NUTRITIONAL CHARACTERISATION OF ROSELLE (*HIBISCUS SABDARIFFA)* CALYCES, EVALUATION OF ITS FUNCTIONAL PROPERTIES AND SENSORY QUALITY OF ITS NOVEL PRODUCTS

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Abstract
Hibiscus sabdariffa L (roselle) is an important flowering plant in the Malvaceae family (FAO). But surprisingly very little fundamental research, in terms of cultivation, agronomy, productivity and various applications, has been carried out in Kenya. The aim of this study was to determine the nutritional, chemical composition and bioactive compounds in the calyces of roselle and evaluate its potential in development of roselle drinks. Properties of roselle extract before and after pasteurization included pH of 3.88 ± 0.00 and 3.42 ± 0.01, total titratable acidity (as Malic acid) of 2.24 ± 0.00 and 2.24 ± 0.03 %, total phenolic content 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 EC_{50} (efficient concentration), were 230.01 ± 2.40 and 235.34 ± 0.79 µg/ml respectively. Iron and calcium contents were 28.5±0.3 and 14.83±0.60 mg/100 g respectively. Product formulations were done and their consumer acceptability determined based on a 9-point hedonic scale. After sensory analysis, the pure roselle drink was generally more acceptable in all the sensory parameters. However, there was no significant differences at ($p>0.05$) with the other drinks mixed with orange, apple and melon. Upscaling cultivation of roselle in Kenya is recommended to enhance its utilization as a product with functional properties. The roselle drinks products generally had high overall acceptability, thus a pointer to market success scenario.

Key words: Roselle, bioactive properties, sensory properties, functional properties, antioxidant activity, processing

1.0 Introduction

Hibiscus sabdariffa L. Family Malvaceae is believed to be native of tropical Africa. It is known by different synonyms and vernacular names such as roselle (Carmden, and Jordan, 2002), Karkade (Abu-tarbuoush, 1997) and Mesta (Rao, 1996). The different parts of roselle are seeds, stems, leaves and calyces. The roselle calyces (the outer whorl of the flower) groups are red, dark red and green types. (Schippers, 2000). Fresh young calyces are eaten raw in salads, refreshing drinks and flavourant (Mahadevan *et al.*, 2009). 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 (Clydescale, 1979). 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(CTA, 2001) Among other uses, strong fibre obtained from the stem (called roselle hemp) is used for various household purposes including making sackcloth, twine and cord (Mungole, 2011).

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. Polyphenols in beverages are common because of their beneficial physiological effects on health (Bravo, 1998; Ina *et al.*, 2002).

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). Anthocyanins are labile compounds that will undergo a number of degradative reactions. Color 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 this study was to determine the nutritional and bioactive composition in roselle calyces and to evaluate its functional properties in food product development. The present study also monitored the effect of pasteurization and storage on the bioactive properties and quality changes of roselle extract. The rationale of studying roselle was to gather information that will provide an incentive for commercial utilization.

2.0 Materials and Methods

2.1 Proximate Composition

Roselle seeds planted in experimental plots at Jomo Kenyatta University of Agriculture and Technology and calyces harvested at maturity stage of plant development. They were washed with tap water, sun-dried to about 10% moisture content and stored before subsequent extractions and analyses. Proximate composition of the extract calyces was determined using AOAC methods (1995).

2.2 Mineral Composition

The ashes obtained were quantitatively transferred to 100ml volumetric flasks using 15 ml of 6 N HC and the solution was topped up to the mark using distilled water. Mineral content was determined using Atomic Absorption Spectroscopy (Model AA-6200, Shimadzu, Corp., Kyoto, Japan). (AOAC, 1995 method). Potassium and Sodium were determined by Flame Emission spectrophotometry using the same machine. Standard solutions were used to quantify the samples.

2.3 Vitamin Composition

Water-soluble vitamins (niacin, thiamin, riboflavin, pantothenic, folic acid, ascorbic acid, pyridoxine were determined by a reversed-phase HPLC method by Ekinci and Kadakal (2005), modified from Cho *et al.,* (2000). Twenty grams of water were added to 5 g of the sample. The mixture was homogenized using a homogenizer at medium speed for 1 minute. The homogenized samples were centrifuged for 10 minute at 14×10^3 g (Centrifuge Model H–2000C Shimadzu Corp., Kyoto, Japan). The sample treatment consisted of solid phase extraction (SPE) with Sep-Pak C_{18} (500 mg) cartridges that enabled separation of water-soluble vitamins and removed most of the

interfering components. The stationary phase preparation involved flushing with 10 ml methanol and 10 ml water (pH 4.2) to activate it. The homogenized and centrifuged samples were then loaded. The sample was eluted with 5 ml acidified water (pH 4.2) then 10 ml methanol at a flow rate of 1 ml min^{-1}. The eluent was collected in a bottle and evaporated to dryness. The residue was dissolved in mobile phase and then filtered through 0.45μm pore size filters and 20 μl of samples was then injected into the HPLC column. The column elute was monitored with a photodiode-array detector at 234 nm for thiamine, 265 nm for ascorbic acid, 266 for riboflavin, 324nm for pyridoxine, 282nm for folic acid, 204nm for pantothenic acid and 261 nm for niacin. Quantification was done by comparing the peak areas on the profiles were against those of the standards.

2.4 Bioactive Properties

Total polyphenol content was extracted using a method developed by Kim and Lee (2002) with slight modifications. Prior to extraction, sun-dried roselle calyces were ground and homogenized. A portion (1 g) was extracted in 40 ml 80% methanol (v/v) in the dark for 1 hour at room temperature (25 ± 2^0C). The extract was centrifuged at 10,000 rpm, filtered with Whatman filter paper (No. 40), re-extracted with 80% methanol and concentrated with a rotary evaporator (Model RE.100, by Bibby Sterlin Ltd, UK). The phenolic extract was used for all the phytochemical analyses.

2.5 Antioxidant Activity

The radical-scavenging capacity was determined using 1-1 diphenyl picryl hydrazyl radical (DPPH) according to Ayoola *et al,* (2006). The following concentrations of the extracts were prepared, 0.05, 0.1, 0.5, 1.0, 2.0 and 5 mg/ml in methanol in cuvette placed in the spectrophotometer (Analar grade). Ascorbic acid was used as the antioxidant standard at concentrations of 0.02, 0.05, 0.1, 0.2, 0.5 and 0.75 mg/ml. One ml of the extract was placed in a test tube, and 3 ml of methanol added followed by 0.5 ml of 1 mM DPPH in methanol. The mixture was shaken vigorously and left to stand for 5 min. A blank solution was prepared containing the same amount of methanol and DPPH. The absorbance of the resulting solution was measured at 517 nm with a spectrophotometer. The radical scavenging activity was calculated using the following formula:

% inhibition = {[Ab-Aa]/Ab} x 100

Where Ab is the absorption of the blank sample and Aa is the absorption of the extract. All tests were run in triplicate, and analyses of all samples run in duplicate and averaged.

2.6 Indices for Polymeric Colour Degradation

The roselle fruit-flavored drinks were packed in plastic bottles for the purpose of analysis. Tristimulus colorimeter was used to take colour measurements (Simple Spectrophotometer NF 333-Model 99061, Nippon Nenshoku Ind., Tokyo, Japan). The instrument expresses colour measurement in the CIELAB (L*, a*, b*) form. The instrument was first calibrated using standard black and white plates (with transparent papers placed on the standard plates). After calibration colour measurements were randomly taken in triplicates. 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, 80 and 100^0C. The products were stored at ambient and cold storage conditions for 60 days.

The hue angle of difference (HD) which describes the visual sensation according to an area which appears to be similar to one or proportions of two of the perceived colours, red yellow, green, and blue was calculated according to the formula below.

Hue angle of difference (HD) = $[\tan^{-1}(\text{'b*'}_x / \text{'a*'}_{x)} - \tan^{-1}(\text{'b*'}_o / \text{'a*'}_o)]^{0.5}$

Where 'L^*'$_o$, 'a^*'$_o$, and 'b^*'$_o$ are values at the time zero (time for calibration) and 'L^*'$_x$, 'a^*'$_x$ and 'b^*'$_x$ are at time x (of object analysis).

2.7 Product Development and Sensory Evaluation

Three beverage product categories of roselle orange drink (ROD), roselle apple drink (RAD) and roselle melon drink (RMD) were formulated from roselle extract and fruit juice pulp in the ratios 1:1, 3:1 and 3:2 for each product category. The developed products were then randomly subjected to sensory evaluation to determine the most preferred. This was done by a team of 15 untrained panelists. Each panelist recorded their degrees of likes and dislikes using a nine point hedonic scale. (1= dislike extremely and 9= like extremely) (Ihekoronye & Ngoddy, 1985).

Before each sample testing the panelists rinsed their mouth with water to avoid cross interaction of product sensorial properties. Sensory evaluation was carried out under natural light at 25ºC.

2.8 Statistical Analysis

All tests were determined in triplicate, and analyses of all samples run in duplicate and averaged to determine the mean and standard deviation. The results were analyzed using Genstat 12th edition for Analysis of Variance (ANOVA) with a significance level of α=0.05%. Duncan Multiple Range comparisons of the means was also done at α=0.05%.

3.0 Results and Discussion

3.1 Proximate Composition

Table 1 shows macro-nutrient compositions of the roselle calyces. Carbohydrate was the most abundant nutrient 58.7%; this was followed by crude fibre with 14.6%. The calyces had appreciable amounts of protein (5%) and ash of 12%. These results were in agreement with those found by other researchers Babalola (2000) and Ojokoh (2003). The high carbohydrate content which is mainly sugars is important in product development where low calories are recommended like in diabetic diet management. The results obtained lend further support to the assertion of Omemu (2006).

3.2 Mineral Composition

Table 2 shows the mineral composition of dried roselle calyces. Potassium was the most abundant mineral (101.5 mg /100 g) in roselle calyces, followed by magnesium (100 mg /100 g). The mineral occurring in the least quantity was zinc (0.2 mg /100 g).

3.3 Vitamin Composition

Table 3 shows the amounts of water-soluble vitamins in the fresh and dried roselle calyces. Ascorbic acid was the most abundant vitamin (6.701 mg) followed by niacin (3.8mg) and pyridoxine (1.5 mg). All the other vitamins were at concentrations less than 1 mg/100g. 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 B_6, B_3 and ascorbic acid retention in comparison to conventional fruits and vegetables (Omemu, 2005).

3.4 Bioactive Properties

Table 4 presents the quality and bioactive properties of roselle extract before and after pasteurization and storage for 90 days. The results in the present study revealed that that the total phenolic compounds in roselle extracts are considerable. Pasteurization significantly ($p< 0.05$) lowered the pH and flavonoids of the roselle extract. Total acidity, total phenolic content and tannins did change significantly ($P <0.05$) after pasteurization; however the
There was no significant change in pH, TTA, tannins and flavonoids of the roselle extract ($P < 0.05$) at the storage conditions, however, there was a significant increase in total phenolic content and (EC50) at the same storage conditions. Storage at 27^0C for 90 days significantly increased ($p < 0.05$) total phenolic content. The antioxidant activity expressed as EC_{50} of the product was more in the activity compared to that at cold storage. This study suggested that storage at 5^0 C provided greater retention in bioactive properties of products compared to storage at 27^0 C.

The combination of organic acids present and other bioactive components could have the influence on the ability to scavenge for the radical. There's a connection between pH and flavonoids. 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 5 mg/ml. As compared to the ascorbic acid radical scavenging capacity, it suggested the potential to offer antioxidant activities in comparison to ascorbic acid.

Total phenolic content in roselle was compared with their DPPH radical scavenging capacity. As expected, they showed a close relationship (R^2 = 0.95). The total phenolic content and DPPH radical scavenging capacity of roselle was high because of bioactive composition this is in agreement with a study by Marc *et al*.,(2004) 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 0.99. Gansch (2009) tested total phenolic content in raspberry cultivars and recorded values ranging from 342.0 to 875.3 mg of GAE/100 g of fresh weights. The relatively lower levels of total phenolic content in *Hibiscus sabdariffa* L. could be attributed to the type of food matrix. Roselle is a flower while raspberry is a fruit. Fruits are more concentrated in total polyphenol content (Gansch, 2009)

3.5 Indices for Polymeric Color

The roselle extracts were monitored for 60 days for colour change. Figure 2 shows how the monomeric color of roselle extract changed after 60 days 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 3.

The color changed gradually from red to dark red. This was anticipated because of the decrease in pH, co-pigmentation, oxidation and thermal processing of the products. Several studies have reported a logarithmic course of anthocyanin destruction with an arithmetic increase in temperature (Wrolstad, 2005). Color degradation in roselle was as a result of anthocyanin degradation. However, this colour darkening can be managed by modifying the medium of roselle in terms of temperature, pH, and storage material.

3.6 Sensory Evaluation

Sensory analysis of the various formulations for ROD, RAD and RMD are presented in Table 5. After sensory analysis it was envisaged that RD (1:0) was most preferred, followed by ROD, RAD and then RMD. There were no significant differences ($p < 0.05$) in the formulation ratios within the product categories with respect to pure roselle drink (RD). However, appearance of the product was significant ($p < 0.05$) according to the panelists' responses, this was in agreement with the general knowledge about a consumers' judgment on the color of the product. Taste, aroma, consistency and flavour were not significantly different for RAD and ROD. However RD and RMD exhibited significant differences ($p < 0.05$) in the sensorial properties. 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.

Conclusion

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. The processing regimes and 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 drink. Consumers rated highly roselle fruit flavored drinks, hence roselle has a great chance of market success. The brilliant red luster of roselle could be added to red wine and/or used in natural polymeric color development in food industry.

Acknowledgements

Kenya Industrial Research and Development Institute is credited for the financial and technical support.

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Table 1: Proximate composition of roselle calyces on dry weight basis (dwb)

Parameter	Amount
Carbohydrate	58.7±0.7
Crude Fibre	14.6±0.5
Ash	12.2±0.3
Protein	4.7±0.1
Fat	2.2±0.1

Table 2: Mineral composition in dried roselle calyces

Metal	Amount(mg/100g)	RDA
Potassium	101.5±0.1	2g
Magnesium	100.7±0.4	240mg
Sodium	72.1±0.1	500mg
Phosphorus	35.3±0.1	700mg
Calcium	14.8±0.6	1g
Manganese	10.8±0.1	2mg
Iron	8.5±0.3	27mg
Copper	3.6±0.1	1mg
Zinc	0.2±0.0	10mg

[a]Mean ± SD
SD……Standard deviation
RDA Relative Daily Allowance

Table 3: Water soluble vitamins (mg/100 g dry-matter-basis) in fresh and dried roselle calyces

Constituent Vitamins	Calyces (fresh)mg/100g	Calyces (Dried)mg/100g	RDA (mg/100g)
Vitamin C	6.7±0.1	4.7±0.1	45.0
Niacin (B3)	3.8±0.0	2.6±0.0	16.0
Pyridoxine (B6)	1.6±0.1	1.1±0.1	1.5
Panthothenic (B5)	0.3±0.1	0.2±0.0	5.0
Riboflavin (B2)	0.3±0.0	0.2±0.0	1.1
Thiamin(B1)	0.2±0.0	0.1±0.0	1.1
Folic acid	0.2±0.0	0.1±0.0	0.4

[a]Mean ± SD 95% confidence interval for mean
RDA Relative Daily Allowance
Rows with different letters are significantly different at $p<0.05$

Table 4: Quality and bioactive properties of roselle calyces extracts

Quality changes and bioactive properties	Before pasteurization	After pasteurization	Storage for 90 days	
			5^0C	27^0C
pH	3.88±0.0[b*]	3.42±0.0[a]	3.45±0.0[a]	3.45±0.0[a]
TTA[x]	2.24±0.0[a]	2.24±0.0[a]	2.23±0.0[a]	2.25±0.0[a]
TPC[x x]	6.06±0.2[a]	5.82±0.0[a]	3.3±0.4[b]	3.58±0.4[c]
Tannins [xxx]	2.26±0.0[a]	3.21±0.0[a]	4.17±0.0[b]	4.07±0.0[b]
Flavonoids [xxxx]	6.3±0.34[a]	5.57±0.05[b]	9.33±0.3[c]	7.33±0.3[c]
EC 50 ug/ml	230.01±2.4[d]	235.34±0.7[c]	359.96±0.9	390.63±4.8[a]

[x] Means±standard deviation,
[a] Rows with the same letters are not significantly different at (p>0.05)
[xx]Determined as malic acid (mg/g)
[xxx] Total polyphenol content determined as gallic acid (mg/g)
[xxxx]Determined as mg catechin/g extract,
[xxxx]Determined as Quercetin (mg/g)
The concentration of dried Roselle extract (µg/ml) needed for 50% decreasing in the initial DPPH concentration.

Table 5: Sensory evaluation of roselle drinks

	Roselle extract ratio to fruit juice	Appearance	Taste	Aroma	Consistency	Flavour	General Acceptability
ROD	1:0	7.7[a]	5.7[b]	6.7[a]	6.1[a]	6.3[a]	6.2[a]
	1:1	6.2[b]	6.7[a]	6.5[a]	6.8[a]	6.2[a]	6.4[a]
	3:1	6.9[ab]	6.7[a]	6.4[a]	6.4[a]	6.4[a]	6.8[a]
	3:2	6.6[b]	7.1[a]	6.2[a]	6.4[a]	6.1[a]	6.7[a]
RAD	1:0	7.7[a]	6.5[a]	6.4[a]	6.7[a]	6.8[a]	6.9[a]
	1:1	6.1[b]	6.9[a]	6.3[a]	6.0[a]	7.1[a]	6.9[a]
	3:1	6.3[b]	6.7[a]	5.7[a]	5.3[a]	6.5[a]	6.8[a]
	3:2	6.0[b]	6.8[a]	6.6[a]	6.1[a]	6.8[a]	6.3[a]
	1:0	7.3[a]	6.0[a]	6.0[a]	5.7[a]	6.0[a]	5.8[a]

RMD	1:1	5.6[b]	4.0[a]	4.1[a]	4.8[a]	4.8[a]	4.8[a]
	3:1	6.1[b]	4.5[a]	5.7[a]	4.9[a]	5.8[a]	5.3[a]
	3:2	6.2[b]	4.8[a]	6.6[a]	5.0[a]	4.9[a]	5.2[a]

Where; Like extremely....9, Like very much...8, Like moderately ...7, Like slightly.....6, Neither like nor dislike..5, Dislike slightly...4, Dislike moderately ...3, Dislike very much..2 Dislike extremely ...1. Means in columns with the same letters for each product category are not significantly different (p>0.05)

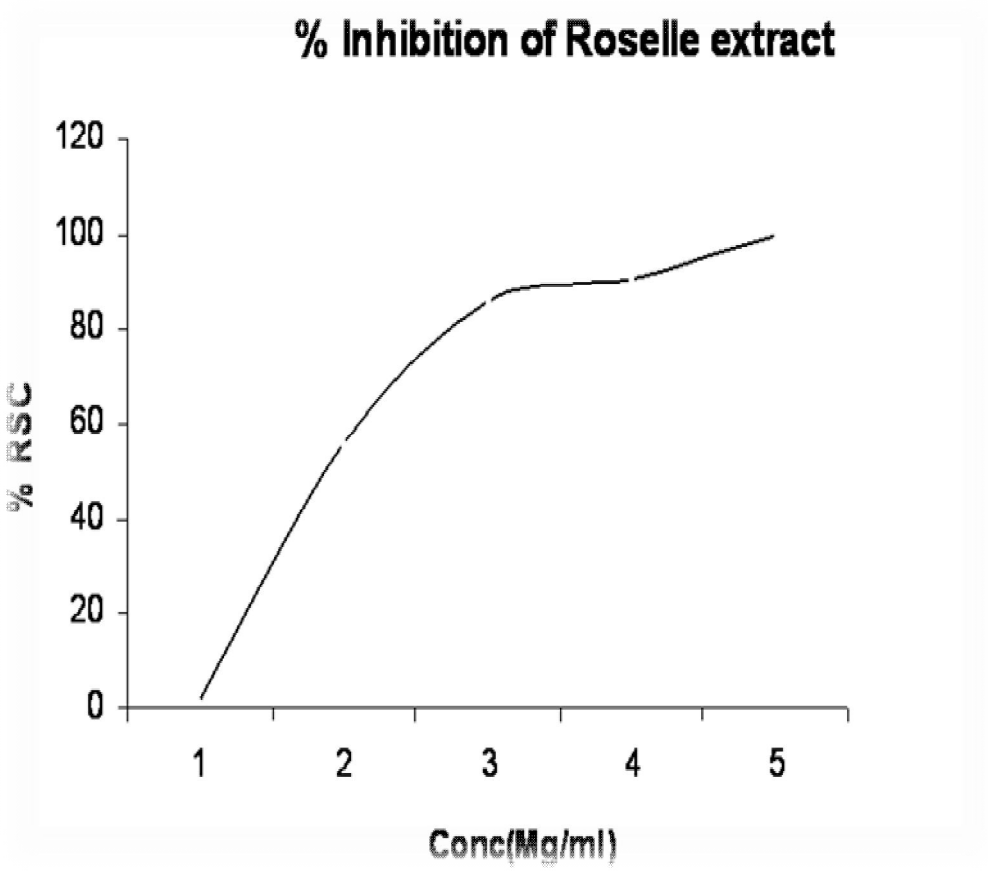

Figure 1: Percentage inhibition of roselle as depicted by the absorbance values at 517nm using UV-visible spectrophotometer

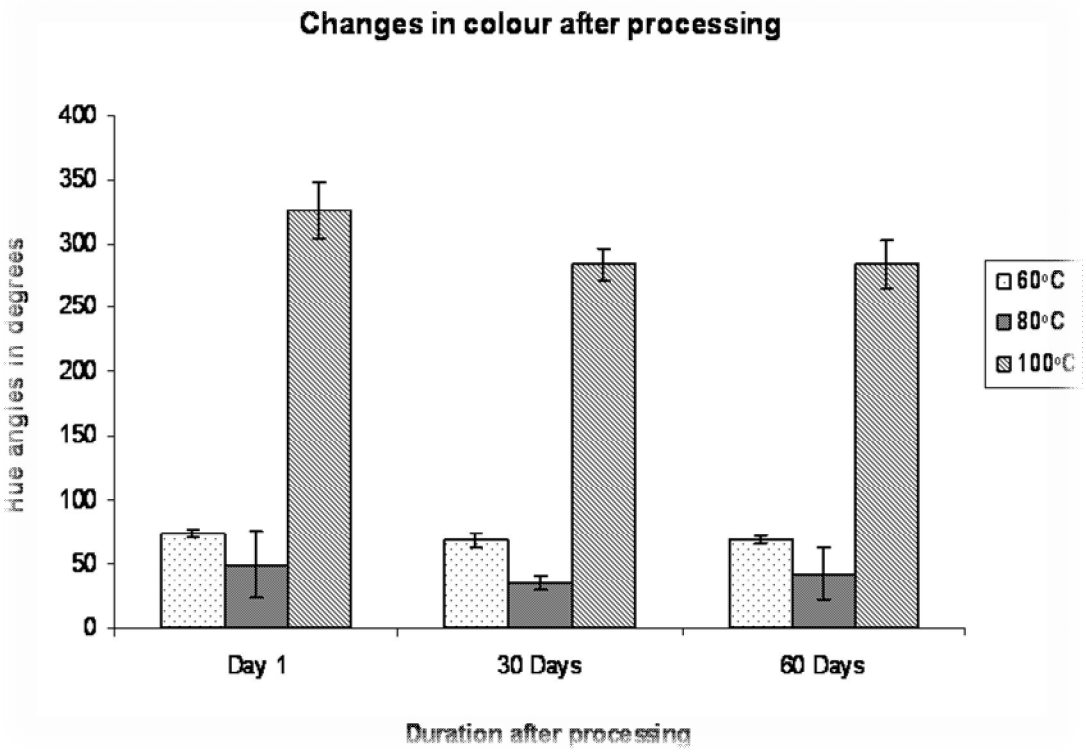

Figure 2: Hue angle of the Roselle drink after pasteurization at 60, 80 and 100^{0}C for 30, 15 and 5 minutes respectively, and stored for 60 days

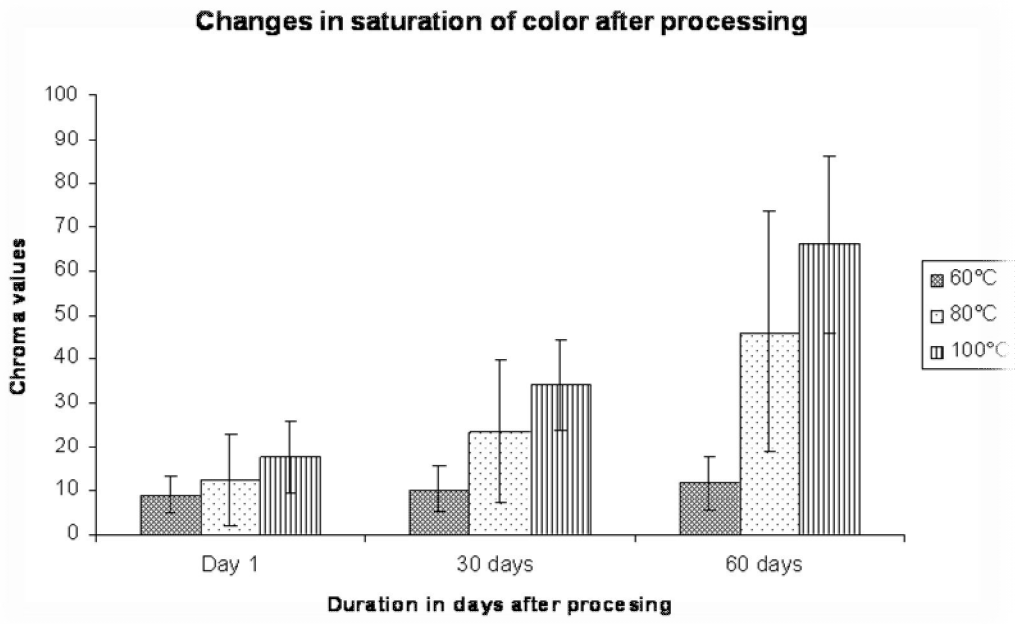

Figure 3: Chroma values for the Rosella drink at pasteurization temperatures of 60, 80 and 100^{0}C monitored over time