



RESEARCH ARTICLE

# The effects of various drying techniques on physicochemical and nutritional qualities of roselle (*Hibiscus sabdariffa* L.) calyx

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## Abstract

The calyx of the roselle plant (*Hibiscus sabdariffa* L.) has been extensively studied for its anthocyanins. Roselle calyces are exceedingly perishable, making the process of drying and extracting them without sacrificing quality quite difficult. Similarly, there are several commercial drying procedures available, each with its own set of benefits and drawbacks on nutritional and physicochemical qualities in the final product. The goal of this study was to examine the effects of sun, oven and freeze drying on the physicochemical and nutritional properties of roselle calyx. The antioxidant (%RSA), total anthocyanin content (TAC) and their HPLC identification, physicochemical as well as proximate composition were determined. The results showed that freeze drying increased the drying rate significantly and retained the antioxidant activity (32 mg/100 g QE) then fresh calyx, sun and oven drying, freeze-dried calyx also have higher TAC (176.9 mg/l) than sun and oven dried calyx, the HPLC analysis revealed two anthocyanidin cyanidine glucoside and cyanidin sambubioside. phytochemicals and proximate of roselle calyces were higher in freeze-dried than oven and sun drying techniques employed. The findings demonstrated that freeze drying the roselle calyces preserve their quality characteristics.

## Keywords

antioxidants, phytochemical, calyx, drying, cyanidin glucoside

## Introduction

*Hibiscus sabdariffa* L. Many nations in the tropics and subtropics, including India, Indonesia, Sudan, Egypt, Mexico and Malaysia, commercially cultivate (Roselle) (1). The rich red colour of the roselle calyx is due to anthocyanins such cyanidin-3-sambubioside, cyanidin-3-glucoside and delphinidin-3-glucoside (1). It's used in everything from jam and jelly to ice cream and pastries to preserves and herbal drinks (2). Drinking roselle tea has been connected to many medical advantages, primarily because of its antioxidant properties (2). Antioxidant properties and roselle calyx anthocyanin have been linked in the literature (3). Delphinidin-3-glucoside is used to express the total anthocyanin content of roselle calyces (4). Ascorbic acid is also found in roselle extracts. Anthocyanins have greater antioxidant activity than ascorbic acid (1). As a result, anthocyanins are primarily responsible for the roselle extract's antioxidant properties. Anthocyanins in food items faced issues due to their susceptibility to oxygen, heat and light during pro-

cessing and storage. Mazza and Miniati ascertained that anthocyanin thermal degradation of roselle was rapid at temperatures beyond 100 °C (5, 6).

Typically, the roselle calyx is picked with a high moisture level (85%). Thus, drying is a critical post-harvest process that decreases the moisture content and extends the shelf life of produce. Dried calyxes are available for purchase due to the drying process, which involves the simultaneous transmission of heat and mass, which removes the calyxes' water content or humidity while maintaining their nutritional properties, notably their ascorbic acid concentration (7-10).

One strategy for the shelf-life extension of the fresh calyx is to dry them. There are several commercial drying procedures available, each with its own set of benefits and drawbacks and nutritional and physicochemical qualities in the final product (11). Furthermore, proper drying techniques can greatly improve product quality. Spray drying, freeze drying, solar thermodynamic drying, fixed bed drying, sun drying, and oven drying were among the methods used to dry the roselle flower (11). Drying settings and circumstance influence the scent and flavour of dried roselle. Generally, drying processes ensure microbiological stability, extend product shelf life and make packing and distribution easier. It demonstrates that the freeze-drying approach is greatly beneficial for the production of power-driven fruit. Much research shows that freeze-drying maintains the fruit's biologically important chemicals more effectively than other preservation methods, but the process is expensive (12, 13). As a result, research into various drying procedures is needed to ensure that the final product meets the specified standards (12). As such this study focus on the effects of various drying techniques on physicochemical and nutritional qualities of (*Hibiscus sabdariffa* L.) roselle calyx.

## Materials and Methods

### Research design

Roselle calyx fully matured 6 weeks post-transplanting was used. About 2 kg of the calyx each was dried using sun, oven and freeze-drying method. The quality characteristics was analyzed in 3 replicates.

### Sample preparations

Fresh samples of roselle (*Hibiscus sabdariffa* L.) calyxes UKMR-1 were obtained from the experimental farm of the Faculty of Bioresources and Food Industry Besut (FBIM, UNISZA.) 6 weeks post transplanting. The seed was removed, and the fresh calyx was manually sought and washed thoroughly in a running tap for dirt removal, then excess water was drained and weighed. Subsequently the calyx was dried using drying methods oven, freeze and sun-drying respectively.

### Sun drying

About (2 kg) of fresh roselle calyxes of each were placed on a plate and placed on a table in direct sunshine between 9 and 4 pm.; respectively, for 7 days. A data logger RH /

Temp (EL-USB-2-LCD+, USA) was used to monitor the drying temperature (averaged 34.9 °C) and ensure that the calyxes were evenly agitated (2, 14).

### Oven drying

About 2 kg of fresh roselle calyxes were placed on a clean metal plate and set in the oven at 60 °C for 24 hrs.

### Freeze drying

About (2 kg) of the calyxes were cleaned with tap water and then frozen and preserved at -20 °C. Before using frozen material, it was thawed overnight in a refrigerator (4 °C). Drying was performed beyond 48 hrs inside a freeze dryer (Freeze dry System- LABCONCO free zone 18, Kansas City, MO, USA) at -40 °C and pressure  $3.3 \times 10^{-3}$  mbar (3).

### Total anthocyanin content (TAC)

TAC was established by comparing the pH differences across samples (Giusti and Wrolstad, 2001). In a nutshell, a pH 1.0 solution (0.025 M KCl) and a pH 4.5 solution (0.025 M KCl) were created (0.4 M CH<sub>3</sub>COONa). It was necessary to dilute the extract samples. They were shaken for 15 min in the dark, after which they were diluted to a pH of 1.0 and 4.5 respectively. Each sample's absorption peak wavelength (A vis-max) was then used to compute its specific TAC value. The absorbance values of the samples were then measured by spectrophotometer at 700 nm (A700). The following formulae were used to express each TAC value in mg cyanidin-3-glucoside (C3G) equivalents per litre of concentrate determined according to the following equations (15).

$$\text{Absorbance (A)} = (A_{\lambda \text{ vis-max}} \times A700 \text{ pH } 1.0) - (A_{\lambda \text{ vis-max}} \times A700 \text{ pH } 4.5)$$

$$\text{TAC (mg/L)} = (A \times \text{MW} \times \text{DF} \times 1000) / (\epsilon \times l)$$

MW: the molecular weight, calculated as cyanidin-3-glucoside (449.2); DF: the dilution factor;

l: the cuvette radius, 1 cm;  $\epsilon$ : the molar absorptivity, calculated as cyanidin-3-glucoside (26900) [Anggraini et al 2019](#).

### HPLC Preparation of anthocyanin from the calyx

The extract was produced via the standard procedures (16). For 24 hours at 4 °C, 100 g of roselle calyxes' powder was combined with 200 ml acidic methanol and 0.1 percent trifluoroacetic acid (v/v). Cotton and Whatman paper were used to filter the macerate. A dry extract is produced during low-pressure vacuum evaporation of methanol at 38 °C in a BÜCHI Rotavapor R-114. After mixing the dried extracts with 200 ml distilled water, the resultant aqueous solution was filtered through gel XAD-7 to remove sugar and chlorophyll pigments. One hundred millilitres (100 ml) trifluoroacetic acidified methanol 0.1 % (v / v) was transferred over gel XAD-7. The obtained methanol deposit was evaporated once more using low-pressure vacuum evaporation at 38 °C in a BÜCHI Rotavapor R-114. Prior to dissolving the dry extract, 100 ml of distilled water was added. The aqueous solution was lyophilized using a freeze drier.

### HPLC analysis for the determination of anthocyanin in roselle calyx

The analysis was carried out using the high-performance liquid chromatography (HPLC) technique (17). "Around 10 mg of freeze-dried calyx extracts were dissolved in 5 ml of methanol in a blender overnight at 4 °C. Centrifuged for 10 min at 3000 rpm, using a Millipore membrane, the supernatant was collected and filtered (0.45 m) membrane. Two dilutions of the filtrate with purified distilled water were performed. The analysis was carried out on a Windows XP Workstation with an HPLC (Agilent) model-LC 1100 series equipped with a degasser, an autosampler automated injector, a high-pressure pump and a UV/Visible detector working at several wavelengths. For the HPLC analyses, a reversed-phase C18 column (Prontosil, 250 x 4.0 mm, 5 m, Bischoff) was utilised. A binary gradient eluent was utilised as the mobile phase (solvent A, 0.1% trifluoroacetic acid in water; solvent B, 0.1 % trifluoroacetic acid in acetonitrile). Acetonitrile was purchased from Sigma/Aldrich and degassed in an ultrasonic bath before to use. A Milli-Q system was used to distilled the water (Millipore). With a flow rate of 0.8 ml/min<sup>-1</sup>, the elution programme was 5-20 % B (0-5 min), 20-35 % B (5-10 min), 35-100% B (10-25 min) and 100% B (25-40 min). Chromatograms were measured at a wavelength of 521 nm. The identification and peak assignments of anthocyanins are based on their retention durations, comparison of UV-VIS spectra to standards and published data. The anthocyanins were quantified using cyanidin 3-glucoside" as an external standard.

### DPPH method

A diphenyl phosphate (DPPH) test was performed using the technique described in a previous research (18). It was made daily, and the working solution of DPPH was prepared by diluting the sample and mixing it with 1 ml of the active ingredient. After vortexing, the mixture was allowed to rest for 10 min in a dark environment at room temperature. The spectrophotometric measurement of the absorbance at 517 nm was then carried out. As a reference standard, quercetin was used in this study. The formulation was used to compute the % reduction in DPPH (6, 12, 19)

$$\% \text{DPPH reduction} = \frac{\text{AC-AS}}{\text{AS}} \times 100$$

### Calyx colour

"Ten roselle calyces was used to measure colour indices using Minolta Chroma Meter (Model R200 Trimulus Colour Analyzer, Minolta camera Co. Ltd., Japan). The colour was measured on the 3 sides of the roselle calyx. The colour data for Calyx was represented in the values L \*, a \*, and b\*. L \* defined a lightness coefficient ranging from 0 (black) to 100 (white). A \* ranged from -60 to + 60 colour, which is red (+ 60) and green (-60). In the meantime, b \* ranged from 60 to + 60, (yellow) and blue (-60) A \* and b \* are also used to calculate the colour angle ( $h^\circ = \tan^{-1} b^*/a^*$ ) for the interpretation of colours. Hue angle ( $h^\circ$ ) were red-violet (0°), yellow (90°), bluish-green (180°) and blue" (20).

### Titrateable acidity determination

Ruck's technique for determining titrateable acidity was used to make the measurement (21). The blended Roselle fraction of one gramme was weighed and placed in a 50 ml centrifuge tube. Each tube was dissolved with about 10 ml of distilled water and then flitted. A volume of about 1 ml of each solution was transferred to another 50 ml centrifuge tube and 10 ml of distilled water was added to dilute the sample due to its intense colour. Approximately 10 ml of the diluent was titrated against 0.1N NaOH solution using phenolphthalein (2 drops) as an indicator until the mixture became reddish. As malic acid, the percentage titrateable acidity was computed (22).

### pH determination

Standard buffer solutions 4.0 and 7.0 were used to calibrate the pH metre (type BA 350 EDT devices). The pH was determined by immediately placing the electrodes into a 10 ml beaker containing the sample.

### Total soluble solids concentration (TSS)

Total soluble solids (TSS) were measured and expressed at 25 °C using an Atago 8469 hand refractometer (Atago Co. LTD., Tokyo, Japan). At 25 °C, glucose, fructose and sucrose were determined and expressed as percentages using an A-Brix Digital refractometer HI 96811 (Hanna instruments). The maximum soluble sugar concentration was determined using the phenol-sulfuric acid technique.

### Proximate analysis

#### Ash content determination

The ash content of roselle calyx was assessed using the AOAC technique (23) with slight modifications. A (3 g) of calyx was placed in a crucible and heated in a fume hood until no fumes were produced. The sample was heated overnight in a cooled muffle furnace to 450–500 °C. Ash weight (g) = (crucible weight + ash) – (crucible weight + ash) (weight of crucible).

$$\text{Ash Content \%} = \frac{\text{Weight of ash}}{\text{Weight of the sample}} \times 100$$

#### Crude fibre content determination

The crude fibre content was evaluated utilising a fibre-bag as a container after consecutive extraction of pre-dried calyx samples (0.1 g) with 1.25 % H<sub>2</sub>SO<sub>4</sub> (200 ml) and 1.25 % NaOH (200 ml). For drying and ashing, the crucible containing the sample was dried in an oven at 105 °C for 5 hrs and cooled in desiccators; this is weighed as M1. The crucible with the sample was then ashed at 525 °C overnight and cooled in desiccators; this is weighed as M2. The weight of the crucible containing the sample after drying and ashing was determined as well as the crude fibre content (23).

$$\% \text{ Fibre content} = \frac{\text{M1} - \text{M2}}{\text{Weight of the sample}} \times 100$$

### Crude fat determination

Analyses of fat content, the fats were extracted from the roselle powder using the AOAC-recommended technique (23). About 2 g of pre-dried roselle powder was weighted into a pre-dried extraction thimble, about 200 ml hexane was put into a pre-dried boiling flask, and the boiling flask was filled with 200 ml hexane for 2 to 2.5 hrs. The flask was then dried for a further hour at 105 °C. After cooling in a desiccator, it was weighed.

$$\text{Fat Content (\%)} = \frac{[(\text{Weight of flask + fat}) - (\text{Weight of flask})]}{\text{Weight of sample (mg)}} \times 100$$

### Crude protein determination

Crude protein content of roselle was analyzed using the method described by AOAC (23) with slight modifications as recommended via Kjeltac 2300 (Foss Analytical, Denmark). As a starting point, a 4 g pre-dried sample was placed in each tube for digestion. Each vial has 2 Kjeltabs Cu 3.5 (catalyst salts) in it. A 12 ml of concentrated sulfuric acid (H<sub>2</sub>SO<sub>4</sub>) was poured into the tube and shake gently. An InKjel 625M digestion block (Behr, Germany) was preheated to 420°C and used for 60 min to produce a clear blue/green solution during the digestion phase. Samples were cool for 10 to 20 min after being digested. A Kjeltac 2300 distillation unit was used to complete the distillation process" (Foss, Denmark).

"The % of protein was calculated by the given formular: (24)

$$\% \text{ Crude protein} = \frac{0.1 (\text{volume of sample titration} - \text{volume of blank titration})}{\text{Weight of the sample} \times 1000} \times 14.007$$

### Data Analysis

The data was analyzed using one way ANOVA (SPSS 23.0) significant difference, and the result was presented as ± standard deviation.

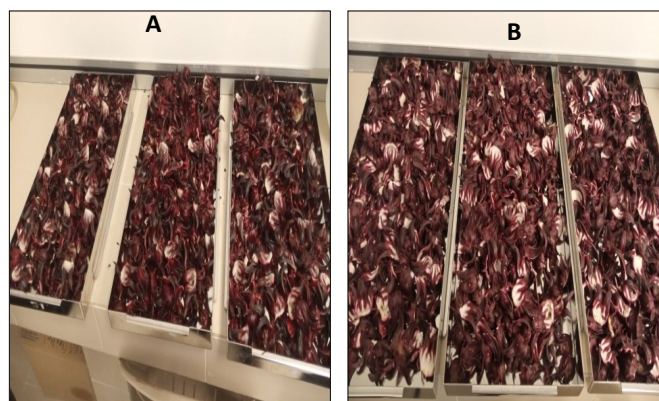
### Results

Roselle withers rapidly when fresh and, due to that, cannot be kept for long or exported over long distances. However, in studies, the three drying methods carried out in this study are; Sun Drying (SD), Oven Drying (OD) and Freeze-Drying (FD). Freeze drying showed higher dry weight than the oven and sun-drying with lower drying time than the sun drying Table 1. Freeze drying also retains the initial colour and shape of the fresh calyx than oven and sun dy-

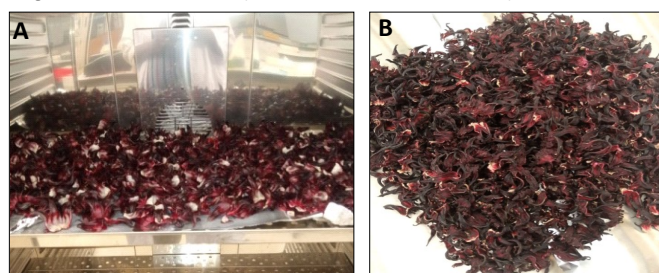
**Table 1.** The three drying methods of roselle calyx

S/NO	Drying methods	Fresh weight kg	Dried weight g	Drying hours
1.	Sun	2	174.1	120
2.	Oven	2	175.3	48
3.	Freeze	2	226.5	31

ing (Fig. 1, 2). However, oven drying also has a slightly higher dried weight and slower drying time than sun drying.



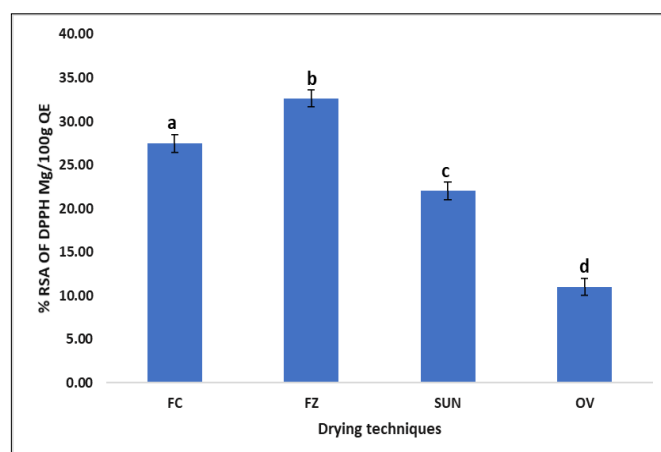
**Fig. 1.** (A) Fresh roselle calyx; (B) Freeze-dried roselle calyx



**Fig. 2.** (A) Oven drying of roselle calyx; (B) Oven dried roselle

### Radical scavenging activity of DPPH

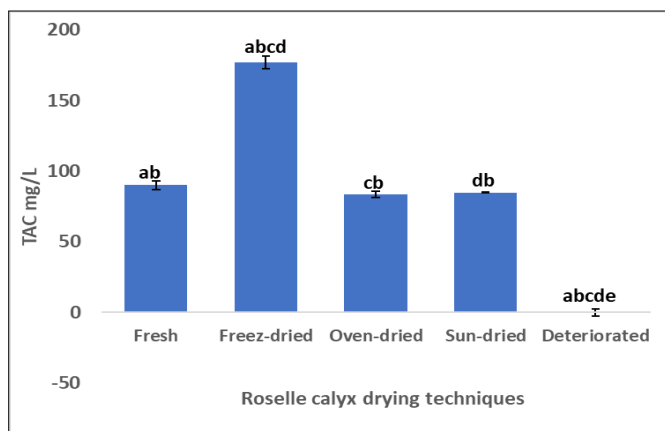
The anthocyanin content is responsible for the major antioxidant activity in roselle calyx. Roselle calyx is known to have higher antioxidant content; therefore, the % RSA in this study revealed that freeze-dried calyx has a higher % RSA with (32 mg/100 g), followed by fresh, sun and oven drying, respectively (27, 22 and 11 mg/100 g QE). Roselle calyx is known to have higher antioxidant content therefore, the % RSA in this study revealed that freeze dried calyx has a higher % RSA with (32 mg/100 g), followed by fresh, sun and oven drying respectively (27, 22 and 11 mg/100 g QE). Roselle calyx are is known to have higher antioxidant content therefore the % RSA in this study revealed that freeze dried-dried freeze calyx has a higher % RSA with (32 mg/100 g), followed by fresh, sun and oven drying respectively (27, 22 and 11 mg/100 g QE) Fig. 3.



**Fig. 3.** % Radical scavenging activity of DPPH of various methods and fresh as control.

### Total Anthocyanin Content of Roselle

*Hibiscus sabdariffa* calyx drying procedures were evaluated using the pH differential method to determine the total anthocyanin content. When comparing the anthocyanin concentration, it was discovered that FD roselle calyx has significantly higher TAC, followed by FC, SD and OD has the least TAC (Fig. 4). This is because freeze drying is one of



**Fig. 4.** Total anthocyanin content TAC mg/l of roselle calyx dried using various drying techniques.

the most effective preservation methods; the high content of TAC in FZD could be that high temperature is not used during the drying as the high temperature is one of the factors influencing anthocyanin stability. However, high temperatures may lower anthocyanin levels in OD and SD (Table 2). In general, high anthocyanin level is promoted by low temperature.

**Table 2.** Roselle anthocyanin retention time, peak area and concentrations of different drying techniques.

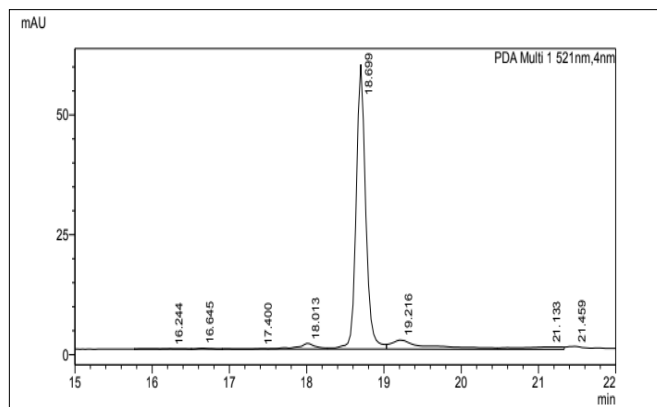
Sample	Retention Time	Peak Area	Concentration $\mu\text{g/ml}$
Sun	18.268	370352	2.426
Oven	18.274	514487	4.293
Freeze	18.299	800575	6.126
Standard (cy-3-G)	18.699	800575	50.000

### Characterization of roselle anthocyanin of various drying techniques using HPLC

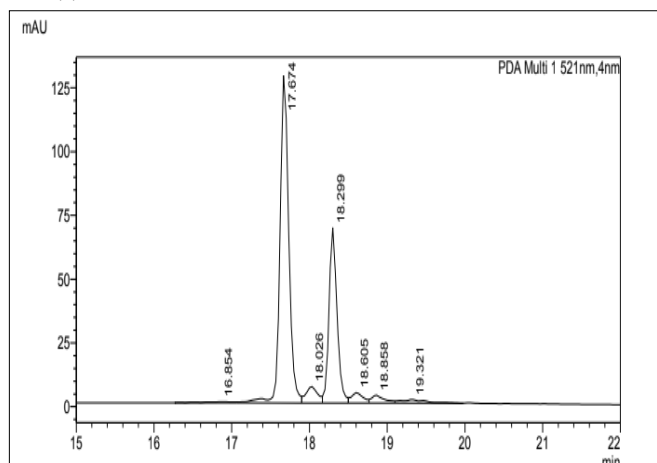
High performance liquid chromatography was used to characterize roselle calyx dried using different drying techniques (Sun, Oven and Freeze-dried). Cyanidine glucoside was used as reference standard. The standard revealed at retention time 18.699 as shown in (Fig. 4). among the drying method analysed freeze dried calyx shown to have high concentration of anthocyanin followed by oven and sun-dried roselle calyx. The retention time, peak area and was explain in Table 4. The anthocyanin revealed were cyanidin glucoside with retention time of 18+++ and cyanidine sambubioside retention time 17+++

### Physicochemical properties of roselle calyx dried differently

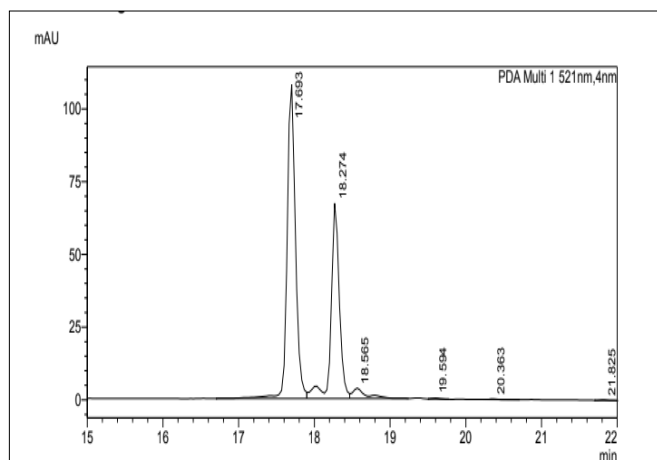
The result of total soluble solute was slightly different across various drying methods and fresh calyx (Table 4) with TSS ranges from 1.7-268 and fresh sample 2.28 Brix. The TSS was higher in freeze-dried calyx, then sun, oven dried and fresh calyx. A product's PH value is regarded one



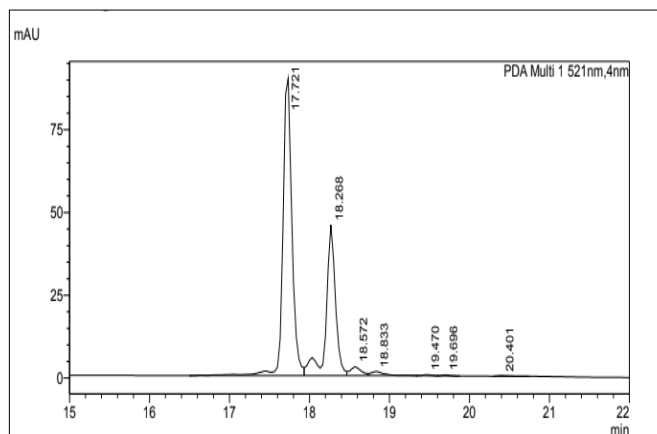
**Fig. 5.** Chromatogram of the external standard anthocyanin cyanidin glucoside (1)



**Fig. 6.** Chromatogram of freeze-dried roselle calyx anthocyanin (1) cyanidin sambubioside while (2) cyanidin glucoside.



**Fig. 7.** Chromatogram of oven-dried roselle calyx anthocyanin (1) cyanidin sambubioside while (2) cyanidin glucoside.



**Fig. 8.** Chromatogram of anthocyanin in Sun-dried roselle calyx (1) cyanidin sambubioside while (2) cyanidin glucoside.

of the most important elements in determining whether or not it was accepted by customers. The pH levels of all roselle of different drying method were slightly different in the range 2.19 to 2.86 for fresh calyx, oven, sun and freeze-dried calyx respectively. The result for total titratable acidity values of the calyx among various drying methods and fresh calyx revealed were slightly different ranges between 18.8-23.4 g malic acid/100g dw (Table 3).

The colour characteristics of the roselle calyx dried using oven, sun, freeze-dried and fresh calyx were significantly different (Tables 4). The *a* (lightness) values of various drying process of roselle calyx were the highest fresh calyx and freeze-dried calyx then sun and oven-dried calyx.

**Table 3.** Physicochemical effect of total soluble solids, pH, titratable acidity and colour on stored roselle calyx.

Sample	TSS (Brix)	TA 100 g/ malic acid dw	pH	Moisture content%
FC	2.28±0.1	23.4	2.7±1.1	78±01
FD	2.68±1.0	22.3	2.19±0.2	14.27±0.1
OD	1.77±1.01	19.01	2.86±2.1	15.75±02
SD	1.70±.001	18.8	2.35±2.01	12.033±0.2

**Table 4.** Colour measurement among various drying techniques.

Sample	L	a	b
FC	14.4±0.8	25.05±4.1	5±0.6
FD	9.42±6.2	24.71.90±5.6	6±0.6
OD	5±9.5	18.87±2.8	5±0.6
SD	11.92±13.6	17.62±2.3	5.33±1.3

### Proximate Analysis

The result of the proximate analysis among various drying techniques revealed that the % ash content was higher in freeze-dried samples with 17.40% followed by oven and sun-dried with 6.76 and 10.36% respectively. The fibre content % was also higher in freeze-dried calyx than in oven and sun-dried with 17.5 and 18.8% respectively. However sun-dried calyx has lowest % fat with 0.37, then oven and freeze-dried with 0.87 and 1.24%. Finally, the % crude protein revealed that freeze dried has higher protein content with 9.2% then sun-dried and oven-dried calyx with 7.8 and 6.9% respectively Table 5.

**Table 5.** Proximate composition of different drying techniques of roselle calyx.

Samples	%Ash	%Fibre	%Fat	% Protein
Sun-dried	6.76	17.5	0.87	7.8
Oven-dried	10.36	18.8	0.37	6.9
Freeze-dried	17.40	20.5	1.24	9.2

### Discussion

Due to its multiple benefits, freeze-drying was more suitable for drying roselle calyx than oven and sun-drying. The calyx is dried without heat or high temperature, as high temperature affects the general qualities of the bioactive compounds found in roselle calyx, particularly anthocyanin.

The higher antioxidant activity in freeze-dried roselle might be because no heat is applied while drying all the bioactive components were preserved because thermal affects the stability of anthocyanin. Moreover, thermal treatment is capable of causing the nutritional and organoleptic decline and modifications in the levels of carotenoids, ascorbic acid and phenolic compounds, leading to decreased antioxidant capacity and other effects on biologically active compounds (4, 13).

The prevalent of cyanidin and delphinidin in roselle is in agreement with the findings of many authors (25, 26) that have reported two significant anthocyanins compounds (delphinidin-3-sambubioside and cyanidin-3-sambubioside) and 2 minor compounds (delphinidin-3-glucoside and cyanidin-3-glucoside) present in the calyces of roselle (25, 27). Moreover, the results were consistent with (28). Environmental factors, including solar radiation, temperature and nitrogen level, are essential for regulating anthocyanin accumulation in plants. The freeze-dried roselle proved superior colour stability and prevent anthocyanin degradation (25). Several variables, including pH and temperature, enzymes, metal ions, light and oxygen have been shown to deteriorate anthocyanins in nature. Anthocyanin degradation may also produce aldehydes with benzene rings, which could harm human health (4). Among the most common food processing activities are thermal treatment and pH adjustment. When it comes to preserving industrial beverages, heat treatment is the most used procedure. Our HPLC analysis of anthocyanin (Fig. 4-7 and Table. 3) is similar to the findings of an earlier study (29). The finding of cyanidine sambubioside with 17++ in our studies correspond the finding of (30). The HPLC identification of anthocyanin in our study indicated that freeze-dried roselle calyx has significant concentration, retention time closer to the reference standard cyanidine glucoside than oven and sundried roselle calyx.

The physicochemical properties in this finding revealed that, the amount of total soluble solids (TSS) and the pH value of roselle calyces are critical to their quality. The low pH value and high TSS levels are markers of the product's quality. Table 4 the increase level of TSS may be due to hydrolysis of polysaccharides to monosaccharides and oligosaccharides (31). The finding on TSS in this study is in agreement with an earlier report (32, 33) where they reported TSS value ranges from 2.60 – 4.10 and 2.90 - 4.60 with slight increase due to storage. The findings of Ph level in the current study agreed with values reported (34) for the pH of roselle from Malaysia (2.49) similar to another report (35) who reported 2.16±0.02. The titratable acidity in the present study is in agreement with an earlier report (35, 36). Organic acid findings were attributed to the presence of acids in the roselle extract, including citric acid, hydroxy citric acid, hibiscus acid, malic and tartaric acid as main constituents and oxalic and ascorbic acid as minor compounds (37). Finally, these findings corroborate with another study (38). Additionally, reports are on the fact that (39), roselle has a large amount of acid, with a titratable acidity value of 2.49%. The acid that makes roselle unique is hibiscus acid, or (+)-hydroxy citric acid, abbrevi-

ated (+) HCA. This inhibited fat formation and resulted in a decrease in body weight experiments carried out on rats (38).

Colour is one of the most critical quality aspects influencing customer acceptance of food since it conveys the initial impression of food quality to the consumer (38). There have been many studies on the colour qualities of roselle extract, with the reported values varying according to the drying procedure, duration and temperature used (40, 41). The present investigation discovered that the colour values varied according to the drying procedures used. The calyx characteristics were aided by the colour qualities. Our results on colour measurement are consistent with those of with a few exceptions (36).

The results of proximate composition viz, moisture content, crude protein, crude fat, crude fibre and total ash of among various drying process including sun, oven and freeze-dried roselle calyx are presented in Table 4 and are discussed.

The result was similar with the literature values (35, 42-44) who reported the moisture content of fresh Roselle calyces as 86.50% (w.b.), 85.00% and 86.50%. In the present investigation, the result of the average moisture content of fresh calyces of Roselle was found to be  $78\pm 0.1\%$ . The average crude protein content of fresh calyces of Roselle was found to be  $2.95\pm 0.14\%$ . The results obtained were slightly lower than the earlier studies reported (44), who reported the protein content

## Conclusion

The effects of different drying techniques including freeze, oven and sun drying of roselle calyx were tested in the present study. The freeze dried showed higher dry weight, slower drying time, higher antioxidant and anthocyanin content compared to HPLC analysis revealed two roselle major anthocyanins, cyanidin glucoside and cyanidin sambubioside accumulated in the freeze dried. Therefore, the freeze-drying method was determined to be the best method for preserving roselle calyx hence might be utilized for future production and commercialization of dried roselle calyx.

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## Authors contributions

The authors' contributions are as follows: NSM came up with the research topic and oversaw the manuscript's drafting; AAL performed all the analysis and write the manuscript; MMK re-evaluated the paper and made changes, while NFHN proofread it and contributed to the manuscript report AMD review the statistics.

## Compliance with ethical standards

**Conflict of interest:** The authors declare no Conflict of

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**Ethical issues:** None

## References

- Djaeni M, Utari FD, Sasongko SB, Kumoro AC. Evaluation of food drying with air dehumidification system: A short review. IOP Conf Ser Earth Environ Sci. 2018;102(1).
- Castañeda Ovando A. Síntesis hidrotermal de ferritas de plata. Una opción para depurar aguas residuales y recuperar plata en la industria minera. 2002.
- Duan X, Liu T, Zhang D, Su X, Lin H, Jiang Y. Effect of pure oxygen atmosphere on antioxidant enzyme and antioxidant activity of harvested litchi fruit during storage. Food Res Int [Internet]. 2011;44(7):1905-11. Available from: <http://dx.doi.org/10.1016/j.foodres.2010.10.027>
- Tsai, Jen P, McIntosh, John, Pearce, Philip et al. Anthocyanin and antioxidant capacity in Roselle (*Hibiscus sabdariffa* L.) extract. Food Res Int. 2002;35(4):351-56.
- Mazza G Miniati E. Anthocyanins in fruits, vegetables and grains. CRC Press. 2018.
- Juhari NH, Martens HJ, Petersen MA. Changes in physicochemical properties and volatile compounds of roselle (*Hibiscus sabdariffa* L.) calyx during different drying methods. Molecules. 2021;26(20).
- Hahn F, Hernandez G, Hernandez J, Perez C, Vargas JM. Optimization of Roselle drying time and drying quality. Can Biosyst Eng., 2011;53:3.1-3.8.
- Sucherman O. Efektivitas formulasi insektisida nabati marigold (*Tithonia diversifolia*) terhadap *Empoasca flavescens*, hama utama pada tanaman teh. J Penelit Teh dan Kina., 2012;15(2).
- Meza-Jimenez J, Ramirez-Ruiz J, Diaz-Nunes J. The design and proposal of a thermodynamic drying system for the dehydration of Rosell (*Hibiscus sabdariffa*) and other agro-industrial products. African J Agric Res. 2008;3(7):477-85.
- Daniel DL, Huerta BEB, Sosa IA, Mendoza MG. Effect of fixed bed drying on the retention of phenolic compounds, anthocyanins and antioxidant activity of roselle (*Hibiscus sabdariffa* L.). Ind Crops Prod [Internet]. 2012;40(1):268-76. Available from: <http://dx.doi.org/10.1016/j.indcrop.2012.03.015>
- Hirunpanich V, Utaipat A, Norales NP, Bunyapraphatsara N, Sato H, Herunsale A, Suthisisang C. Hypocholasterolamic and antioxidant effects of aqueous extracts from the dried calyx of *Hibiscus sabdariffa* L. in hypercholesterolemic rats. Journal Ethnopharmacol. 2006;(103):252-60.
- Sharma A, Chen CR, Vu Lan N. Solar-energy drying systems: A review. Renew Sustain Energy Rev. 2009;13(6-7):1185-210.
- Gonzalez-Palomares S, Estarrón-Espinosa M, Gómez-Leyva JF, Andrade-González I. Effect of the temperature on the spray drying of Roselle extracts (*Hibiscus sabdariffa* L.). Plant Foods Hum Nutr. 2009;64(1):62-67.
- Hirunpanich V, Utaipat A, Morales NP, Bunyapraphatsara N, Sato H, Herunsale A et al. Hypocholasterolemic and antioxidant effects of aqueous extracts from the dried calyx of *Hibiscus sabdariffa* L. in hypercholesterolemic rats. J Ethnopharmacol. 2006;103(2):252-60.
- Mazza G, Miniati E. Anthocyanins in fruits, vegetables, and grains. CRC Press, Boca Raton, FL, USA. 1993.
- Kovinich N, Kayanja G, Chanoca A, Otegui MS, Grotewold E. Abiotic stresses induce different localizations of anthocyanins in *Arabidopsis*. Plant Signal Behav. 2015;10(7).
- Alexandra Pazmio-Durán E, Giusti MM, Wrolstad RE, Glória MBA. Anthocyanins from banana bracts (*Musa X paradisiaca*) as potential food colorants. Food Chem. 2001;73(3):327-32.

18. Wu HY, Yang KM, Chiang PY. Roselle anthocyanins: Antioxidant properties and stability to heat and pH. *Molecules*. 2018;23(6).
19. Ramírez-Rodrigues MM, Plaza ML, Azeredo A, Balaban MO, Marshall MR. Phytochemical, sensory attributes and aroma stability of dense phase carbon dioxide processed *Hibiscus sabdariffa* beverage during storage. *Food Chem*. 2012;134(3):1425-31.
20. McGuire RG. Reporting of objective color measurements. *HortScience*. 1992;27:1254-55.
21. Ruck JA. Chemical methods for analysis of fruits and vegetables products. Stn Summerl Can Res Board, Dep Agric Canada.
22. Ashaye OA, Adeleke TO. Quality attributes of stored Roselle jam. *Int Food Res J*. 2009;16(3):363-71.
23. AOAC. Official methods of analysis of AOAC International (17th ed.). Gaithersburg, MD, USA AOAC Int.
24. J.S.H. Association of official agricultural chemists. *J Franklin Inst*. 1935;219(2):236.
25. Garg A, Garg S, Zaneveld LJD, Singla AK. Chemistry and pharmacology of the *Citrus bioflavonoid* hesperidin. *Phyther Res*. 2001;15(8):655-69.
26. Lewicki PP. Design of hot air drying for better foods. *Trends Food Sci Technol*. 2006;17(4):153-63.
27. Martínez-Navarrete N, Salvador A, Oliva C, Camacho MM. Influence of biopolymers and freeze-drying shelf temperature on the quality of a mandarin snack. *Lwt* [Internet]. 2019;99:57-61. Available from: <https://doi.org/10.1016/j.lwt.2018.09.040>
28. Gu KD, Wang CK, Hu DG, Hao YJH. How do anthocyanins paint our horticultural products?. *Scientia Horticulturae*. 249:257-62.
29. Anaya-Berrios M del PG-FEO-RBB-OG-PA-B del PG-FEO-RBB-OG-P. New Pyranoanthocyanins Synthesized from Roselle (*Hibiscus sabdariffa* L.) Anthocyanins. *J Mex Chem Soc*. 2016;60(1):13-18.
30. Salazar-González, Claudia, Vergara-Balderas TF, Ortega-Regules EA et al. Antioxidant properties and color of *Hibiscus sabdariffa* extracts.
31. Bhardwaj RL, Pandey S. Juice blends-a way of utilization of under-utilized fruits, vegetables, and spices: A review. *Crit Rev Food Sci Nutr*. 2011;51(6):563-70.
32. Waskar DP, Gaikwad RS. Packaging and storage studies on pomegranate, kokum and mango based blend. *Agric Sci Dig*. 2004;24(3):174-77.
33. Gomaa, R. B. A., & Nahed MR. Post-harvest studies on reducing losses and maintaining quality of packaging Roselle calyxes. *J Sustain Agric Sci*. 2016;42(4).
34. Wong JWC, Xiang L, Chan LC. pH requirement for the bioleaching of heavy metals from anaerobically digested wastewater sludge. *Water, Air, Soil Pollution*,. 2002;138(1):25-35.
35. Shruthi VH, Ramachandra CT, Nidoni U, Hiregoudar S, Naik N, Kurubar AR. Physico-chemical, nutritional and functional properties of Roselle (*Hibiscus sabdariffa* L.). *Int J Curr Microbiol Appl Sci*. 2017;6(12):2976-82.
36. Sukkhaeng S, Promdang S, Doung-ngern U. Fruit characters and physico-chemical properties of roselle (*Hibiscus sabdariffa* L.) in Thailand-A screening of 13 new genotypes. *J Appl Res Med Aromat Plants* [Internet]. 2018;11(May):47-53. Available from: <https://doi.org/10.1016/j.jarmap.2018.10.001>
37. Da-Costa-Rocha I, Bonnlaender B, Sievers H, Pischel I, Heinrich M. *Hibiscus sabdariffa* L. - A phytochemical and pharmacological review. *Food Chem*. 2014;165:424-43.
38. Fasoyiro S., Ashaye O., Adeola A, Samuel F. Chemical and storability of fruit-flavoured (*Hibiscus sabdariffa*) drinks. *World J Agric Sci* [Internet]. 2005;1(2):165-68. Available from: [http://www.idosi.org/wjas/wjas1\(2\)/11.pdf](http://www.idosi.org/wjas/wjas1(2)/11.pdf)
39. Wong, Peng Kong, Yusof Salmah, Ghazali HM, Man CYB. Physico-chemical characteristics of Roselle (*Hibiscus sabdariffa* L.). *Nutr Food Sci*. 2002;32(2):68-73.
40. Chumsri P, Sirichote A, Itharat A. Studies on the optimum conditions for the extraction and concentration of roselle (*Hibiscus sabdariffa* Linn.) extract. *Songklanakarin J Sci Technol*. 2008;30(SUPPL. 1):133-39.
41. Park HS, Chung MS, Shin DS, Jung YW, Park JS. Accessible and informative sectioned images, color-coded images, and surface models of the ear. *Anat Rec*. 2013;296(8):1180-86.
42. Babalola SO, Babalola A, Aworh OC. Compositional attributes of the calyces of roselle (*Hibiscus sabdariffa* L.) Babalola, *Bioline Int*. 2001;01046(1977):2-4.
43. Langová J, Jaisut D, Thuwapanichayanan R, Phowong C, Štencl J, Jovanovic D et al. Modelling the moisture sorption isotherms of roselle (*Hibiscus sabdariffa* L.) in the temperature range of 5-35 °C. *Acta Univ Agric Silvic Mendelianae Brun*. 2013;61(6):1769-77.
44. Miah M, Bhowmik S, Kumar Sarker A, Yusuf Miah M, Bhattacharjee S, Sultana A et al. Evaluation of amino acid profile of jackfruit (*Artocarpus heterophyllus*) seed and its utilization for development of protein enriched supplementary food. *J Noakhali Sci Technol Univ* [Internet]. 2017;1(1):77-84. Available from: <https://www.researchgate.net/publication/317168972>

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