods

The Roselle calyces were obtained from those cultivated at JKUAT farm. They were subjected to postharvest operations that included washing with tap water, sun-drying to 10% moisture content, then they were stored for subsequent extractions and analyses. Proximate composition of the extract calyces was determined using the AOAC methods (1995). Water Soluble Vitamins (WSV) were determined by a reversed-phase HPLC method by Ekinci and Kadakal (2005), modified from Cho *et al.*, (2000). The sample treatment consisted of solid phase extraction (SPE) with Sep-Pak C₁₈ (500mg) cartridges that enabled separation of water-soluble vitamins and removed most of the interfering components. Total phenolics were extracted using a method developed by Kim and Lee (2002) with slight modifications. Prior to extraction, sundried roselle were ground and homogenized. A 1g sample was extracted in 40ml of 80% methanol (V/V) in the dark for 1 hour at room temperature. The extract was centrifuged at 10,000rpm, filtered with whatman filter paper, re-extracted with 80% methanol and concentrated with a rotary evaporator. The phenolic extract was used for all the phytochemical analyses. The radical-scavenging capacity (RSC) was determined using 1-1 diphenyl picryl hydrazyl radical (DPPH) according to Ayoola *et al.*, (2006). All tests were run in triplicate, and analyses of all samples run in duplicate and averaged. Wrolstad (2005) approach was used to monitor color changes as affected by processing temperature and storage conditions for 60 days. The pasteurization temperatures were 60^o, 80^o and 100^oC. The products were stored at ambient and cold storage conditions for a period of 60 days.

Three beverage product categories of rosella orange drink (ROD), rosella apple drink (RAD) and rosella melon drink (RMD) were formulated in the ratios of (roselle extract: fruit juice pulp) 1:1, 3:1 and 3:2 for each category respectively. The developed products were then randomly subjected to sensory evaluation to determine the most

preferred. This was done by a team of 15 trained panelists who represent the common consumers most likely to use the product. Each panelist recorded their degrees of likes and dislikes using a nine point hedonic scale (Ihekoronye and Ngoddy, 1985). Before each sample testing the panelists rinsed their mouth with water to avoid cross interaction of product sensorial properties. The assessment was carried out under natural light at a temperature of 25°C.

3.0 Results and Discussions

3.1 Proximate Composition

The proximate analysis of roselle calyces is presented in Table 1. The roselle calyces were relatively high in carbohydrates, crude fibre and ash. The carbohydrate content of the calyces is high. The high carbohydrate content obtained lends further support to the assertion of Babalola (2000) and Ojokoh (2003) that the Roselle calyces contain high carbohydrate contents.

Table 1: Proximate analysis of Roselle calyces (g/100g) dry mater

Parameter	Value
Ash Content	12.2
Fat Content	2.0
Crude Fibre	14.6
Protein Content	4.7
Moisture Content	7.6
Carbohydrate Content	68.7

3.2 Mineral Determination

Roselle calyces were found to be relatively high in K, Na, Mg, Ca and Fe as presented in Table 2. These values are relatively high implying that roselle can be a useful source in enriching other food products that are not rich in essential minerals. Potassium was found to be the most abundant mineral. Potassium is not only for the chief electrolytes but also essential for the nervous systems, maintenance of fluid volume in the body, contractile mechanism of muscles, maintenance of correct rhythm of heart beat, clothing of blood(Shahnaz *et al.*, 2003).

Table 2: Mineral in dried Roselle in mg/100g,

Metal	Value
Potassium	101.5±0.1
Sodium	72.1±0.1
Magnesium	100.7±0.4
Calcium	14.8±0.6
Iron	8.5±0.3
Manganese	10.8±0.1
Copper	3.6±0.1
Zinc	0.2±0.0
Phosphorus	35.3±0.1

Values are presented as means±SD

3.3 Vitamin Determination

HPLC determination of water soluble vitamins (WSV) is presented in Table 3. The vitamins that were relatively high include niacin, ascorbic acid and pyridoxine as analyzed at a wavelength of 261nm, 265nm and 324nm respectively. Thiamin, riboflavin, panthothenic acid and folic acid also were present in appreciable amounts.

However, there was 25-30% reduction in vitamins upon drying the roselle calyces this because vitamins are labile and degrade upon exposure to light, oxidation and thermal processes. Roselle calyces are relatively stable in vitamin B₆, B₃ and ascorbic acid retention in comparison to conventional fruits and vegetables.

Table 3: Water soluble vitamins in fresh and dried calyces of roselle before processing

Constituent vitamins	Calyces (fresh)mg/100g	Calyces (Dried)mg/100g
Niacin	3.765	2.644
Thiamin	0.177	0.123
Riboflavin	0.277	0.194
Panθοthenic	0.324	0.227
Folic acid	0.122	0.092
Ascorbic acid	6.701	4.690
Pyridoxine	1.546	1.080

3.4 Bioactive Properties

The quality and bioactive properties of product before and after pasteurization and storage for 90 days are shown in Table 5.

Pasteurization and storage conditions significantly effected the total phenolic content (TPC) and Inhibition capacity (EC₅₀) of the product. Storage at 27⁰C for 90 days significantly effected on the reduction of TPC. The antioxidant activity expressed as EC₅₀ of the product was less in the activity compared to that at cold storage. The combination of organic acids present and other bioactive components could have the influence on the ability to scavenge for the radical. This study suggested that storage at 5⁰C provided greater retention in bioactive properties of products compared to storage at 27⁰C.

Table 5: Quality and bioactive properties of Roselle extract product before and after pasteurization and storage at 5⁰C and 27⁰C for 90 days

Quality and bioactive properties	Before pasteurization	After pasteurization	Storage for 90 days	
			5 ⁰ C	27 ⁰ C
pH	3.88±0.00 ^b	3.42±0.01 ^a	3.45±0.01 ^a	3.45±0.01 ^a
Total acidity(as malic acid)	2.24±0.00 ^a	2.24±0.03 ^a	2.23±0.04 ^a	2.25±0.04 ^a
Total phenolic content (mg) gallic acid/g extract)	6.06±0.18 ^a	5.82±0.01 ^a	3.3±0.41 ^b	2.58±0.43 ^c
Tannins (Mg/g)	2.26	3.21	4.17	4.07
EC 50 ug/ml	230.01±2.40 ^d	235.34±0.79 ^c	359.96±0.89	390.63±4.75 ^a

NB: Means ± standard deviation in each row with the same letters are not significantly different (p > 0.05) EC50: The concentration of dried Roselle extract (µg/ml) needed for 50% decreasing in the initial DPPH concentration.

There was decrease in pH after pasteurization and during storage. Similarly there was a decrease in total acidity. There's a connection between pH and antioxidant activity. According to Azizah *et al*, (1999) the value of the pH affects antioxidant activity of products, as it has an effect on the type of compounds extracted from the raw material and their changes during heating or storage.

The ability to inhibit radical oxidation was demonstrated by roselle at various levels of concentrations as presented in Figure 1, at low concentration, inhibition was almost maximum at 100% decreasing gradually to less than 5% at 5mg/ml. As compared to the ascorbic acid Radical Scavenging capacity (RSC), it suggested the potential to offer antioxidant activities in comparison to ascorbic acid. As noted earlier, this is because of the bioactive components in roselle particularly anthocyanins.

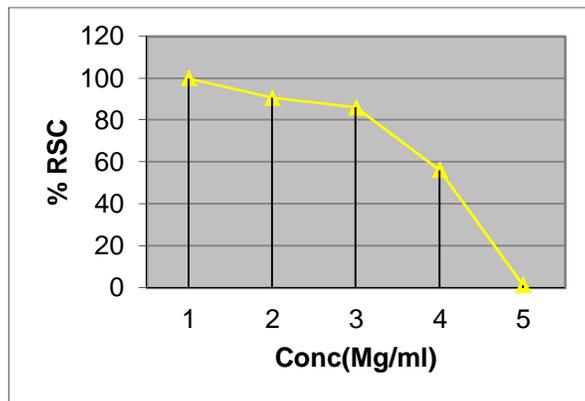


Figure 1: Percentage inhibition of roselle as depicted by the absorbance values at 517nm using UV-visible spectrophotometer

3.5 Total Phenolic Content (TPC)

Total phenolic content (TPC) in Roselle was measured by Folin Ciocalteu method and compared with their DPPH Radical Scavenging Capacity. As expected, they showed a close relationship (0.95). The TPC and DPPH RSC of Roselle was high because of bioactive composition. There was direct correlation between Gallic acid concentration and spectrophotometer absorbance at 760 nm with a gradient of 0.7936 and correlation coefficient (R^2) of 0.9857 as shown in Figure 2. The sample readings generally increased with increase in temperature 51.7mg/g, 62.5mg/g and 73.58mg/g of dry matter for 60°C, 80°C, 100°C respectively. Gansch (2009) tested TPC in Raspberry cultivars and ranged from 342.0 to 875.3 mg of GAE/100g of fresh weights. The relatively lower levels of TPC in *Hibiscus sabdariffa* could be attributed to the type of food matrix; roselle is a flower while raspberry is a fruit. Fruits are more concentrated in phenolics.

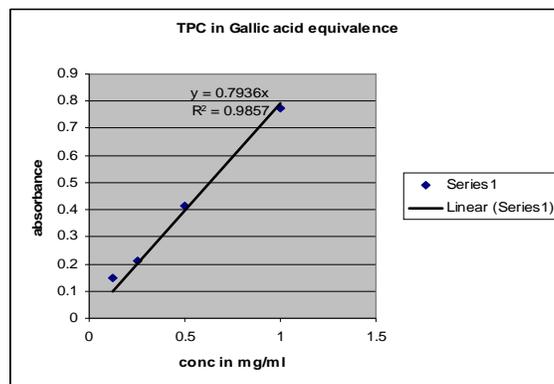


Figure 2: Plot for GAE versus absorbance at UV-V Spectrophotometer at a wavelength of 760 nm

3.6 Indices for Polymeric Colour

The products developed were monitored for a period of 60 days in terms of color changes. Figure 3 shows how the monomeric color of roselle drink changed in a period of two months as depicted by the calculated hue (h^*) angle, i.e., $\arctan(b^*/a^*)$. The intensity of the pigment degradation was depicted by plotting the chroma (c^*) calculated as $(a^{*2} + b^{*2})^{1/2}$ values against time as shown in Figure 4. The color changed gradually from red to dark red. This is anticipated because of the decrease in pH, copigmentation, oxidation and certainly the thermal processing of the products. Several studies have reported a logarithmic course of anthocyanin destruction with an arithmetic increase in temperature (Drdak and Dancik 1990). Color degradation in roselle was as a result of anthocyanin degradation. For commercial production of roselle drink, processing time, temperature regimes will be critical to maintain color stability, quality and bioactive properties.

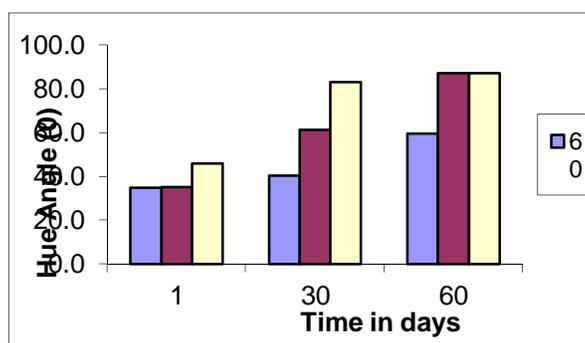


Figure 3: Hue angle of the Roselle drink at pasteurization temperature of 60,80 and 100 degrees as monitored for a period of 60 days

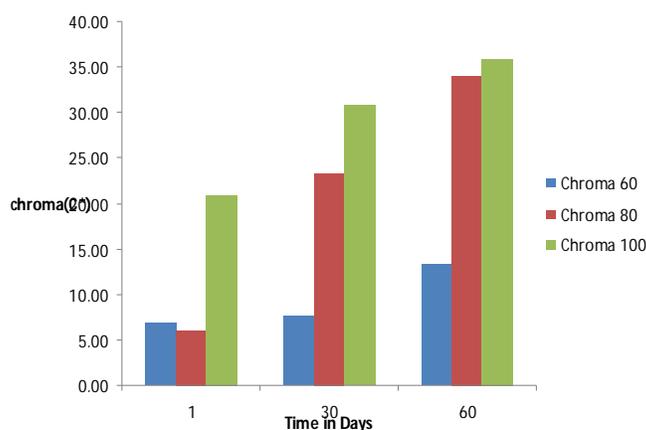


Figure 4: Chroma values for the Rosella drink at pasteurization temperatures of 60, 80 and 100 degrees as monitored over time

3.7 Sensory Analysis

After sensory analysis it was envisaged that pure rosella drink (RD) was most preferred, followed by ROD, RAD and then RMD. Tables 5, 6 and 7 present the sensory analysis of the various ratios for each product category ROD, RAD and RMD respectively. There were no significant differences in the formulation ratios within the product categories with respect to pure rosella drink (RD). However, appearance of the product was significant according to the panelists' responses, this was in agreement with the general knowledge about a consumers' judgment on the color of the product.

Generally all product categories were acceptable to consumers except RMD which was rated low; this could be due to the aroma and taste of the drink. The order of product acceptability based on 9-point hedonic scale was RD>ROD>RAD>RMD.

Table 5: Rosella orange drink (ROD) sensory Analysis based on 9-point hedonic scale

Products code	Appearance	Taste	Aroma	Consistency	Flavour	General Acceptability
100	7.7 ^a	5.7 ^b	6.7 ^a	6.1 ^a	6.3 ^a	6.2 ^a
300	6.2 ^b	6.7 ^a	6.5 ^a	6.8 ^a	6.2 ^a	6.4 ^a
301	6.9 ^{ab}	6.7 ^a	6.4 ^a	6.4 ^a	6.4 ^a	6.8 ^a
305	6.6 ^b	7.1 ^a	6.2 ^a	6.4 ^a	6.1 ^a	6.7 ^a

NB/=Means in columns with the same letters are not significantly different ($p>0.05$)
100=RD, 300=1:1,301=3:1,305=3:2.

Table 6: Sensory evaluation of Rosella apple flavored drink (RAD) at different rations with reference to pure rosella drink (RD=100)

Products code	Appearance	Taste	Aroma	Consistency	Flavour	General Acceptability
100	7.7 ^a	6.5 ^a	6.4 ^a	6.7 ^a	6.8 ^a	6.9 ^a
300	6.1 ^b	6.9 ^a	6.3 ^a	6.0 ^a	7.1 ^a	6.9 ^a
301	6.3 ^b	6.7 ^a	5.7 ^a	5.3 ^a	6.5 ^a	6.8 ^a
305	6.0 ^b	6.8 ^a	6.6 ^a	6.1 ^a	6.8 ^a	6.3 ^a

NB/=Means in columns with the same letters are not significantly different ($p>0.05$)
100=RD, 300=1:1,301=3:1,305=3:2.

Table 7: Rosella Mellon Drink (RMD) sensory Analysis based on 9-point Hedonic scale

Products code	Appearance	Taste	Aroma	Consistency	Flavour	General Acceptability
100	7.3 ^a	6.0 ^a	6.0 ^a	5.7 ^a	6.0 ^a	5.8 ^a
300	5.6 ^b	4.0 ^a	4.1 ^a	4.8 ^a	4.8 ^a	4.8 ^a
301	6.1 ^b	4.5 ^a	5.7 ^a	4.9 ^a	5.8 ^a	5.3 ^a
305	6.2 ^b	4.8 ^a	6.6 ^a	5.0 ^a	4.9 ^a	5.2 ^a

NB/=Means in columns with the same letters are not significantly different ($p>0.05$)
100=RD, 300=1:1,301=3:1,305=3:2.

4.0 Conclusions and Recommendations

The findings from this study suggest that the compounds in *Hibiscus sabdariffa* could potentially provide health benefits and support the ethnomedicinal use of roselle because of the depicted antioxidant activities. There was monomeric color degradation through polymerization as indicated by the hue and chroma indices of the processed products. From the foregoing processing temperature/time regimes & conditions of storage are critical in maintaining the bioactive compounds particularly total phenols. Roselle calyces could find applications in the food industry in the manufacture of a refreshing soft drink (Rosella drink-RD). Representative of Kenyan consumers rated highly rosella fruit flavored drinks RD>ROD>RAD. Preliminary research also shows that roselle could be applied in red wine and polymeric color development in food industry. To realize greater impact of the project however, cooperation at both institutional and national level is required in terms of resource commitment to upscale cultivation and utilization. The project is still in progress for conclusive outputs.

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