Assessment of Antioxidant and Phenolics Content of Various Drying Techniques (*Hibiscus sabdariffa roselle L.*) Calyx UKMR-1

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Abstract
Providing food, clothing, shelter, and medicine, plants have played a critical role in human existence for mil lennia. The utilization of roselle (Hibiscus sabdariffa L.) calyx as a source of anthocyanins has been studied through rigorous investigations. Due to its perishable feature, the transformation of roselle calyces into dried without decreasing in their quality is highly difficult. This research was aim to determine effect various drying techniques of roselle sun, oven and freeze drying. Antioxidant, phenolic, flavonoids and anthocyanin content were determined. The studies revealed that freeze drying retained the antioxidant qualities, rate of drying increased significantly than oven and sun drying. It is concluded in other to retain the roselle calyces extract quality freeze drying methods should be employed.

***Index Terms*—Aantioxidants, phytochemical, calyx, drying and, cyanidin glucoside**

I. INTRODUCTION

Hibiscus sabdariffa L. (Roselle) is commercially cultivated in many tropical and subtropical countries like India, Indonesia, Sudan, Egypt, and Mexico including countries like India, Indonesia, Sudan Egypt, Mexico, and Malaysia[1]. The roselle calyx is brilliant red due to the existence of anthocyanins, such as cyanidin-3-sambubioside, cyanidin-3-glucoside, and delphinidin-3-glucoside [1]. Jam, jelly, ice cream, pastries, preserves, as well as herbal beverages, are all made with the calyx [2]. Drinking roselle tea has been connected to many medical advantages, primarily because of its antioxidant properties [3]. Antioxidant properties and roselle calyx anthocyanin have been linked in the literature [4]. The total anthocyanin content of roselle calyces is 2.52 g/100 g, expressed as delphinidin-3-glucoside [5]. Ascorbic acid is also found in roselle extracts. According to Wang et al., anthocyanins have greater antioxidant activity than ascorbic acid [1]. As a result, anthocyanins are primarily responsible for the roselle extract's antioxidant properties. Due to their sensitivity to oxygen, heat, and light during manufacturing and storage, anthocyanins in food products encountered difficulties [6]. Mazza and Miniati ascertained that anthocyanin thermal degradation of roselle was rapid at temperatures beyond 100°C [7]. The calyces are frozen or dried in the sun /artificially for out of season supply [3]. Compared to other drying procedures, cell structure retained the best by freeze-drying, and samples resembled fresh samples the most [3, 8].

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. Furthermore, proper drying techniques can greatly improve product quality [3]. Various methods of drying, such as spray-drying [3], freeze-drying [9], solar thermodynamic drying, fixed bed drying [8], sun-drying, and oven-drying [3], has been applied to the roselle flower. Drying settings and circumstance influence the scent and flavour of dried roselle [10]. Generally, drying processes ensure microbiological stability, extend product shelf life, and make packing and distribution easier [11-13].[3], 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. As a result, research into various drying procedures is needed to ensure that the final product meets the specified standards. Roselle UMKR-1 hasn't been the subject

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of any experiments examining various drying methods. Drying procedures for roselle calyx, antioxidants, and bioactive components focus on this study.

II. Procedure for Paper Submission

The roselle calyx UMKR-1wereharvested freshly at a completely mature stage from the Faculty of Bioresources and Food Industry Farm Besut Campus, UNISZA, Malaysia. 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.

A. *Sun Drying*

The fresh roselle calyces of each were placed on a plate and placed on a table in direct sunshine between 9 and 4 p.m.; respectively, for seven 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 calyces were evenly agitated[2, 3].

B. *Oven Drying*

Two kilograms of fresh roselle calyces were placed on a clean metal plate and set in the oven at 60 °C for 24 hours.

C. *Freeze drying*

After being washed with tap water, the calyces were frozen and stored at -20^{0}C. Frozen material was thawed by placing it in a refrigerator (4^{0}C) overnight before use. Drying was performed beyond 48 h inside a freeze dryer (Freeze dry System- LABCONCO free zone 18, Kansas City, MO, USA) at -40 ^{0}C and pressure 3.3× 10-3 mbar [4].

D. *Total Phenolic Content*

To make 100 ml of methanol, one "gram of methanol extract was added to 15 ml of methanol and extracted three times by maceration for 2 hours, then filtrated. 10mg of garlic acid was added to 100 ml of alcohol (100 micrograms per millilitre) and then combined to yield 6, 25, 12, 5, 25 or 50 micrograms per millilitre. Each dilution was diluted with 10 ml of distilled water to yield a final volume of one millilitre. Folin Ciocalteu's reagent was then added and incubated for five minutes at room temperature. Four ml of $Na_2 CO_3$ (20% w/w) was added, and the mixture was stirred and allowed to stand for 30 minutes at room temperature before being centrifuged. The standard's absorbance was measured at 765 nm with a UVIS spectrophotometer against a blank, which is distilled water, to determine its consistency and reliability.

The standard curve of garlic acid was used to measure the amount of garlic acid in the sample (Tan and Kassim, 2011). To calculate the results in terms of weighted average percentages, we utilized the procedure shown below: %

$$(\% \ w/w) = GAE\times V\times D\times 10^{-}6 \times 100/W, \text{"}GAE.$$

E. *Total Flavonoids Content*

Ten milligrams of quercetin were dissolved in 100 ml of methanol at a concentration of 100 g/ml; then the solution was diluted to 10, 20, 40, 80, or 160 g/ml." For each of the standard solutions, separate mixtures were made out of (0.5 mg) of aluminium chloride, (0.1 mg) of aluminium chloride (10 percent), (0.1 mg) of 1 M potassium acetate, and (2.8 mg) water. After 30 minutes of incubation at room temperature, a UV/VIS spectrophotometer was used to detect the absorbance at 415 nm. Approximately 10% of the aluminium chloride was replaced with distilled water. 25 ml of methanol was used to extract 1 g of methanol extract. "Aluminum chloride was used to determine the flavonoid concentration of a 0.5 ml methanol extract, as stated above" (Tan and Kassim, 2011). Quercetin was measured using a standard curve. Calculated values are given in weight-to-weight ratios. Percent w/w flavonoids content

$$(QE) = QE+V+D+10+6+100/W$$

F. Total Anthocyanin Concentration (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 CH3COONa). It was necessary to dilute the extract samples. They were shaken for 15 minutes 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 [5]. 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 by [6].

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G. *DPPH method*

An assay for diphenyl phosphate (DPPH) was carried out in accordance with the method of an earlier study [Wu and colleagues 2015]. It was made daily, and the working solution of DPPH was prepared by diluting the sample and mixing it with 1mL of the active ingredient. After vortexing, the mixture was allowed to rest for 10 minutes 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:[7-9]

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

III. RESULTS AND DISCUSSION

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 shows a 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 dying fig. The fresh calyx than oven and sun dying figs 1 and 2. However, oven drying also has a slightly higher dried weight and slower drying time than sun drying.

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

Due to its multiple benefits, freeze-drying will be 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.

Figure 1. Oven drying of roselle calyx (a) and Oven-dried roselle calyx(b)

Figure. 2 Fresh roselle calyx (a) and Freeze-dried roselle calyx (b)

Total phenolic and flavonoids content of different dried roselle calyx

According to the current findings, fresh roselle calyx has the highest phenolic content (487mg GAE/100g), followed by freeze-dried roselle calyx (428mgGAE/100g), oven-dried roselle calyx (165 mg GAE/100g), sun-dried roselle calyx (113 mg GAE/100g), and finally deteriorated roselle calyx (60 mg GAE/100g) fig 3. This study's results are comparable to those of Wu et al. (2018), who discovered that dried roselle calyces contain total polyphenol content (TPC) of around 683.13 mg (GAE)/100. While flavonoids concentration, surprisingly sun-drying shown to have higher flavonoids content (193 mg/ml QE/100g) followed by freeze-dried calyx (109 mg/ml QE/100g) fig. 4, then oven (108 mg/ml QE/100g) and fresh calyx shown to have lower flavonoids content (43mg/ml QE/100g) unlike phenolic content. This finding for the higher flavonoids in sun-drying than other drying method utilized in this study is in line with the explanation of [8] that might be due to the haze occurrence, that made the sun drying slow as well as partial, and the results for sun-dried samples tested, therefore not be generalised [8].

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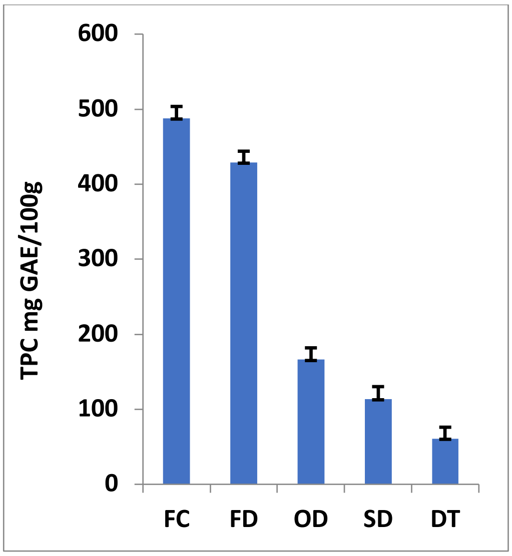

Figure 3. The total phenolic content mg GAE/100g

Key: FC=Fresh calyx, FD= Freeze dried, SD= Sun-dried, OD= Oven-dried.

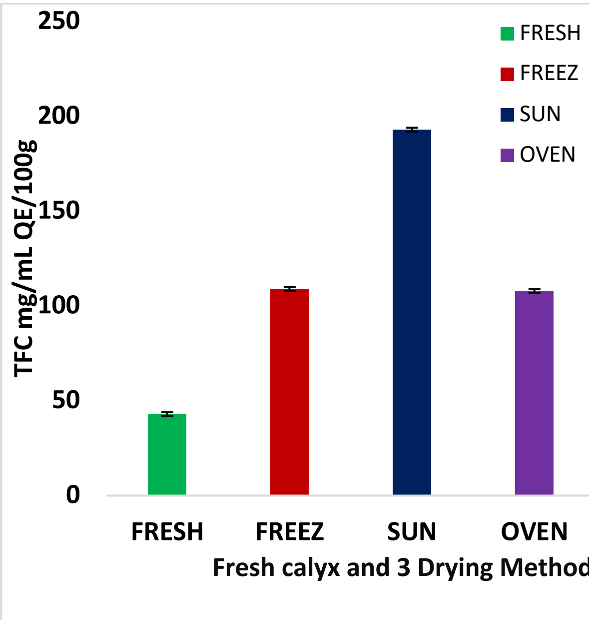

Figure 4. Total Flavonoids Content of different drying methods

% Radical scavenging activity of DPPH

The anthocyanin content is responsible for. The major antioxidant activity in roselle calyx. The anthocyanin content is responsible for the major antioxidant activity in roselle calyx 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/100g), followed by fresh, sun and oven drying, respectively (27, 22 and 11 mg/100g 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/100g), followed by fresh, sun and oven drying, respectively (27, 22 and 11 mg/100g 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/100g), followed by fresh, sun and oven drying, respectively (27, 22 and 11 mg/100g QE). Therefore, 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[5, 10].

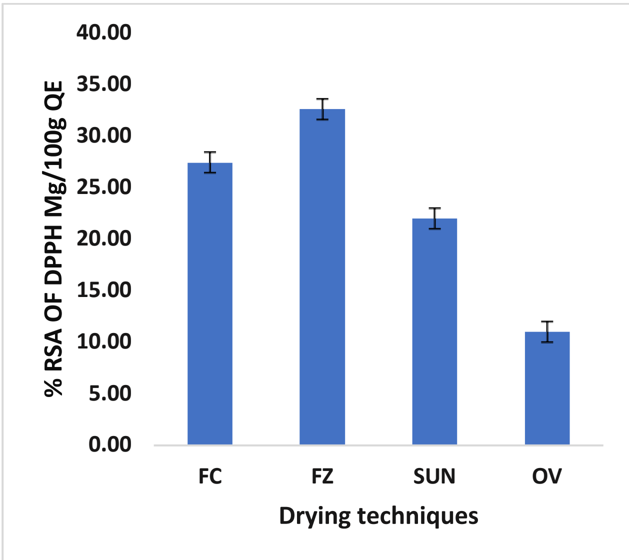

Figure 5. % Radical scavenging activity of DPPH of various methods and fresh as control

Total anthocyanin content of roselle

The total anthocyanin content of different *Hibiscus sabdariffa* calyx drying methods was measured using the pH differential method. When comparing the anthocyanin concentration, it was discovered that FD roselle calyx has the highest TAC, followed by FC, SD, and OD has the least TAC (Table.2). This is because Freeze drying is one of 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

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temperatures may lower anthocyanin levels in OD and SD (Table 2). In general, high anthocyanin level is promoted by low temperature. This study identified three anthocyanidins, two of which are the most abundant anthocyanidin in Hibiscus sabdariffa delphinidin-3- sambubioside, Cyanidin-3-sambubioside, and then cyanidin-3-Glucoside. All the anthocyanidin present has higher TAC in FZD samples than FC, SD, and OD. The prevalent of cyanidin and delphinidin in roselle is in agreement with the findings of many authors, among which are [11], [12] and [13] have reported two significant anthocyanins compounds (delphinidin-3-sambubioside and cyanidin-3-sambubioside) and two minor compounds (delphinidin-3-glucoside and cyanidin-3-glucoside) present in the calyces of roselle [14] and [14]; [12].

TABLE.2 TOTAL ANTHOCYANIN CONTENT OF FOUR DIFFERENT DRYING METHODS

ID	Cyanidin-3-Glucoside mg/L	Cyanidin-3-Sambubioside mg/L	Delfinidin-3-sambubioside mg/L
FC	115.24	158.7	162
FZ	231	317.7	284
SD	114.6	157.43	161.5
OD	85.91	117.9	145.2

Key: FC=Fresh calyx, FD= Freeze dried, SD= Sun-dried, OD= Oven-dried.

Moreover, the results were consistent with (Gu et al., 2019). 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 [11]. 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 [5]. 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.

CONCLUSION

The roselle calyx was analysed in this research using different drying techniques freeze, oven and sun drying. The Freeze has been shown to have higher dried weight, slower drying time, higher phenolic content, higher antioxidant and anthocyanin content. After drying, freeze-drying was determined to be the best method for preserving cell structure. The quality of the dried roselle calyx varied depending on the drying method employed, and this study might be utilized to help determine the best drying process for future production and commercialization of dried roselle calyx.

CONFLICT OF INTEREST

No conflicts of interest have been declared by the authors. However, it appears the funders were not involved in any way in study design or data collection and analysis, writing of this publication, or decision to publish results.

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