Songklanakarin J. Sci. Technol.
30 (Suppl.1), 133-139, April 2008

Songklanakarin Journal
of Science and Technology

http://www.sjst.psu.ac.th

Original Article

Studies on the optimum conditions for the extraction and concentration of roselle (*Hibiscus sabdariffa* Linn.) extract

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Received 29 December 2006; Accepted 6 June 2007

Abstract

The present study aimed to: (a) study the physical and chemical properties of fresh roselle calyxes; (b) study the optimum conditions for water extraction of roselle; and (*e*) compare the methods of evaporation (vacuum, atmospheric) for processing of concentrated roselle extract. This study found that the *L**, *a** and *b** values of fresh roselle calyxes were 0.09±0.01, 0.02±0.01 and 0.05±0.01. The pH, total acidity and total soluble solids content were 2.16±0.05, 4.20±0.01% as malic acid and 5.83±0.04°Brix, respectively. The yields of fresh and dried roselle calyxes production were 47.45±0.71% and 9.58±0.77%. The optimum conditions for fresh roselle calyxes, fresh calyxes to water ratio was 1:2, with the extraction temperature of 50°C for 30 min. For dried roselle calyxes, the optimum conditions were 1:10 ratio of dried calyxes to water and the extraction temperature of 50°C for 30 min. The method of evaporation under the vacuum of 44 cmHg at 70°C was an appropriate selected method for both concentrated fresh and dried roselle extracts. The pH, total acidity and total soluble solids contents of concentrated fresh roselle extract were 2.77±0.02, 12.73±0.09% as malic acid and 25.07±0.10 °Brix, respectively. The total anthocyanin, total phenolic contents and EC_{50} (DPPH radical scavenging assay) were 37.67±0.02 mg/100 g fresh roselle calyxes, 31.26±0.75 mg gallic acid/g and 39.37±0.61 mg/ml (n=9). The pH, total acidity and total soluble solids contents of concentrated dried roselle extract were 2.89±0.05, 11.96±0.34% as malic acid and 25.07±0.10 °Brix. The total anthocyanin, total phenolic contents and EC_{50} were 340.97±0.15 mg/100 g dried roselle calyxes, 31.18±0.62 mg gallic acid/g and 47.53±0.85 mg/ml (n=9), respectively.

Keywords: roselle extract, water extraction, antioxidant activity, concentrated roselle extract

1. Introduction

Roselle (*Hibiscus sabdariffa* Linn.) is a tropical plant widely cultivated in Thailand and locally known as Krachiap Daeng. Roselle produces red edible calyxes which primarily can be used for making brilliant red color jam, jelly, preserve and juice (Hirunpanish *et al*., 2006). Since the early 1970s, roselle has received great considerable attention as a potential source of natural food colorant, pharmaceuticals and cosmetics (Mazza and Miniati, 1993). It is also claimed as a Thai traditional medicine for kidney stones and can be used as an antibacterial, antifungal, hypocholesterolemic, diuretic, mild laxative and antihypertensive substance (Farnworth and Bunyapraphatsara, 1992). The calyxes contain brilliant red pigments of four anthocyanins including dephinidin 3-sambubioside or hybiscin and cyanidin 3-sambubioside as the major pigments and delphinidin 3-glucoside and cyanidin 3-glucoside as the minor ones (Du and Francis, 1973; Wong *et al*., 2002). Roselle anthocyanins can

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also contribute benefit to health as a good source of antioxidants as well as a natural food colourant. As anthocyanins are derivatives of the basic flavylium cation structure, which has an electron deficient nucleus, they generally are highly reactive. The reactions usually involve decolorization of the anthocyanin pigments. The rate of anthocyanin destruction depends on many factors such as pH, temperature, intermolecular copigmentation, ascorbic acid, oxygen, etc. The reactions are usually undesirable in juice processing. However, an extraction technique for roselle anthocyanins also plays a major role in an antioxidant activity of the extract. Water extraction of calyx has considerable economic potential and produces a brilliant red color extract, rich in anthocyanins and hibiscus acid (Al-Kahtani and Hassan, 1990). In addition, the antioxidant activity of roselle extract is also pH dependent (pH 2 to 7), the activity decreases as pH increases. However, at a constant pH, only a relatively small decrease in antioxidant activity and total phenolic content is observed (Sukhapat *et al*., 2004). Mazza and Miniati (1993) reported that roselle anthocyanin extract produced by using water at 50^{o}C for 4 hours with a calyx/solvent ratio of 1:10 behaved as a first-order kinetics of thermal degradation which was rapid at temperatures above 100^{o}C, and virtually instantaneous at 165^{o}C to 170^{o}C. Currently, only a few studies have been reported (Tsai *et al*., 2002) with regard to the effects of processing techniques, such as extraction and concentration, on the properties especially antioxidant activity of roselle extract. Therefore, this study aimed: (a) to study the chemical and physical properties of fresh roselle calyxes; (b) to study the optimum conditions for water extraction of roselle; and (c) to compare the method of evaporation (vacuum, atmospheric) for production of concentrated roselle extract, in which optimum conditions and method of evaporation were selected based on the major antioxidant property of concentrated extracts.

2. Materials and Methods

2.1 Physical and chemical properties of fresh roselle calyxes

1) Preparation of fresh roselle calyxes

Fresh roselle fruits (*Hibiscus sabdariffa* Linn.), cultivar Sudan, were harvested from Amphur Namom, Songkhla Province. After harvesting the fruits were washed with tap water three times, then the seeds were removed to obtain fresh roselle calyxes.

2) Preparation of dried roselle calyxes

Fresh roselle calyxes were dried in a rotary air dryer at 50^{o}C for 36 hr to 10% moisture content. Dried roselle calyxes were immediately packed in nylon-bags sealed with an impulse sealer, and kept in a stylofoam box until used.

3) Determination of %yields of fresh and dried roselle calyxes

The %yield of fresh roselle calyxes was calculated as X = (weight of fresh roselle calyxes after removing the seeds/weight of fresh roselle fruits) x 100. The %yield of dried roselle calyxes was calculated as Y = (weight of fresh roselle calyxes before drying/weight of dried roselle calyxes after drying in a rotary air dryer) x 100.

4) Determination of physical and chemical properties of roselle calyxes

Fresh roselle calyxes were placed into juice extractor (Moulinex, France) to separate the juice and residues. The color of fresh roselle juice was measured by using a Colorimeter (Model ColorQuest XT, HunterLab, U.S.A.) expressed as CIE LAB L^*, a^*, b^* values. The total acidity content of the juice as malic acid (%) was determined according to the method of AOAC (2000). The pH was measured with a glass-electrode pH-meter, the total soluble solids content (^{o}Brix) was measured using a refractometer (N1 Brix 0~32%, Atago, Tokyo, Japan) according to the method of AOAC (2000).

2.2 Optimum conditions for water extraction of roselle

1) Optimum ratio of roselle calyxes to water

1.1) Preparation of fresh roselle extract with various ratios of fresh roselle to water

The extraction with various different ratios of fresh roselle calyxes to water including 1:2, 1:5 and 1:10 g/ml were conducted in a water bath at a constant temperature of 60^{o}C for 60 min. Fresh roselle extracts were filtered with a cheesecloth bag. The color, pH, total acidity (as malic acid, %) and total soluble solids contents were measured in the same manner as described in 2.1(4). The total anthocyanin contents expressed as cyanidin-3-galactoside (mg/100 g roselle calyxes) were determined according to the method described by Fuleki *et al*.(1968). Total phenolic content (as gallic acid, %) was also analyzed according to the method described by Miliauskas *et al*.(2004). The antioxidant activity, expressed as EC_{50} (the amount of sample needed for 50% decrease of the initial DPPH concentration) was determined according to the DPPH radical scavenging assay (Yamasaki *et al.,* 1994). The optimum water extraction ratio of fresh roselle calyxes to water was selected.

1.2) Preparation of dried roselle extract with various ratios of dried roselle to water

Dried roselle calyxes were ground for 3 second using a blender (Panasonic MX-895M, Japan). The extraction with various different ratios of dried roselle calyxes to water including 1:5 and 1:10 g/ml was conducted in a water bath at constant temperature of 60°C for 60 min. Dried roselle extracts were filtered with a cheesecloth bag. The properties of dried roselle extract were determined as described in 2.1(4) and 2.2(1.1). The optimum ratio of dried roselle calyxes to water was selected.

2) Optimum temperature and time for water extraction

2.1) Preparation of fresh roselle extract with various temperatures and times for extraction

The optimum ratio of fresh roselle calyxes to water was selected from 2.2(1.1) and used for extraction with various temperatures of 50°C and 60°C, and various times for 30 and 60 min, respectively. The properties of fresh roselle extract were then determined as described in 2.1(4) and 2.2(1.1). The optimum conditions of fresh roselle calyxes to water were selected.

2.2) Preparation of dried roselle extract with various temperatures and times for extraction

The optimum ratio of dried roselle calyxes to water from 2.2(1.2) was selected for water extraction of dried roselle extract with various temperatures of 50°C and 60°C for various times of 30 and 60 min. Then properties of dried roselle extract were determined as described in 2.1(4) and 2.2(1.1). The optimum conditions of dried roselle calyxes to water were selected.

2.3 Comparison the method of evaporation for production of concentrated roselle extract

1) Preparation of concentrated fresh roselle extract

Fresh roselle extract was produced with the optimum ratio of fresh roselle calyxes to water and the optimum temperature and time for water extraction of fresh roselle extract (selected from 2.2(1.1) and 2.2(2.1). Fresh roselle extract was filtered and concentrated to the total soluble solids content of 25°Brix with a vacuum steam jacket evaporator at 70°C, 44 cmHg, as compared to the atmospheric evaporation at 90°C using a steam kettle. The properties of concentrated fresh roselle extracts were determined as described in 2.1(4) and 2.2(1.1).

2) Preparation of concentrated dried roselle extract

Dried roselle extract was produced with the optimum ratio of dried roselle calyxes to water and the optimum temperature and time for water extraction of dried roselle extract (selected from 2.2(1.2) and 2.2(2.2). Dried roselle extract was filtered and concentrated in a vacuum steam jacket evaporator at 70°C, 44 cmHg as compared to the atmospheric evaporation at 90°C using a steam kettle. The properties of concentrated dried roselle extracts were determined as described in 2.1(4) and 2.2(1.1).

2.4 Statistical analyses

All experiments were conducted in triplicate. Data from 2.2(1.1), 2.2(2.1) and 2.2(2.2) were analyzed using one-way analysis of variance (ANOVA). The experimental design was a Complete Randomized Design (CRD). The significance level was established at $p \leq 0.05$. DMRT was used to separate treatment means. Data from 2.1(1.2), 2.3(1) and 2.3(2) were analyzed using t-test to determine statistically significant differences at the $p \leq 0.05$. All analysis was conducted using SPSS for Window Version 10.5.

3. Results and Discussion

3.1 Physical and chemical properties of fresh roselle calyxes

1) The yields of fresh roselle and dried roselle calyxes

The %yield of fresh and dried roselle calyxes were 47.45±0.71% and 9.58±0.77%, respectively, as shows in Table 1. Dried roselle calyxes contained of approximately 10% moisture content contributed to less in %yield compared to that of fresh one.

2) Physical and chemical properties of fresh roselle calyxes

Table 2 shows physical and chemical properties of fresh roselle calyxes. From the results, the CIE LAB color was measured with the following color coordinate: lightness (L^*), redness (a^*, red-green) and yellowness (b^*, yellow-blue).

Table 1. %Yields of fresh and dried roselle calyxes

Roselle calyxes	%Yields*
Fresh	47.45±0.71
Dry	9.58±0.77

*Determination was done in triplicate

Table 2. Physical and chemical properties of fresh roselle calyxes

Physical and chemical properties	Values*
L^*	0.09±0.01
a^*	0.02±0.01
b^*	0.05±0.01
pH	2.16±0.05
Total acidity, as malic acid (%)	4.20±0.01
Total soluble solids (°Brix)	5.83±0.04

*Determination was done in triplicate

Table 3. Properties of fresh roselle extracts with various ratios of fresh roselle to water at 60°C for 60 minutes

Properties of fresh roselle extract	Ratios of fresh roselle calyxes to water (g:ml)		
	1:2	1:5	1:10
L^*	6.93±0.30 c	27.02±1.79 b	40.18±2.78 a
a^*	36.09±0.40 c	61.23±1.77 b	68.70±0.85 a
b^*	11.79±0.52 c	48.28±0.94 b	70.54±2.33 a
pH	2.26±0.02 c	2.32±0.01 b	2.44±0.03 a
Total acidity, as malic acid (%)	1.43±0.07 a	0.68±0.06 b	0.36±0.05 c
Total soluble solids (°Brix)	2.12±0.04 a	0.80±0.00 b	0.40±0.00 c
Total anthocyanin contents (mg/100 g fresh roselle calyxes)	65.12±0.07 a	38.86±0.05 b	35.60±0.05 c
Total phenolic contents, as gallic acid (mg/g)	22.39±0.02 a	22.12±0.10 b	22.08±0.04 b
EC_{50} (mg/ml)	42.15±0.18 c	51.52±0.79 b	60.82±1.23 a

Means±standard deviation in each row with the same letters are not significantly different ($p>0.05$)

Table 4. Properties of fresh roselle extracts with various temperatures and times for extraction

Properties of fresh roselle extract	Extraction temperatures (°C)/times (min)			
	50°C/30 min	50°C/60 min	60°C/30 min	60°C/60 min
L^*	14.08±0.16 a	13.24±0.87 a	5.88±0.76 b	4.10±0.79 c
a^*	47.69±0.29 a	46.09±1.61 a	33.45±2.15 b	28.74±2.63 c
b^*	24.25±0.25 a	22.74±1.52 b	10.12±1.31 c	7.06±1.37 d
pH	2.33±0.01 c	2.38±0.00 a	2.35±0.01 b	2.30±0.01 d
Total acidity, as malic acid (%)	1.58±0.08 a	1.60±0.16 a	1.63±0.09 a	1.64±0.22 a
Total soluble solids (°Brix)	1.40±0.00 b	1.40±0.00 b	1.80±0.00 a	1.80±0.00 a
Total anthocyanin contents (mg/100 g fresh roselle calyxes)	45.13±0.08 a	38.64±0.04 b	35.52±0.07 c	35.18±0.08 d
Total phenolic contents, as gallic acid (mg/g)	22.25±0.31 a	21.21±0.97 b	21.06±0.52 b	20.75±0.57 b
EC_{50} (mg/ml)	27.87±1.27 c	37.13±1.58 b	42.39±0.58 a	43.34±0.67 a

Means±standard deviation in each row with the same letters are not significantly different ($p>0.05$)

L^*, a^* and b^* values of roselle were 0.09±0.01, 0.02±0.01 and 0.05±0.01, respectively. The pH, total acidity (as malic acid, %) and total soluble solids contents were 2.16±0.05, 4.20± 0.01% and 5.83±0.04°Brix, respectively. The results showed that fresh roselle calyxes contained natural constituents of organic acids such as malic, citric and 3-indolyl acetic acids (AL-Kahtani and Hassan, 1990) which played an important role in giving brilliant red color of juice sample. It has been demostrated that in acidic media, four anthocyanin structures, including the flavylium cation, the quinonoidol base, the carbinol pseudobase and the chalcone, exist in equilibrium. And at pHs below 2, the anthocyanin exists primarily in the form of the red flavylium cation. As the pH is raised (≥4.5), a rapid proton loss occured to yield blue quinonoidal forms (Mazza and Miniati, 1993).

3.2 Optimum conditions for water extraction of roselle

1) Optimum ratios of fresh roselle calyxes to water

Table 3 shows the properties of fresh roselle calyx extracts from calyxes to water ratios of 1:2, 1:5 and 1:10 at the temperature of 60°C for 60 min. The results from this study showed that the greater the ratio of fresh calyxes to water the more the red color intensity observed. As the water portion for extraction increased the pH, total acidity, total soluble solids, total anthocyanins and total phenolic contents of the extracts decreased. At the ratio of 1:2, the extract had greatest value in antioxidant activity expressed as the lowest EC_{50} at 42.15±0.18 mg/ml, which was significantly greater antioxidative effect than those ratios of 1:5 and 1:10. Therefore, fresh calyxes to water ratio of 1:2 was selected.

2) Optimum conditions for water extraction of fresh roselle

Table 4 shows the properties of fresh roselle calyx extracts using 1:2 ratio of fresh calyxes to water and various temperatures-times for extraction. The results showed that temperatures of 50°C and 60°C, and times for 30 min and 60 min significantly ($p<0.05$) affected almost all properties of the extracts. It was found that greater extraction temperature

Table 5. Properties of dried roselle extracts with various ratios of dried roselle to water at 60°C for 60 minutes

Properties of dried roselle extract	Ratios of dried roselle calyxes to water (g:ml)	
	1:5	1:10
L^*	0.03±0.02 b	0.75±0.16 a
a^*	0.28±0.09 b	5.22±1.16 a
b^*	0.06±0.04 b	1.24±0.27 a
pH	2.79±0.02 b	2.86±0.02 a
Total acidity, as malic acid (%)	3.29±0.14 a	1.85±0.05 b
Total soluble solids (°Brix)	9.80±0.52 a	5.33±0.16 b
Total anthocyanin contents (mg/100 g dried roselle calyxes)	400.67±0.82 b	445.02±1.54 a
Total phenolic contents, as gallic acid (mg/g)	17.53±0.56 b	40.39±0.72 a
EC_{50} (mg/ml)	50.48±0.94 a	45.05±0.67 b

Means±standard deviation in each row with the same letters are not significantly different (p>0.05)

and time contributed to less brillent red in color and also less in the amount of total anthocyanin contents. Bridle and Timberlake (1997) reported that in acidic media, heating process caused four anthocyanin structures shift towards the colourless carbinol base and chalcone forms which became paler in color. However, anthocyanin destruction also occured depending on many factors such as pH, temperature, intermolecular copigmentation, ascorbic acid, oxygen, etc. The results demonstrated that pH of the extracts was in the range of 2.30 to 2.38. Not only significant differences in pH values were observed from all treatment, but the amounts of total anthocyanin contents were also found to be different ($p<0.05$). Even though the extracts from this study had quite narrow range of pH values, the anthocyanin destruction caused by pH effect was considerable (Francis, 1985). In addition, other phenolic compounds found in roselle extract such as catechin could also react with anthocyanins resulting in complex formation which led to color changes (Tsai and Huang, 2004). As a result, extraction process of fresh roselle calyxes with the shortest time of 30 min at 50°C provided the extract greatest in the amount of total anthocyanin and total phenolic contents as well as antioxidant activity.

3) Optimum ratios of dried roselle calyxes to water

Table 5 shows properties of extracts using dried roselle calyxes (powder form) to water ratios of 1:5 and 1:10, at 60°C for 60 min. It was found that properties of both extracts were significantly different ($p<0.05$). The greater portion of water for extraction, 1:10 ratio of dried calyxes to water, contributed to a brighter red and higher amount of total anthocyanin and total phenolic contents as well as antioxidant activity, expressed as EC_{50}. In contrast, lower acidity and total soluble solids contents were observed as compared to the ratio of 1:5. Properties of the extract from 1:10 extraction ratio of dried calyxes to water also demonstrated pretty close to those from 1:2 extraction ratio of fresh roselle calyxes to water (Table 3). Therefore, dried calyxes to water ratio of 1:10 was selected for extraction process providing desirable extract quality in terms of total anthocyanin and total phenolic retention and antioxidant activity.

4) Optimum conditions for water extraction of dried roselle

The properties of dried roselle calyx extracts using 1:10 ratio of dried calyxes to water at various temperatures of 50°C and 60°C, and various times of 30 min and 60 min are shown in Table 6. Similar results from fresh roselle calyx extracts (Table 4) were observed. The optimum extraction temperature of 50°C for 30 min had the brightest red color and greatest amount of total anthocyanin content. Dried roselle calyxes had 10% moisture content, therefore, providing extracts almost 10 times greater total anthocyanin contents compared to fresh ones. It was found that greater extraction temperature and time contributed to a less brillent red color and also a lower total anthocyanin content. The greatest antioxidant activity of extract was observed from the extraction condition of 50°C for 30 min. The results also implied that antioxidant activity did not have direct correlation with the amount of total anthocyanin and total phenolic contents. It may possibly be due to not only anthocyanins and phenolic compounds such as quercitin but other various constituents such as hibiscus, protocatecuic acid and L-ascorbic acid also found in roselle calyxes and contributing to roselle antioxidant activity (Hirunpanish *et al.*, 2006). Francis (1985) also reported that decoloration of anthocyanins in the presence of ascorbic acid may be accelerated by actual condensation reactions with these compounds. The degradation compounds produced in these reactions are probably quite complex.

Table 6. Properties of dried roselle extracts with various temperatures and times for extraction

Properties of dried roselle extract	Extraction temperatures (°C)/times (min)			
	50°C/30 min	50°C/60 min	60°C/30 min	60°C/60 min
L^*	0.75±0.10 a	0.24±0.15 d	0.54±0.08 b	0.39±0.05 c
a^*	5.14±0.69 a	1.86±1.10 d	3.95±0.55 b	2.75±0.33 c
b^*	1.23±0.13 a	0.40±0.25 c	0.80±0.13 b	0.65±0.10 b
pH	2.95±0.01 a	2.93±0.02 b	2.91±0.02 b	2.91±0.01 b
Total acidity, as malic acid (%)	1.71±0.02 c	1.96±0.12 ab	2.00±0.02 a	1.90±0.03 b
Total soluble solids (°Brix)	5.97±0.08 c	6.23±0.08 a	6.07±0.16 bc	6.13±0.10 ab
Total anthocyanin contents (mg/100 g dried roselle calyxes)	502.33±0.52 a	498.93±0.41 b	457.74±0.04 c	455.08±0.09 d
Total phenolic contents, as gallic acid (mg/g)	43.00±0.97 a	43.06±0.53 a	41.72±0.49 b	41.78±0.47 b
EC_{50} (mg/ml)	44.78±0.49 a	45.02±0.63 a	44.77±0.50 a	44.96±0.72 a

Means±standard deviation in each row with the same letters are not significantly different (p>0.05)

Table 7. Properties of concentrated fresh roselle extracts using vacuum and atmospheric evaporation

Properties of concentrated fresh roselle extract	Methods of evaporation	
	vacuum	atmospheric
pH	2.77±0.02b	2.79±0.02a
Total acidity, as malic acid (%)	12.73±0.09a	11.58±0.24b
Total soluble solids (°Brix)	25.07±0.10a	25.07±0.10a
Total anthocyanin contents (mg/100 g fresh roselle calyxes)	37.67±0.02a	32.56±0.02b
Total phenolic contents, as gallic acid (mg/g)	31.26±0.75a	29.22±0.33b
EC_{50} (mg/ml)	39.37±0.61b	51.55±0.98a

Means±standard deviation in each row with the same letters are not significantly different (p>0.05)

Table 8. Properties of concentrated dried roselle extracts using vacuum and atmospheric evaporation

Properties of concentrated dried roselle extract	Methods of evaporation	
	vacuum	atmospheric
pH	2.89±0.05b	3.01±0.04a
Total acidity, as malic acid (%)	11.96±0.34a	10.37±0.30b
Total soluble solids (°Brix)	25.07±0.10a	25.10±0.09a
Total anthocyanin contents (mg/100 g dried roselle calyxes)	340.97±0.15a	318.68±0.16b
Total phenolic contents, as gallic acid (mg/g)	31.18±0.62a	29.16±0.68b
EC_{50} (mg/ml)	47.53±0.85b	52.94±1.20a

Means±standard deviation in each row with the same letters are not significantly different (p>0.05)

3.3 Comparison methods of evaporation for processing of concentrated roselle extract

1) Properties of concentrated fresh roselle extract

Table 7 shows properties of concentrated fresh roselle extracts (25°Brix) using vacuum and atmospheric evaporation. Under vacuum evaporation of 44 cmHg, 70°C, the concentrated fresh roselle extract had greater antioxidant capacity ($p<0.05$) than that of atmospheric one at 90°C. The pH was in the range of 2.77 to 2.79. Under vacuum evaporation with less oxygen, lower temperature at 70°C and shorter time, approximately 2.0 hr, provided concentrated fresh roselle extracts greater in the amount of total anthocyanin, total phenolic contents and antioxidant activity (EC_{50}) than those from the atmospheric evaporation (approximately 2.5 hr).

2) Properties of concentrated dried roselle extract

Table 8 shows properties of concentrated dried roselle extracts using vacuum and atmospheric evaporation. Similar results were observed as previous study from concentrated fresh roselle extracts. Dried roselle calyx powder contained 10% moisture content resulting in greater amount of used dried roselle calyxes for extraction as compared to fresh ones. As a consequence, dried roselle calyx extracts from vacuum and atmospheric evaporation had total anthocyanin contents expressed as cyanidin 3-galactoside at 340.97±0.15 and 318.68±0.16 mg/100g dried roselle calyxes, respectively. Asen *et al.* (1972) reported that at pH 3.16 the absorbance of cyanidin 3,5-diglucoside at λ_{max} increased 300-fold when the concentration increased from 10^{-4} to 10^{-2} *M* or 100-fold. This study found that vacuum evaporation provided better properties of concentrated dried roselle extract with greater in total phenolic content and antioxidation capacity compared to those of atmospheric one.

4. Conclusions

The optimum ratios of roselle calyxes to water extraction included 1:2 ratio of fresh calyxes to water, and 1:10 ratio of dried calyxes to water. Meanwhile, the temperature of 50°C for 30 min was appropriate and selected for both fresh and dried roselle extraction. The vacuum evaporation method at 70°C, 44 cmHg to obtain 25°brix concentrated fresh and dried roselle extracts was selected in terms of retaining in the antioxidant activity of concentrated extracts. The roselle concentrated extract may possibly be further applied for functional ingredients in food industry.

Acknowledgments

This study was funded by research grants from the Faculty of Agro-Industry, Prince of Songkla University, under the excellence in Agro-Industry scholarship and the Graduate School, Prince of Songkla University.

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