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Analysis of Physicochemical, Nutritional and Antioxidant Properties of Fresh and Dried Roselle (*Hibiscus sabdariffa* Linn.) Calyces

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ABSTRACT

The present investigation aimed to assess the physicochemical, nutritional and antioxidant properties of fresh and dried roselle calyces. The fresh and dried (oven-dried and sun-dried) roselle calyces had considerable amount of crude fiber, crude protein, crude fat, total carbohydrate, minerals (Fe, Ca, K, Mg, Zn), ascorbic acid, total phenol, total flavonoid, total anthocyanin and antioxidant properties. Different chromatic parameters such as L, a*, b*, H*, C* values of fresh and dried calyces were also investigated. The present investigation showed that both fresh and dried roselle calyces are generally acceptable as good source of nutrients and antioxidant potentials.*

Key words: *Physicochemical, Nutritional, Antioxidant, Roselle calyces, Chromatic parameters.*

INTRODUCTION

Roselle (*Hibiscus sabdariffa* Linn.) belongs to the family Malvaceae, is an annual or biennial plant cultivated in tropical and subtropical regions for its stem, fibers, calyces, leaves and seeds. The plant is widely grown in tropics like Caribbean, Central America, India, Africa, Brazil, Australia, Hawaii, Florida and Philippines, Saudi Arabia, Malaysia, Indonesia, Thailand, Philippines, Vietnam, Sudan, Egypt and Mexico. In Sudan, it is a major crop of export especially in western part where it occupies second place area wise after pearl millet followed by sesamum[13].

Indian common names of roselle are silot-sougre (Manipuri), tengamora, mesta tenga (Assamese), anthur-rahrep (Mizo), amta (Arunachali), lal-mesta, chukar (Bengali), lal-ambari, patwa (Hindi), lal-ambadi, tambdi-ambadi (Marathi), erragomgura, erragonkaya (Telegu), pulachakiri, pundibija, kempupundrike (Kannada), polechi, pulichchai (Malayalam), ambasthaki (Sanskrit).

The chemical components contained in the flowers of *Hibiscus sabdariffa* include anthocyanins, flavonoids and polyphenols[23]. The petals are potentially a good source of antioxidant agents as anthocyanins and ascorbic acids[18]. Roselle calyx contains a rich source of dietary fiber, vitamins, minerals and bioactive compounds such as organic acids, phytosterols, and polyphenols, some of them with antioxidant properties. The phenolic content in the plant consists mainly of anthocyanins like delphinidin-3-glucoside, sambubioside, and cyaniding 3-sambubioside mainly contributing to their antioxidant properties.

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Recently, the biological activities of anthocyanins, such as antioxidant activity and anticarcinogenic activity have been investigated[2].

Roselle fruits are available as seasonal surpluses during certain parts of the year in different regions and are wasted in large quantities due to absence of facilities and know-how for proper handling, distribution, marketing and storage. Roselle calyces can be dried in large quantities and store for processing. Dried calyces are more easy to handle, light and deteriorate less when exposure to the environment. The dried calyces can be further processed into cordial drinks, jams, jellies, sauces, liqueurs, wines, and preserves[14].

The main purpose of this study was to focus on determining the physicochemical, nutritional and antioxidant properties of fresh and dried roselle calyces. Results of these studies can be used to determine that the fresh and dried roselle calyces have potent antioxidant properties and rich in nutrients. Hence, it is necessary to explore the possibility of utilizing the roselle calyces for the preparation of different processed products and the application of calyces in a variety of food products as good source of nutrients.

MATERIALS AND METHODS

Collection of sample

The fresh, mature roselle (*Hibiscus sabdariffa* L.) fruits were collected from the experimental farm of Assam Agricultural University.

Sample preparation

The seed capsules of roselle fruits were removed. The calyces were washed thoroughly in filtered water and then divided into two portions of equal size. One portion was oven dried at 55°C for 24 hours and the other portion was sun dried for 5 days at 28-33°C and 65% relative humidity. Dried calyces were ground using a blender and stored in airtight containers for analysis. All the reagents used in this study were of analytical grade.

Chemical and instrumental analysis

Determination of moisture content

Moisture content of dried roselle calyx was measured by using AND MX-50 moisture analyzer (A&D Co. Ltd., Tokyo, Japan).

Determination of crude fiber content

The crude fiber content was determined by Digestion flask method[8]. Percentage of crude fiber content was calculated by using the following equation:

$$\text{Crude fiber}(\%) = \frac{(\text{Weight of residue} - \text{Weight of ash} \times 100)}{\text{Weight of the sample}}$$

Determination of protein content

The protein content was determined by micro Kjeldahl method[8]. Percentage of nitrogen (N) was calculated by using the following equation:

$$\text{Nitrogen}(\%) = \frac{(S - B) \times N \times 14.007 \times D \times 100}{W \times V}$$

Here, S=Sample titre value, B=Blank titre value, N=Normality of HCl, D=Dilution factor, W=weight of sample, 14.007 is the equivalent weight of nitrogen. Protein was estimated by multiplying the corresponding total nitrogen content by a Conventional factor of 6.25. Thus crude protein (%) = % of N × 6.25.

Determination of fat content

The fat content was determined by Soxhlet extraction method[8]. Percentage of fat content was calculated by using the following formula:

$$\text{Fat}(\%) = \frac{\text{Weight of fat in sample} \times 100}{\text{Weight of dry sample}}$$

Determination of ash content

Ash content was determined in muffle furnace at 600°C for 4-6 hours[8]. The ash content was calculated by using the following formula:

$$\text{Fat}(\%) = \frac{\text{Weight of the ash (g)}}{\text{Weight of sample (g)}} \times 100$$

Determination of carbohydrate content

The carbohydrate content was determined by Difference method[8]. It was calculated by using the following formula:

Carbohydrate = 100-(moisture + ash + protein + fat +crude fiber)

Determination of mineral content

Magnesium (Mg) and zinc (Zn) contents were determined on aliquots of the ash solutions were established according to the method of AOAC[7] by using the Atomic Absorption Spectrophotometer (WFX-210 AAS Spectrometer). Iron (Fe) content was determined according to the method described by Wong[24] by using the UV- Visible Spectrophotometer (Varian-Cary 100 UV-Vis spectrophotometer). Potassium (K) and calcium (Ca) were estimated by using the flame photometer (The Model 410 Classic Flame Photometer) [6, 10].

Determination of p^H

The p^H was determined by using pH meter (Denver Instruments, Model 215) and it was standardized with standard buffer solution 4.0 and 7.0. Powdered roselle calyces (5g) were added with 10 ml of distilled water and mixed until getting a uniform paste; an electrode was submerged directly in the sample[2]. In case of fresh calyces the p^H was measured by submerged the electrode in the fresh extract.

Determination of titratable acidity

Titratable acidity was determined according to the method described by AOAC[7]. One (1g) of each sample (powdered roselle calyces, fresh roselle extract) taken into 50 ml centrifuge tube. 10 ml of distilled water was added to each tube to dissolve the sample and then filtered. 1 ml aliquot of each solution was taken into another 50 ml centrifuge tube and 10 ml of distilled water was added to dilute the highly colored sample. 10 ml of the diluted sample was titrated against 0.1N NaOH solution using phenolphthalein (2 drops) indicator. Acidity was calculated using the following formula:

$$\%\ \text{acidity as citric acid} = \frac{\text{Titre value} \times \text{Normality of alkali} \times \text{Equivalent wt. of citric acid} \times \text{Vol. made up} \times 100}{\text{Volume of sample taken for estimation} \times \text{Wt. or vol. of sample taken} \times 1000}$$

Determination of ascorbic acid content

Vitamin C was determined by the 2, 6-dichlorophenol-indophenols dye method[9]. Percentage of ascorbic acid content was calculated by using the following formula:

$$\text{Ascorbic acid (mg/100g)} = \frac{\text{Titre value} \times \text{Dye factor} \times \text{vol. made up}}{\text{Aliquot of extract taken for estimation} \times \text{Wt. or vol. of sample taken for estimation}} \times 100$$

Determination of total phenolic content

Total phenolic content in extracts were determined according to the Folin–Ciocalteu procedure[20]. Methanolic extracts of roselle calyx (100 µl) were transferred into different test tubes, and then mixed with 0.4 ml of 10% Folin–Ciocalteu reagent. After 3 min for allowing the reaction to take place, 0.8 ml of 10% sodium carbonate (Na_2CO_3) was added. The test tubes were allowed to stand for 1 h at ambient temperature, and the absorption was measured at 725 nm using spectrophotometer (Varian Cary 50 Scan) against a blank, which contained 100 µl of methanol in place of sample. Gallic acid was used as calibration standard, and the total phenolic content was expressed as gallic acid equivalent in mg/100g.

Determination of total flavonoid content

Flavonoid content in the methanolic extract was determined by aluminum chloride colorimetric method[11]. The each extract (1 ml)

was diluted with 4 ml of distilled water in a 10ml volumetric flask. Initially, 0.3 ml of 15% sodium nitrite ($NaNO_2$) solution was added to each volumetric flasks, after 5 mins, 0.3 ml of 10% aluminium chloride ($AlCl_3$) was added; and after 6 min, 2 ml of 1.0M sodium hydroxide (NaOH) was added to each reaction flask and properly mixed. Absorbance of the reaction mixture was read at 510 nm after 30 mins. The result was expressed as mg of quercetin equivalents (QE)/100 g of dry weight.

Determination of total anthocyanins content

Total anthocyanins content was determined according to the pH differential spectroscopic method[16]. One ml of each sample extract was diluted to 10 ml with distilled water. Therefore, one ml of this solution was diluted to 5 ml with buffer pH 1.0 into test tubes (wrapped with aluminum foil). Another 1 ml of the sample solution was diluted to 5 ml with buffer pH 4.5. Mixtures were allowed to stand for 30 min at room temperature and then absorbance was measured, at 520 and 700 nm, using a UV-visible spectrophotometer (Varian Cary 50 Scan), in 4 ml spectrophotometer glass cells. Results were expressed as equivalents of cyanidin-3-glucoside per 100 g of sample calyces, according to the following equation.

$$CA = (A \times MW \times DF \times V \times 100) / (\varepsilon \times L \times w)$$

Where, CA is the concentration of anthocyanins (mg/100 g), A is the absorbance difference ($A=[A_{520nm}-A_{700nm}]_{pH=1}-[A_{520nm}-A_{700nm}]_{pH=4.5}$), MW is the molecular weight of cyanidin-3-glucoside (449.2 g/mole), DF is the dilution factor, V is the total volume of extract (ml), w is the weight of the sample used in the extraction (g), L is the cell width (1 cm), ε is the coefficient of molar extinction for cyanidin-3-glucoside (26,900 L/mole-cm), and 100 is the conversion factor for obtaining mg/100 g of sample.

Buffer for anthocyanin determination were prepared according to Wrolstad[25] as per the following composition:

pH 1.0 buffer: 125 ml of 0.2M KC1 (14.9 g/L) + 385 ml of 0.2N HCl.

pH 4.5 buffer: 400 ml of 1 M sodium acetate (136 g/L) + 240 ml of 1 N HC1 (83.0 ml conc. HC1/L) + 360 ml distilled water.

The pH of the buffers was adjusted as required to obtain final pH values of 1.0 and 4.5.

Determination of DPPH inhibition (%)

Free radical-scavenging was evaluated using the 1, 1-diphenyl- 2-picrylhydrazyl (DPPH) free radical according to the method of Shimada *et al*[19]. One ml of freshly prepared 1 mM DPPH in methanol was added to test tubes containing 5 ml of the sample extracts. A control was prepared by adding 1 ml of DPPH solution to 5 ml of 80% methanol. Following storage in the dark for 30 min, the absorbance was read at 517 nm using a UV–visible spectrophotometer against a blank containing 80% methanol. The % inhibition was calculated by the following formula:

$$\text{DPPH inhibition } (\%) = \frac{AC - AS}{AC} \times 100$$

Where, AC = Absorbance of control at 517 nm
AS = Absorbance of sample at 517 nm

Determination of chromatic (CIE Lab) parameters

CIE (Commission Internationale de l'Eclairage) Lab parameters, i.e. L*, a* and b* values were determined in a HunterLab Color Quest XE Colorimeter and Hue and Chroma were calculated from following equations:

$$\text{Hue} = \tan^{-1} b/a$$

$$\text{Chroma} = \sqrt{a^2 + b^2}$$

Statistical analysis

The data obtained was subjected to statistical analysis using 'completely randomized design' with 4 replications. The results were statistically evaluated by one-way analysis of variance (ANOVA).The significance differences of the treatment values was determined at the 0.05 probability level by using LSD test using SPSS software. Values are means of four (4) replications ± standard deviation. Means within a row with the same superscript were not significantly different ($P<0.05$).

RESULTS AND DISCUSSION

The results of the present investigation on "Analysis of Physicochemical, Nutritional and Antioxidant Properties of Fresh and Dried Roselle Calyces (*Hibiscus sabdariffa* Linn.)" are presented and discussed in this chapter.

The proximate composition of the roselle calyces (fresh sample, oven-dried sample and sun-dried sample) are shown in Table 1. Drying reduced the moisture content from roselle calyces. Oven-dried sample was recorded lowest moisture content of 11.08% while sun-dried sample had 15.62% moisture content. The higher moisture in sun-dried sample was probably due to the high and controlled temperature in the oven dryer relative to the sun peak temperature[12]. However, the increased in crude protein content and decreased in crude fiber, crude fat, and total carbohydrate were recorded in dried samples. The increased in crude protein is due to the increased in dry matter contents and concentration of soluble solids. The decreases in the crude fiber, crude fat and crude carbohydrate contents of the dried samples on dry weight basis could be linked to the increases in the other components. The trend in change in the proximate composition of fresh and dried okra samples at different drying conditions agrees with the work of Eze and Akubor[12].

Table 1: Proximate composition of fresh and dried roselle calyces

Composition (%)	Fresh calyces	Oven-dried calyces	Sun-dried calyces
Moisture	$88.09^{a} \pm 0.05$	$11.08^{b} \pm 0.03$	$15.62^{c} \pm 0.01$
Crude fiber	$8.11^{a} \pm 0.02$	$7.52^{b} \pm 0.02$	$6.22^{b} \pm 0.01$
Crude protein	$10.28^{a} \pm 0.03$	$15.52^{c} \pm 0.04$	$14.22^{b} \pm 0.02$
Crude fat	$4.16^{a} \pm 0.05$	$1.44^{c} \pm 0.03$	$1.22^{c} \pm 0.02$
Ash	$7.8^{a} \pm 0.01$	$11.21^{b} \pm 0.03$	$10.56^{b} \pm 0.02$
Total carbohydrate	$46.26^{a} \pm 0.05$	$43.68^{c} \pm 0.02$	$44.67^{c} \pm 0.03$

Table 2: Mineral contents of fresh and dried roselle calyces

Parameters	Fresh calyces	Oven-dried calyces	Sun-dried calyces
Iron (mg/100g)	$26.0.05^{a} \pm 0.02$	$35.68^{b} \pm 0.03$	$28.98^{b} \pm 0.05$
Calcium (mg/100g)	$50.14^{a} \pm 0.04$	$58.89^{b} \pm 0.03$	$53.19^{b} \pm 0.02$
Potassium (mg/100g)	$20.16^{a} \pm 0.05$	$23.16^{c} \pm 0.03$	$21.11^{c} \pm 0.01$
Magnesium (mg/100g)	$21.96^{a} \pm 0.03$	$22.98^{b} \pm 0.01$	$22.11^{c} \pm 0.02$
Zinc (mg/100g)	$4.12^{a} \pm 0.01$	$6.68^{b} \pm 0.03$	$5.52^{b} \pm 0.02$

The mineral contents of roselle samples are shown in Table 2. Drying increased the mineral contents in roselle calyces. The ash content is a measure of the total amount of minerals present within a food, whereas the mineral content is a measure of the amount of specific inorganic components present within a food. Minerals are not destroyed by heating and they have a low volatility compared to other food components[3]. The general increase in ash and mineral contents with increase with drying temperature is attributable to concentration factor due to moisture removal, which resulted in higher level of total soluble solid[4].

The physicochemical parameters of fresh and dried roselle calyces are shown in Table 3. The total phenolic contents were increased in dried sample and the highest total phenolic content was recorded in oven-dried sample. The increased in total phenol is possibly due to the liberation of phenolic compounds from the matrix during the drying process[21].

Total flavonoids, total anthocyanins and DPPH inhibition (%) were decreased after drying of roselle calyces**.** It was observed that drying processes were also responsible for flavonoids degradation. The degradation of flavonoids not only depends on temperature and magnitude of heating; it may depend also on other parameters such as pH, phytochemicals, structure and even the presence or absence of oxygen[5].

Anthocyanins are more sensitive to heat than colorless phenolic compounds[15]. The loss of anthocyanins has been attributed to

many factors or combination of factors such as pH and acidity, phenolic compounds, sugar and sugar degradation products, oxygen, and ascorbic acid [1]. The drying processes were also influenced in the ascorbic acid contents of dried roselle calyces. This may be due the facts that ascorbic acid is very unstable when exposed to ultra violet radiation and high temperature[21]. Drying affects the antioxidant activity of plant materials. Increased temperature and water removal generally cause a loss of chemical compounds with antioxidant properties[17]. In case of chromatic parameters it was observed that the dried roselle calyces were darker (lower L^* values) than the fresh roselle calyces. Redness values (a^*) was found to be higher in oven-dried samples and yellowness values (b^*) values was found to be higher in sun-dried samples. The highest hue angle (H^*) was recorded in sun-dried sample. Similar trends were also observed in dried apricot pestil[21].

Table 3: Physicochemical properties of fresh and dried roselle calyces

Parameters	Fresh calyces	Oven- dried calyces	Sun-dried calyces
p^H	$2.07^a \pm 0.01$	$2.71^b \pm 0.02$	$2.67^b \pm 0.01$
Titratable acidity (% Citric acid)	$20.22^a \pm 0.03$	$18.82^b \pm 0.02$	$19.12^b \pm 0.03$
Ascorbic acid (mg/100g)	$66.52^a \pm 0.04$	$58.48^c \pm 0.01$	$52.42^c \pm 0.02$
Total phenol (mg/100g)	$1018^a \pm 0.32$	$2019^b \pm 0.34$	$2010^b \pm 0.32$
Total flavonoids (mg/100g)	$388^a \pm 0.11$	$322^b \pm 0.16$	$310^b \pm 0.12$
Total anthocyanins (mg/100g)	$326^a \pm 0.12$	$298^c \pm 0.13$	$282^c \pm 0.14$
DPPH inhibition (%)	$92.12^a \pm 0.10$	$86.14^b \pm 0.14$	$79.28^b \pm 0.13$
Color			
L^*	$28.76^a \pm 0.01$	$23.74^b \pm 0.01$	$21.76^c \pm 0.02$
a^*	$18.82^a \pm 0.04$	$15.82^b \pm 0.03$	$14.78^c \pm 0.01$
b^*	$3.22^a \pm 0.02$	$5.38^b \pm 0.02$	$6.49^c \pm 0.01$
H^*	$9.73^a \pm 0.04$	$18.79^b \pm 0.03$	$23.76^c \pm 0.04$
C^*	$19.09^a \pm 0.03$	$16.70^b \pm 0.02$	$16.14^c \pm 0.02$

CONCLUSION

Roselle crops are abundantly grown in North-East India. Roselle calyces are used to prepare jam, jelly, chutney, and pickle in household levels. Roselle calyces are perishable in nature and susceptible to post harvest losses. Glut of seasonal crops during the peak production periods not only lowers the market price, but also leads to heavy losses. The results of this study showed that both oven-dried and sun-dried calyces were richest source of nutrients and had potent antioxidant properties. From this investigation it can be concluded that oven-drying and proper sun-drying methods not only reduces the post harvest losses, but also retains the valuable nutrients of the roselle calyces.

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