

Article

Processing of Herbal Tea from Roselle (*Hibiscus sabdariffa* L.): Effects of Drying Temperature and Brewing Conditions on Total Soluble Solid, Phenolic Content, Antioxidant Capacity and Sensory Quality

Quang Vinh Nguyen¹ and Hoang Van Chuyen^{2,*} 

¹ Institute of Biotechnology and Environment, Tay Nguyen University, Buon Ma Thuot City, Daklak 630000, Vietnam; vinh12b@yahoo.com

² Faculty of Agriculture & Forestry, Tay Nguyen University, Buon Ma Thuot City, Daklak 630000, Vietnam

* Correspondence: vanchuyen.hoang@uon.edu.au or baobien35@gmail.com

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Abstract: Roselle (*Hibiscus sabdariffa* L.) is an annual shrub popularly grown in tropical and subtropical areas. Although Roselle fruit has been used for a long time as a herbal tea due to its high content of bioactive compounds like phenolic acids and anthocyanins, the studies on the effects of the drying process and brewing conditions on the content of beneficial components and bioactivities of Roselle tea is limited. In this study, the influence of drying temperature on the retention of total phenolic content (TPC) and antioxidant capacity (AC) of the dried Roselle and the effects of brewing conditions including water temperature, liquid-solid ratio and brewing time on total soluble solid (TSS) content, total phenolic content, and antioxidant capacity of Roselle tea were investigated. The drying at 80 °C produced the dried Roselle with the highest retention of TPC (25.196 mg GAE/g) and AC (IC₅₀ of 2.2 mg/mL for 2,2-diphenyl-1-picrylhydrazyl (DPPH) inhibition) and the highest sensory quality compared to the drying at other temperatures. The brewing process using hot water at 90 °C with the solid-liquid ratio of 1:10 (g/mL) for 30 min resulted in the highest TSS content (5.4 °Bx), TPC (12.7 mg GAE/g dried Roselle) and AC (73.3% DPPH inhibition) of the Roselle tea. Thus, the drying at 80 °C combined with the brewing of dried Roselle for 30 min using 90 °C hot water with the solid-liquid ratio of 1:10 (g/mL) are recommended to produce Roselle tea with the highest content of beneficial ingredients.

Keywords: *Hibiscus sabdariffa* L.; drying; brewing; phenolic; antioxidant

1. Introduction

Roselle (*Hibiscus sabdariffa* L.) is an annual shrub belongs to the Malvaceae family and mostly grows in tropical and subtropical areas like African and Asian countries [1]. The calyces of Roselle have been used in traditional medicine to help to treat many diseases like hypertension, diabetes and liver disorders [2,3]. Studies on the phytochemical compositions of Roselle have found that the calyces of Roselle contain a significant number of bioactive compounds, which may contribute to the beneficial health effects of this material such as phenolic acids, anthocyanins, flavonoids and polysaccharides [4]. The major anthocyanins are delphinidin-3-glucoside and cyanidin-3-sambubioside while quercetin and its glucosides are the principal flavonols and chlorogenic acid is the main phenolic acid present in Roselle [4,5]. Tsai et al. (2002) [6] found that anthocyanins were the principal component contributing to the antioxidant capacity of the extract from Roselle calyces. Roselle extracts also showed antibacterial activity against various species like *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, and *Clostridium sporogenes* [7] and antifungal activity against *Aspergillus*, *Trichophyton* and

Cryptococcus [8]. In a study by Liu et al. (2011) Roselle extract was reported to have anticancer activity via protective effects against oxidative damage induced by tert-butyl hydroperoxide (t-BHP) in rat primary hepatocytes [5].

In addition to the use of folk medicine, Roselle is also commonly used to provide natural purple color for many kinds of food like jellies, beverages, and desserts. The fruit and calyxes of Roselle are also processed to jams, marmalade and, especially, dried to use as herbal tea [6,9]. Previous studies have demonstrated that drying, an essential thermal process in producing herbal teas, may cause significant losses of bioactive ingredients and thereby decrease the health benefits and quality of the products [10,11]. The brewing conditions such as quality, volume, and temperature of water and brewing time have also been reported to significantly affect the content of the desired components in tea infusion [12,13]. The release level of the tasty ingredients and volatile compounds into the tea will decide the sensory quality of the herbal tea. Although there have been some studies on the extraction of Roselle using different types of solvent [14–16] and investigations into the drying processes for this material [17–19], comprehensive studies to establish a convenient procedure of drying and brewing to achieve high-quality Roselle tea infusion are limited.

In this study, the use of a common hot-air dryer for drying of Roselle and the classical brewing method were investigated to find out the most suitable drying and brewing conditions in order to produce Roselle tea with a high content of bioactive, antioxidant activity and sensory quality. The results help to suggest a convenient procedure of drying, along with brewing, which can be easily used at household or small scale to produce high-quality Roselle tea.

2. Material and Methods

2.1. Material

Roselle fruits were harvested at Daklak province in the Central Highland of Vietnam. After being harvested, the edible parts were separated to be used as the material for the drying process.

Gallic acid, Folin-Ciocalteu reagent and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma-Aldrich Pty Ltd. (Singapore City, Singapore). Analytical grade methanol was purchased from Merck Ltd. (Ho Chi Minh City, Vietnam).

2.2. Drying of Roselle

The material was dried at 60, 80, 100, and 120 °C using a hot-air drying oven (UM500, Memmert, Schwabach, Germany) until reaching a moisture content under 8%. During the drying processes, the moisture content of the samples was measured to establish the drying curves at each drying temperature. The dried Roselle samples obtained from different drying temperatures were then analyzed for total phenolic content (TPC), antioxidant capacity and sensory quality.

2.3. Brewing of Dried Roselle

To investigate the effect of brewing temperature on the quality of the Roselle tea, the dried Roselle was brewed using hot water at the temperature of 80, 90, and 100 °C with the ratio of water to dried Roselle of 10:1 (mL/g) for 20 min. The influence of the liquid-solid ratio on the tea quality was investigated by brewing the Roselle at 100 °C for 20 min using different water-Roselle ratios (8:1, 10:1, 12:1 and 14:1). Finally, different brewing periods (20, 30, and 40 min) were used to study the effect of brewing time on tea quality. The Roselle tea samples brewed under different conditions were evaluated for total soluble solid (TSS) content, TPC and antioxidant capacity.

2.4. Measurement of Total Phenolic Content (TPC)

Total phenolic content of the extracts was determined according to the method described by Vuong et al. (2014) [20] with some modifications. An extract of 0.5 mL from Roselle was mixed with 2.5 mL of Folin-Ciocalteu reagent in a test tube and left at room temperature. After 5 min, 2 mL of

7.5% (w/v) Na₂CO₃ and the mixture was left for reacting at room temperature in a dark room for 1 h. The absorbance of the reacted mixture was then measured at 765 nm using a spectrophotometer (V-630, Jasco, Tokyo, Japan). The total phenolic content of the extracts was expressed as mg gallic acid equivalent (mg GAE) based on the standard curve of gallic acid solutions.

2.5. Determination of Antioxidant Capacity

Antioxidant activity of the extracts from Roselle was evaluated by the free radical scavenging activity of the extracts against 2,2-diphenyl-2-picrylhydrazyl (DPPH) radical. The DPPH antioxidant assay was carried out according to a method described by Nguyen and Eun (2011) [21] with some modifications.

The working DPPH solution was prepared by diluting stock DPPH solution (0.24 g/L) with methanol to obtain an absorbance of 1.1 ± 0.02 at 515 nm. A volume of 0.15 mL of Roselle extract was then mixed with 2.85 mL of the DPPH working solution in a test tube and the solution was left for 15 min at room temperature in a dark room. The absorbance of the reacted solution was then measured at 515 nm using a spectrophotometer (V-630, Jasco, Japan). The absorbance of a blank sample that contained the same amount of methanol and DPPH solution was also measured.

Radical scavenging activity was calculated by the following formula:

$$\% \text{ Inhibition} = [(A_b - A_e)/A_b] \times 100$$

where A_b is the absorbance of the test with a blank sample and A_e is the absorbance of the test with Roselle extract solution. The IC₅₀ of the DPPH radical scavenging activity was calculated based on a plot of scavenging ability versus concentration of extracts.

2.6. Sensory Evaluation

To investigate the effect of drying temperature on the dried Roselle samples, Roselle tea was prepared by brewing 1 g of the dried Roselle with 200 mL of hot water at 90 °C for 15 min. The tea was then decanted into a white ceramic cup, added with 0.5 g of stevia sweetener and left to cool down to 50 °C before being evaluated by 20 panelists (10 men and 10 women) with different ages ranging from 22 to 40 years old at Tay Nguyen University. The sensory characteristics including aroma, taste, aftertaste, color, transparency and the overall acceptance were evaluated by the 9-point hedonic scale [22].

2.7. Determination of Total Soluble Solid (TSS) Content

The TSS content of the extracts were determined using a digital refractometer (PAL- α , ATAGO, Tokyo, Japan) according to the method described by AOAC (2000) [23].

2.8. Determination of Moisture Content

Moisture content of the samples were analyzed by drying to a constant weight at 105 °C using a drying oven (UM500, Memmert, Germany) according to the method described by AOAC (2000) [23].

2.9. Statistical Analysis

Experiments were repeated in triplicates and the results were expressed as the mean values \pm standard deviations. The statistical significance for each experiment was determined using the analysis of variance test (ANOVA) and the LSD (Least Significant Difference) post-hoc test was used for the comparison amongst the mean values. Differences were considered to be significant at $p < 0.05$.

3. Results and Discussion

3.1. Effects of Drying Temperature on Drying Time, Total Phenolic Content, Antioxidant Activity and Sensory Quality of Roselle

3.1.1. Drying Time

The drying curves of Roselle at 60, 80, 100, and 120 °C are presented in Figure 1. The drying at 60 °C required 22 h to bring the moisture content of Roselle from 89.9% to 7.1% while the use of drying temperatures at 80, 100, and 120 °C could shorten the time to achieve moisture content of the materials less than 8% to 9, 7, and 3.5 h, respectively.

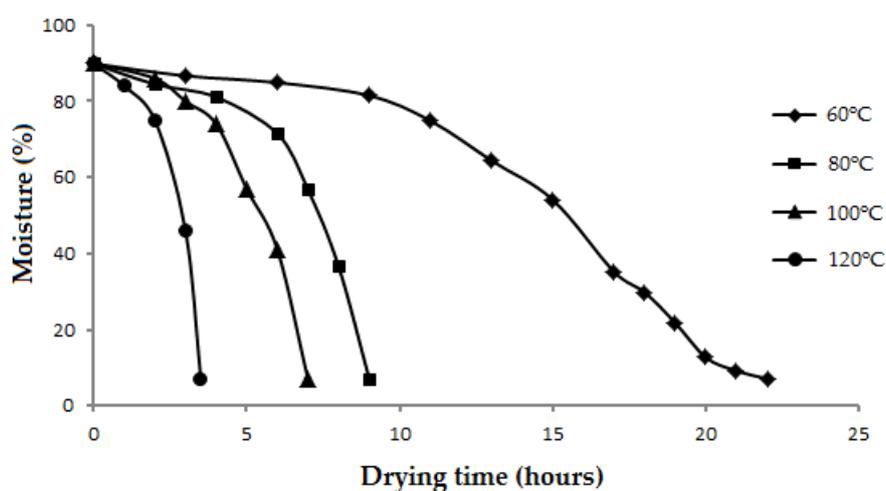


Figure 1. Drying curves of Roselle at different temperatures.

As expected, the higher the drying temperatures used, the higher the rate of moisture loss was achieved. At the beginning of the drying process, the decrease of moisture content in the material was slow but the drying rate rose rapidly until the end of the processes. This phenomenon is different from the change in drying rate for many other herbs or medicinal plants, which usually reaches a high rate at the beginning and becomes slow at the end of the process when the materials reach a low moisture content [24–26]. This difference may be due to the high moisture content in the fresh juicy Roselle (approximately 90%) which requires more heat to reach the evaporation temperature of the water. The reduction in drying rate did not occur at the end of the drying process of the Roselle may be attributed to the soft structure and the thin skin of the material, which allowed moisture to easily escape from the matrix.

3.1.2. Total Phenolic Content in Dried Roselle

Concentrations of total phenolic compounds were significantly different among dried Roselle obtained from different temperatures. The TPC of the sample dried at 80 °C was the highest, followed by the Roselle dried at 60 °C, 100 °C, and 120 °C (Figure 2). As reported in previous studies [4,6], Roselle contains a high level of phenolic compounds which considerably contribute to biological activities of the material but are also very susceptible to processing conditions like high temperature and oxygen.

The thermal drying processes have been reported to cause significant degradation of phenolic compounds in medicinal plants and herbs such as green tea, ginseng and wax apple [27–29]. In this study, a remarkable decrease in TPC of dried Roselle samples was observed for the drying at 100 and 120 °C. Although the drying at 60 °C used the lowest temperature, the long drying time led to the longer exposure to heat, light, and oxygen of the bioactive ingredients in the materials which resulted in greater degradation of those compounds compared to the short time drying at higher temperatures.

The highest retention of TPC in Roselle dried at 80 °C may be caused by the use of middle drying temperature combined with a relatively short time drying (9 h) which was reduced by 60% compared to that of the drying at 60 °C.

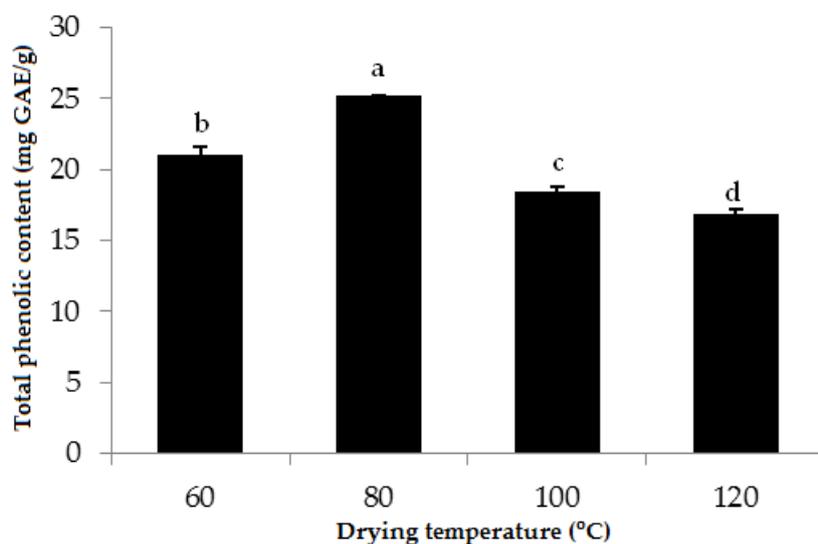


Figure 2. Effect of different drying temperatures on total phenolic content of dried Roselle. (a–d) Different letters express significant differences among values.

3.1.3. DPPH Radical Scavenging Activity of Roselle Dried at Different Temperatures

The variation in DPPH radical scavenging activity of the dried Roselle samples was in agreement with that of the total phenolic content. Table 1 shows that extracts from Roselle dried at 80 °C possess higher antioxidant activities than those produced at other temperatures at all tested soluble solid concentrations. Although at low concentration, the antioxidant activity of Roselle extract dried at 60 °C was lower than those at 100 °C and comparable to the extracts at 120 °C, this increased rapidly and became significantly higher than the others at high concentrations (Table 1). This may be due to the difference in the composition of antioxidants in the Roselle dried at 60 °C compared to that of dried samples at the higher temperatures. This may contain some antioxidants which only show antioxidant activity at high concentration but not at low concentration and those were not present in the samples dried at 100 °C and 120 °C.

Table 1. DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity (% inhibition) of the extracts from Roselle dried at different temperatures.

Drying Temperature	Concentration (mg/mL)					
	0.2	0.4	0.6	1.0	2.0	4.0
60 °C	3.248 ^{a,A}	8.311 ^{a,B}	13.063 ^{a,C}	21.415 ^{a,D}	36.668 ^{a,E}	54.804 ^{a,F}
80 °C	10.29 ^{b,A}	20.903 ^{b,B}	23.816 ^{b,C}	28.548 ^{b,D}	53.577 ^{b,E}	59.334 ^{b,F}
100 °C	5.075 ^{c,A}	10.176 ^{c,B}	21.136 ^{c,C}	25.91 ^{c,D}	40.082 ^{c,E}	45.389 ^{c,F}
120 °C	4.202 ^{a,b,A}	7.489 ^{a,B}	12.083 ^{d,C}	21.752 ^{a,D}	37.85 ^{a,E}	46.654 ^{d,F}

^{a–d} Different lower case letters express significant differences among the values within a column. ^{A–F} Different Upper case letters express significant differences among the values within a row.

As a result, the IC₅₀ for the DPPH radical scavenging ability of the Roselle dried at 80 °C followed by that dried at 60 °C while the IC₅₀ values of samples dried at 100 and 120 °C were the lowest (Figure 3). The same trend in the variation of TPC and antioxidant capacity among the dried Roselle from different drying temperatures is predictable because phenolic acids, anthocyanins, and flavonoids, which all belong to the phenolic group, are the predominant bioactive ingredients in Roselle and

mainly contribute to the antioxidant capacity of this material [4,6]. Thus it is reasonable when the products having higher TPC show stronger DPPH radical scavenging activity.

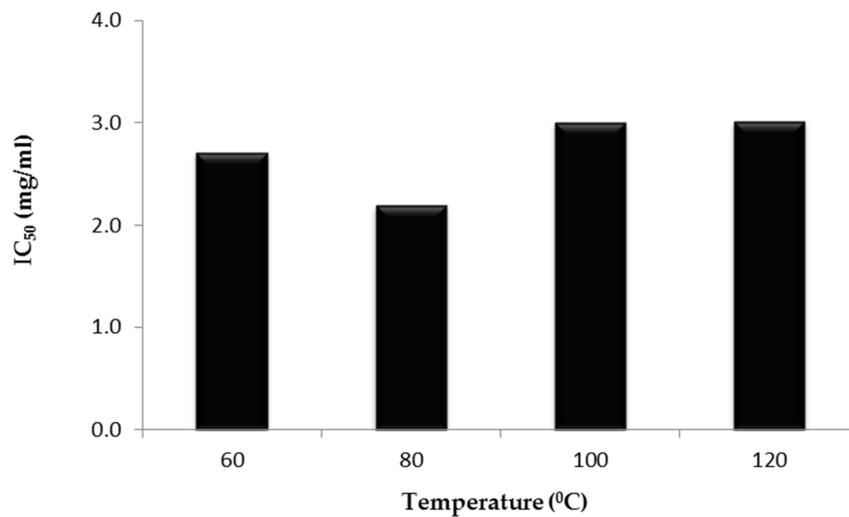


Figure 3. IC₅₀ values for DPPH radical scavenging activity of Roselle dried at different temperatures.

3.1.4. Sensory Characteristics of Roselle Dried at Different Temperatures

The images of dried Roselle products from different drying temperatures are shown in Figure 4. The samples dried at higher temperatures present a brighter appearance while those obtained from lower temperatures were darker. This may be due to the higher concentration of anthocyanins in the samples dried at 60 and 80 °C which were claimed as the principal components contributing to the purple-red color of the Roselle [6].



Figure 4. Dried Roselle samples obtained from different drying temperatures.

The sensory evaluation using the 9-point hedonic scale with 20 panelists showed great variations in the preference of the panelists with the individual characteristics of the dried Roselle samples (Table 2). The samples dried at lower temperatures had higher points for aroma, color, and aftertaste while the dried Roselle from higher drying temperatures achieved higher grades for taste (Figure 5).

Table 2. Effects of drying temperature on the sensory characteristics of the Roselle tea.

	60	80	100	120
Aroma	6.10 ^a	6.10 ^a	5.30 ^b	5.30 ^b
Taste	5.80 ^a	6.20 ^b	6.25 ^b	6.30 ^b
Color	6.65 ^{a,b}	7.60 ^a	6.25 ^b	5.45 ^c
Aftertaste	6.50 ^{a,b}	6.55 ^a	5.70 ^c	6.40 ^b
Overall	6.40 ^a	7.05 ^b	5.85 ^c	7.00 ^b

^{a-c} Different letters express significant differences among the values within a column.

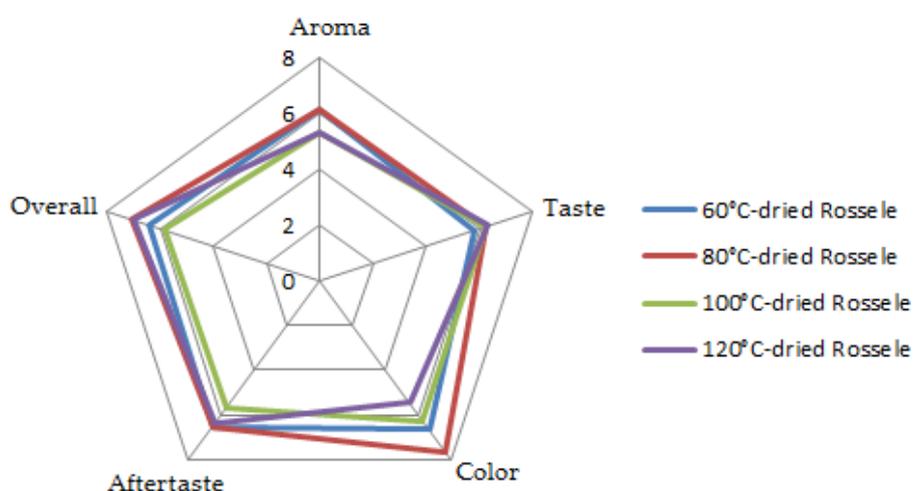


Figure 5. Sensory characteristics of the Roselle tea dried at different temperatures.

Since the phenolic compounds significantly contribute to the bitter taste and astringent mouth feel of foods and drinks [30], the higher concentration of those compounds in Roselle dried at 60 and 80 °C may be the reason of the lower sensory results for the taste. However, the higher TPC resulted in the better aftertaste for those samples compared to the others. The higher retention in the principal color components, anthocyanins, in those samples also contributed to the higher intensity of the Roselle tea infusion which is shown by a very high sensory point for color (7.6) of the Roselle dried at 80 °C. As a result, the Roselle dried at 80 °C also got the highest overall acceptance of the sensory evaluation panel.

3.2. Effects of Brewing Conditions on Total Soluble Solid Content, Total Phenolic Content and Antioxidant Activity of Roselle Tea

3.2.1. Effect of Water Temperature

Table 3 shows that the use of water at 90 °C produces the Roselle infusion with the highest TPC and antioxidant capacity. The increase of water temperature from 80 to 90 °C resulted in an improvement in total soluble solid of the tea but this parameter did not significantly improve when the temperature increased to 100 °C. The use of water at 100 °C for brewing Roselle actually caused a decline in TPC and antioxidant capacity of the tea (Table 3).

Table 3. Effects of brewing water temperature on the characteristics of the Roselle tea.

	Temperature (°C)		
	80	90	100
Total soluble solid (oBx)	4.20 ± 0.06 ^a	4.23 ± 0.05 ^b	4.27 ± 0.11 ^b
Total phenolic content (mg GAE/g)	8.24 ± 0.13 ^a	8.38 ± 0.03 ^b	8.23 ± 0.03 ^a
DPPH inhibition (%)	40.77 ± 0.07 ^a	43.57 ± 0.03 ^b	40.36 ± 0.26 ^a

^{a,b} Different letters express significant differences among the values within a row.

Literature has shown that most of the bioactive compounds, and the phenolic group in particular, from plant sources are very sensitive and very easy to be decomposed under high temperatures [31]. This characteristic of phenolic compounds explained for the reduction of TPC in Roselle tea brewed at 100 °C. DPPH radical scavenging activity of this sample was also lower than that brewed at 90 °C. The literature has shown that phenolic acids, anthocyanins and flavonoids are the major compounds contributing to the antioxidant capacity of Roselle so the reduction of TPC in Roselle tea brewed at 100 °C may be responsible for the lower DPPH radical scavenging activity compared to that brewed at 90 °C [4,6].

3.2.2. Effect of Liquid-Solid Ratio

As reported by many studies related to the recovery of bioactive compounds and phenolics in particular from plant sources, the increase in the volume of solvent would improve the extraction yield of the compounds [20,30]. In the brewing of Roselle, the same trend was observed for the increase in TPC and antioxidant capacity of the Roselle tea. However, the significant improvement of those parameters was only obtained when the liquid-solid ratio increased from 8:1 to 10:1 but not at higher ratios (Table 4).

Table 4. Effects of liquid-solid ratio (ml/g) on the characteristics of the Roselle tea.

	8:1	10:1	12:1	14:1
Total soluble solid (oBx)	5.13 ± 0.06 ^a	4.52 ± 0.05 ^b	3.81 ± 0.03 ^c	3.32 ± 0.02 ^d
Total phenolic content (mg GAE/g)	7.59 ± 0.02 ^a	10.12 ± 0.01 ^b	10.00 ± 0.03 ^c	10.07 ± 0.07 ^{b,c}
DPPH inhibition (%)	40.99 ± 0.87 ^a	50.07 ± 0.55 ^b	50.04 ± 0.31 ^b	49.97 ± 0.39 ^b

^{a-d} Different letters express significant differences among the values within a row.

The improvement in the extraction yield of soluble ingredients from materials with a higher liquid-solid ratio has been well-known to be caused by the higher gap of the concentration of solutes in the material and their concentration in the solvent. This leads to higher concentration gradients and allows the ingredients to diffuse into the solvent faster [32]. However, the diffusion rate could not proportionally increase with the increase of the solvent amount because the amount of the solutes is limited and the rate depends on the distance from the surface of solid material to the diluted areas of solvent. Thus the increasing rate of the extraction yield declines slowly when an excessive amount of solvent is used [32]. This trend can be clearly observed in this study when the liquid-solid ratios of 12:1 and 14:1 were used for brewing Roselle tea. Based on this result, Roselle is suggested to be brewed using water at 90 °C instead of boiling water as commonly recommended for the brewing of other herbal teas.

3.2.3. Effect of Brewing Time

The influence of brewing time on the TSS content, TPC, and antioxidant capacity of the Roselle drinks is shown in Table 5. The TSS content and TPC increased along with the increase in the brewing time from 10 to 30 min but the increase was not significant when the brewing time excess 30 min. The change in the antioxidant capacity of the tea was similar to the increasing trend of TSS content and TPC but then declined when the drying time extended to 40 min.

Table 5. Effects of brewing time (minutes) on the characteristics of the Roselle tea.

	10	20	30	40
Total soluble solid (oBx)	3.67 ± 0.18 ^a	4.97 ± 0.10 ^b	5.40 ± 0.14 ^c	5.47 ± 0.13 ^c
Total phenolic content (mg GAE/g)	8.66 ± 0.057 ^a	11.96 ± 0.25 ^b	12.70 ± 0.03 ^c	12.35 ± 0.23 ^d
DPPH inhibition (%)	46.90 ± 0.04 ^a	58.82 ± 0.18 ^b	73.31 ± 0.60 ^c	62.13 ± 0.88 ^d

^{a-d} Different letters express significant differences among the values within a row.

The literature on the extraction of phenolic compounds reported that the phenolic extraction yield reached the maximum value after a certain extraction time depending on materials and the solvents, then fluctuated at this level or started declining if the extraction time is extended [33,34]. The decrease in antioxidant capacity of the Roselle drink after 40 min might be due to the degradation of other bioactive compounds in the solution such as carotenoids which also contribute to the antioxidant capacity of the solutions but are susceptible and degraded by high temperature combined with a long exposure time of the brewing process [34].

4. Conclusions

In this study, drying temperatures (60, 80, 100, and 120 °C) were used for drying Roselle calyces to investigate their effects on the retention of total phenolic content, antioxidant capacity and sensory quality of the dried Roselle. The drying at 80 °C produced the dried Roselle with the highest retention of TPC (25.196 mg GAE/g), strongest AC (IC₅₀ of 2.2 mg/mL), and the highest sensory quality compared to the Roselle dried at other temperatures. Effects of brewing conditions including water temperature, water-to-Roselle ratio and brewing time on the quality of Roselle infusion were also evaluated. The brewing process using hot water at 90 °C with the solid-liquid ratio of 1:10 (g/mL) for 30 min resulted in the highest TSS content (5.4 °Bx), TPC (12.7 mg GAE/g Roselle), and strongest AC (73.3% DPPH inhibition) of the Roselle tea. Thus, the drying at 80 °C combined with the brewing of dried Roselle for 30 min using 90 °C hot water with the solid-liquid ratio of 1:10 (g/mL) are recommended to produce Roselle tea with the highest content of beneficial ingredients.

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