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Roselle Calyces Particle Size Effect on the Physicochemical and Phytochemicals Characteristics

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Abstract

The effect of average particle size (APS), type of solvent, and extraction times (ET) on the physicochemical (moisture, pH, total soluble solids (TSS), titratable acidity, color, water activity (a_w), density), and phytochemical (total anthocyanins and phenols content)propertiesin *Hibiscus sabdariffa* (Roselle) calyces was analyzed. The phytochemical properties evaluation was performed using a factorial design 2×3×3: two APS (median diameters, d_{50}, of 0.55 ± 0.016 (fine powder) and 0.97 ± 0.034 (ground powder) mm), three solvents (water, 2% citric acid, and 50% ethanol) and three ET (30, 45, and 60 min). All extractions were performed at 50 °C. The APS was determined by sieve analysis using Tyler sieves of different number of mesh. Regarding physicochemical properties, no significant differences ($p > 0.05$) were observed in moisture content, pH, and titratable acidity; however, the 0.55 mm fine powder (FP) of *Hibiscus* calyces had lower a_w(0.37±0.01) and higher TSS (5.53±0.05%) than the 0.97 mm ground powder (GP). The extracts obtained fromGP showed a deeper red color than those of FP. The best combination of independent variables, in order to obtain the highest concentration of anthocyanins (451.4±28.1 mg/100 g d.s.) and total phenols (2016.2 ± 159.8 mg/100 g d.s.) were APS of 0.55 mm, 50% ethanol, and ETof 30 min.

Keywords: *Hibiscus sabdariffa*, average particle size, phytochemicals, anthocyanins, phenolic compounds

1. Introduction

Roselle, also known as sorrel, Jamaica flower, and karkade, has been used by people for preparing soft drinks and in traditional medicine. It has been observed that its components, such as vitamins (C & E), polyphenol acidsand flavonoids, mainly anthocyanins, have functional properties. The Roselle calyces production, in developing countries, is of great importance since its production represents a very important income for people from rural communities (Juliani et al., 2009). Today, several studies have shown that compounds found in aqueous and ethanol Roselle calyces extracts may have antioxidant properties (Josiah et al., 2010). These compounds could work in several ways in humans; for instance, they could have anticancer characteristics (Akim, Chooi, Rahmat, & Amiruddin, 2011). They may also reduce chronic diseases such as diabetes mellitus (Sini, Umar, & Inuwa, 2011), dyslipidemias (Ubani, Joshua, & Oraeki, 2010), hypertension (Herrera-Arellano, Miranda-Sánchez, & Avila-Castro, 2007; Ojeda et al., 2010) and cardiovascular diseases, among others. Some of these compounds (flavonoids and anthocyanins) are natural which have no toxic or mutagenic effects (Ali, Wabel, & Blunden, 2005; Fakeye, 2008).

Particle size of powdered materials is an essential part of their characterization. This is closely linked to the behavior of the material and/or its physicochemical properties (Guerrero-Beltrán, Jiménez-Munguía, Welti-Chanes, & Barbosa-Cánovas, 2009). Therefore, the average particle size, the extraction temperature, the type of solvent, and the solid-solvent ratio have a significant impact on the extraction of bioactive compounds of parts of plants. It has been shown that the yields of anthocyanins, by extraction with solvents, increases as the particle size of Roselle calyces decreases (Cissé et al., 2012). Also, the increase of the solid-solvent ratio improves the yields of phenolic compounds in the extract (Dai & Mumper, 2010). Another important factor is the

temperature of extraction. It has been found that increasing the temperature for extraction, up to 80 °C, increases the yields of total phenols in soy extracts using soy powder with an average particle size of 0.459 mm (Jokić et al., 2010). However, the use of temperatures higher than 70 °C for prolonged periods may cause significant degradation of anthocyanins (Cissé, Vaillant, Acosta, Dhuique-Mayer, & Dornier, 2009; Gartaula & Karki, 2010).

The aim of this study was to determine the effect of the average particle size, type of solvent and extraction time on the physicochemical and phytochemical contents in Roselle calyces.

2. Materials and Methods

2.1 Roselle Calyces

Creole Roselle calyces (long red variety) from Chiautla de Tapia, Puebla, Mexico, were usedin the study. Roselle calyces fine powder (FP) was obtained using a stainless steel Spray Veyco MPV mill model 100 (Mexico) with a mesh of 0.5 mm. Roselle calyces ground powder (GP) was obtained using an industrial JR blender model LM-12 (Mexico) of 12 L of capacity. Roselle calyces powders were analyzed for their physicochemical (average particle size, moisture, pH, water activity, total soluble solids, titratable acidity, color, tap density) and phytochemical (total anthocyanins and phenols content) properties.

2.2 Average Particle Size

The APS was performed using a Tyler Ro-Tap® RX-812 (Mentor, OH, USA) sifter with different meshes (2, 1.4, 1.0, 0.85, 0.6, 0.5, 0.425, 0.3, 0.25 and 0.125 mm). At the bottom of the column of sieves, a dish to collect fines was placed (Gee & Or, 2002). Sieving tests were performed for different times (5, 6, 7, 8, 9, and 10 min) for establishing the most suitable sieving time. 50 g of sample was used; then, powders retained in each sieve were weighed. From this information, the size distribution and cumulative weight curves were plotted as well as the mean diameter, d_{50} (O'Hagan, Hasapidis,Coder, Helsing, & Pokrajac, 2005; Guerrero-Beltrán, Jiménez-Munguía, Welti-Chanes, & Barbosa-Cánovas, 2009).

2.3 Physicochemical Properties

2.3.1 Moisture Content

It was measured according to the Mexican Official Standard NOM-116-SSA1-1994. Sample was dehydrated at 100 °C for 4 hours in a HDP-334 MAPSA oven (Grupo Rocas, S.A., Monterrey, NL, Mexico) at atmospheric pressure.

2.3.2 Titratable Acidity

It was assessed according to 22.061 AOAC (1980) method. One g of sample was weighed and placed in a 100 mL glass beaker. Forty milliliters of distilled water were added, heated to reach 70 °C, and let stand for 1 hour. The supernatant was filtered through Whatman paper No. 4. The Roselle residues were rinsed with two portions of 20 mL of hot distilled water. The filtrate and the washings were transferred to a 100 mL flask, cooled down to room temperature, brought to volume and thoroughly mixed. An aliquot of 25 mL of the extract was titrated with 0.1 N NaOH until reaching pH 8.3. Results are reported as g of malic acid per 100 g of calyces.

2.3.3 Total Soluble Solids (TSS)

An extract was obtained by mixing 10 g of Roselle calyces powder with 100 mL of distilled water. This blend was allowed to stand for 1 hour at room temperature and stirring manually sporadically. Total soluble solids were determined according to 31.011 AOAC (1980) method, using an Atago refractometer (Atago Co. LTD, Tokyo, Japan) model Master-M (range of 0-32°Bx). For correcting reading at 20 °C, a set of tables, found in the Mexican Standard NMX-FF-015- 1982, were used.

2.3.4 pH

It was performed according to 10.035 AOAC (1980) method with some modifications (Mexican Standard NMX-F-317, 1978).Five g of Roselle powders were added with 10 mL of distilled water and mixed until getting a uniform paste, an electrode was submerged directly in the sample. An Orion pHmeter model 420A (Orion Research Inc. Jacksonville, FL, USA) was calibrated with buffer solutions of pH 4, 7, and 10.

2.3.5 Color

A Colorgard system 05 colorimeter (Gardner, Germany) was used in the reflectance mode. For color determination, the colorimeter was previously calibrated using black and white mosaics. For the entire Roselle calyces a plate with anillumination gap of 3.7 cm in diameter was used. Two calyces were placed in a Petri dish of 9.44 cm in diameter. Regarding fine and ground powders, a plate with anilluminationgap of 1.9 cm in

diameter was used. The Roselle samples were placed in a 50 mL beaker until reaching the 20 mL mark (about 16.3 and 9.2 g of fine and ground powders, respectively). For extracts, obtained according to the TSS method, the transmittance was measured placing 20 mL of sample in a quartz cell (BYK-Gardner U 10.0 mm). Color parameters were measured in the *CIELab* scale: *L** (lightness, 0 - 100), *a** (green to red) and *b** (blue to yellow). From these data, purity (*C*, color saturation) and hue (*H*) were calculated according to the follow equations:

$$C=(a^{*2}+b^{*2})^{1/2}$$

$$H=tan^{-1}(b^*/a^*)$$

2.3.6 Water Activity (a_w)

It was measured using an AQUA-LAB hygrometer model 4TE (Decagon Devices Inc., Pullman, Washington, USA). The equipment was calibrated with standard solutions of 6.0 M NaCl (a_w= 0.76±0.003) and 8.57 M LiCl (a_w= 0.50±0.003) at 25±0.2° C (Decagon Devices Inc., 2008).

2.3.7 Density

It was performed according to the 962.37 AOAC (1995) method. For liquid extracts, picnometers of 25 mL were used. For fine and ground powders of Roselle calyces, Grease picnometers were used. Empty (W_1), filled with distilled water (W_2), and filled with sample (W_3) picnometers were weighed. The density (25 °C) was calculated according to the following equation:

$$\rho=[(W_2-W_1)/(W_3-W_1)]*\rho H_2O$$

where ρ(g/mL) is the density of sample and ρH_2O (g/mL) is the density of water at 25 °C.

2.4 Phytochemical Properties

2.4.1 Extracts

A 2×3×3 factorial design was used: two average particle sizes (fine and ground powders), three types of solvents (distilled water, 2% citric acid, and 50% ethanol), and three extraction times (30, 45, and 60 min). Extractions were performed at 50°C at a Roselle:solvent ratio of 1:10according to the Chumsri, Sirichote, and Itharat (2008) method. Mixtures were filtered through Whatman paper No. 4, placed in vials of 40 mL and covered with aluminum foil.

2.4.2 Total Anthocyanins Content (TAC)

It was performed according to the Lee, Durst, and Wrolstad (2005) method. One mL of Roselle extract was diluted to 10 mL with distilled water. Therefore, one mL of this solution was diluted to 5 mL with buffer pH 1.0 into test tubes (wrapped with aluminum foil). Another milliliter of the Roselle solution was diluted to 5 mL with buffer pH 4.5. Mixtures were allowed to stand for 30 min at room temperature and then absorbances measured, at 520 and 700 nm, using a Cary 100 UV-visible spectrophotometer (Varian Inc., Palo Alto, CA, USA), in 4 mL spectrophotometer glass cells. Results were expressed as equivalents of cyanidin-3-glucoside per 100 g of Roselle calyces, according to the following equation:

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

where *CA* is the concentration of anthocyanins (mg/100 g), *A*is the absorbance difference ($A=[A_{520nm}-A_{700nm}]_{pH=1.0}-[A_{520nm}-A_{700nm}]_{pH=4.5}$), *MW*is the molecular weight of cyanidin-3-glucoside (449.2 g/mole), *DF* is the dilution factor, *V* is the total volume of extract (mL), *w* is the weight of the sample used in the extraction (g), *L* is the cell width (1 cm), ε is the coefficient of molar extinction for cyanidin-3-glucoside (26,900 L/mole-cm), and *100* is the conversion factor for obtaining mg/100 g of sample.

2.4.3 Total Phenols Content

It was determined by the spectrophotometric Folin and Ciocalteu method (Singleton & Rossi, 1965). Three mL of distilled water, 15 μL of the extract, and 250 μL of the Folin and Ciocalteu reagent were placed in test tubes (wrapped with aluminum foil). Samples were mixed and let stand for up to 8 minutes before adding 750 μL of 20% Na_2CO_3. Then, distilled water was added to make up 5 mL, totally mixed, and allowed to stand for 2 hours at room temperature (26±1 °C) in the darkness. The absorbance was measured at 765 nm using a Cary 100 UV-visible spectrophotometer (Varian Inc., Palo Alto, CA, USA). Various standard curves were performed with different concentrations of Gallic acid (GA) (Sigma, St. Louis, MO, USA): 0, 0.008 ± 0.001, 0.016 ± 0.001, 0.024 ± 0.002, 0.032 ± 0.003, 0.040 ± 0.004, 0.048 ± 0.004, 0.056 ± 0.005, and 0.064 ± 0.006 mg. The average standard curve was: Abs = 21.014 ± 0.810 (1/mg GA)*X (mg GA) + 0.025 ± 0.006, R^2 = 0.998 ± 0.001. Results were expressed as Gallic acid equivalents per 100 g of Roselle calyces.

2.5 Statistic Analysis

The effect of the average particle size, on the physicochemical properties of Roselle calyces, was performed by analysis of unpaired t test with a significance level of 0.05. Regarding to the phytochemical properties, a multivariate analysis and a Tukey's multiple comparison tests were applied to compare the differences within averages. Differences were considered significant for values of $p \leq 0.05$.

3. Results and Discussion

3.1 Granulometry

According to the sieving preliminary tests, performed for different times, it was observed that the best time for sieving was 7 minutes. Figure 1 depicts the weight of powder retained on each mesh. The greater weight of fine Roselle calyces powder was retained in the sieve of 0.6 mm (57.13 ± 2.42%) and the ground Roselle calyces powder was retained in the sieve of 1.0 (25.45 ± 1.3%) and 1.4 (30.16 ± 2.46%) mm. The cumulative weight curves of retained powders are also shown in Figure 2. From this curve, the median diameter (d_{50}) was obtained by interpolation; the d_{50} values were 0.55 ± 0.016 and 0.97 ± 0.034 mm for fine and ground Roselle calyces powders, respectively.

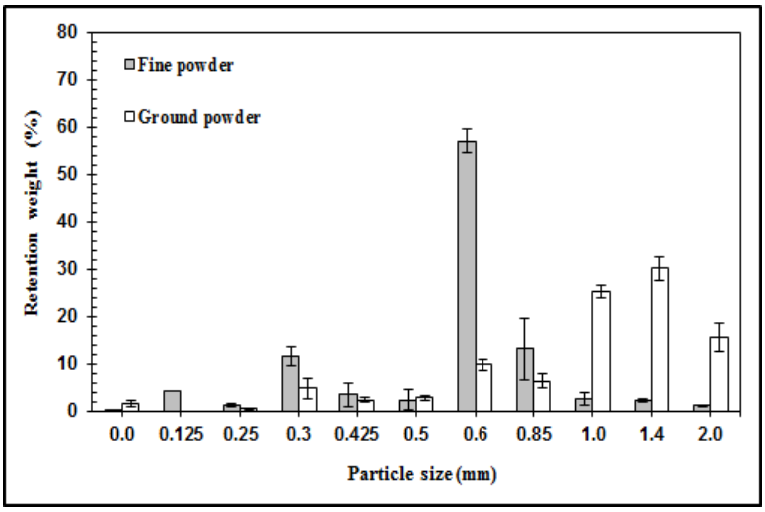

Figure 1. Particle size distribution for fineand ground Roselle calyces powders. Values represent the mean ± standard deviation (n = 3)

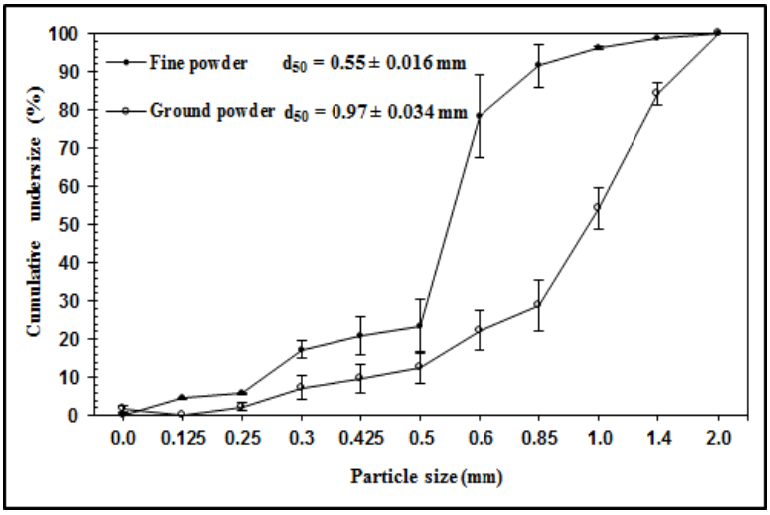

Figure 2. Particle size cumulative weight for fine and ground Roselle calyces powders. Values represent the mean ± standard deviation (n = 3)

3.2 Physicochemical Characteristics

The effect of the average particle size on the physicochemical characteristics of Roselle calyces is shown in Table 1. The moisture content and pH of Roselle are within the range recommended by the Mexican Standard NMX-FF-115-SCFI-2010 (a maximum of 12% of moisture and pH 3). Significant differences ($p \leq 0.05$) were observed only in total soluble solids and water activity. Extracts obtained using fine powder (0.5 mm) had higher content of TSS and lower $a_w$in contrast with those from ground powder (1.0 mm). By having a smaller average particle size, there is more contact surface with the solvent;therefore, there is a much more efficient extraction comparing to a greater average particle size.

Table 1. Effect of average particle size on the physicochemical characteristics of Roselle calyces

	Average particle size	
Characteristic	0.5 mm	1.0 mm
Moisture content (g/100 g)	9.18 ± 0.52a	9.25 ± 0.47a
pH (23.8 ± 3.2°C)	2.27 ± 0.12a	2.16 ± 0.05a
Water activity (25 ± 0.02°C)	0.37 ± 0.01a	0.43 ± 0.00b
Total soluble solids[a] (%) (20°C)	5.53 ± 0.05a	5.09 ± 0.26b
Titratable acidity as malic acid (g/100 g)	20.13 ± 0.77a	20.45 ± 0.70a
Density of extracts[a] (mg/mL) (22°C)	1017.11 ± 1.11a	1013.66 ± 5.30a
Tap density (mg/mL) (25°C)	800.23 ± 31.3a	470.95 ± 27.0b

Values represent the mean ±standard deviation (n = 5). Values that are followed by different letters within the same row are significantly different ($p < 0.05$). [a]Extracts obtained at a ratio of 1:10 of Roselle:water for 60 min.

Suliman, Ali, Idriss-Sharaf, and Abdualrahman (2011) reported a TSS content of 5 and 5.5% in red and white Roselle calyces, respectively. The high acidity in the Roselle calyces extracts is given by organic acids such as citric, malic, tartaric, hibiscus, succinic, oxalic, and ascorbic acids. Cañigueral (2003) reported an acidity between 15 and 30% in Roselle calyces (citric, malic, tartaric, and hibiscus acids). Galicia-Flores, Salinas-Moreno, Espinoza-García, & Sánchez-Feria (2008) reported an acidity (as malic acid) of 15.8, 19.6 and 18.7% and pH of 2.55, 2.36, and 2.15 for samples from Sudan, China and Mexico, respectively. pH values are similar to those obtained in this work as well as the acidity of Roselle calyces from China.Chumsri, Sirichote, & Itharat (2008)reported higher pH (2.86 ± 0.02) and lower acidity (1.85 ± 0.05% malic acid) than values obtained in this work; however, the TSS content was similar (5.33 ± 0.16%). For density, powders with different particle size showed very different values, having higher density the fine powder than the ground powder.

3.3 Color Properties

Table 2 shows color parameters of Roselle calyces powders with different particle size. The Roselle calyces ground powder had higher tone and lower purity than fine powder and whole calyces ($p \leq 0.05$); this indicates that the ground powder had a less intense red color. Galicia-Flores et al. (2008) reported similar results about tone (13.8) for ground Roselle calyces from Guerrero, Mexico; however, their lightness (42.1) and purity (31.8) were different to that obtained in this study. Abou-Arab et al. (2011) reported different color parameter values for Roselle calyces ($L^* = 28.3 \pm 1.0$, $a^* = 71.0 \pm 2.0$, $b^* = 45.9 \pm 2.0$) to those obtained in this study. Furthermore, the extracts obtained from ground powder (1.0 mm) showed higher purity (redder) than those of fine powder (0.5 mm); however, no significant differences ($p > 0.05$) in tone were observed (Table 3). Similar results were reported by Salazar-González, Vergara-Balderas, Ortega-Regules, and Guerrero-Beltrán (2012) in aqueous extracts of calyces using the same Roselle:water ratio (1:10) and same variety of Roselle ($L = 22.83 \pm 0.14$, $a = 45.61 \pm 0.08$, $b = 13.59 \pm 0.11$, $H = 16.59 \pm 0.00$, and $C = 47.59 \pm 0.11$). Chumsri et al. (2008) reported values of L^*, a*, and b^* of 0.75 ± 0.16, 5.22 ± 1.16, and 1.24 ± 0.27, respectively, for Roselle calyces (from Sudan) extracts using a calyces:water ratio of 1:10 and heating at 60 °C for 60 min. They only mention that calyces were ground using a grinder. Differences in color parameter values could be due to the variety of Roselle calyces, the method of extraction, and the average particle size.

Table 2. Effect of particle size on color parameters of Roselle calyces powders

	Average particle size		
Parameter	0.5 mm	1.0 mm	Whole
*L**	38.37 ± 0.20 a	31.06 ± 2.40 b	13.89 ± 1.92 c
*a**	22.76 ± 0.30 a	17.48 ± 2.15 b	25.65 ± 1.34 c
*b**	6.53 ± 0.14 a	5.55 ± 0.56 b	6.95 ± 0.52 a
Hue (H)	15.99 ± 0.22 a	17.65 ± 0.48 b	15.18 ± 1.22 a
Purity (C)	23.68 ± 0.32 a	18.34 ± 2.22 b	26.58 ± 1.32 a

Values represent the mean ± standard deviation (n = 5). Values that are followed by different letters within the same row are significantly different ($p \leq 0.05$).

Table 3. Effect of average particle size in color properties of Roselle calyces extracts

	Average particle size	
Parameter	0.5 mm	1.0 mm
*L**	20.08 ± 0.42 a	22.50 ± 1.00 b
*a**	44.21 ± 0.75 a	49.86 ± 1.70 b
*b**	10.98 ± 0.21 a	12.47 ± 0.69 b
Hue (H)	13.94 ± 0.06 a	14.03 ± 0.32 a
Purity (C)	45.55 ± 0.77 a	51.40 ± 1.81 b

Values represent the mean ± standard deviation (n = 5). Values that are followed by different letters within the same row are significantly different ($p \leq 0.05$).

3.4 Phytochemicals Content in Roselle Calyces

The results of the phytochemicals of Roselle calyces are shown in Table 4.

Table 4. Effect of average particle size (APS), solvent type (ST), and extraction times (ET) on the phytochemical properties of Roselle calyces

			TMA[a]	TP[b]
APS	ST	ET (min)	(mg/100 g of Roselle calyces)	
0.5 mm	Distilled water	30	$451.4 \pm 28.1_{a}$	$2016.2 \pm 159.8_{a}$
		45	$447.3 \pm 22.6_{a}$	$1918.5 \pm 203.3_{a}$
		60	$432.3 \pm 18.1_{a}$	$1804.5 \pm 202.8_{ad}$
	Citric acid (2%)	30	$274.1 \pm 12.9_{ef}$	$1252.4 \pm 98.1_{cg}$
		45	$260.9 \pm 6.8_{egk}$	$973.9 \pm 93.4_{f}$
		60	$259.1 \pm 23.1_{eh}$	$1187.5 \pm 74.7_{bfgh}$
	Ethanol (50%)	30	$299.8 \pm 5.1_{dfm}$	$1250.1 \pm 126.0_{chi}$
		45	$274.6 \pm 12.9_{ejlm}$	$1099.8 \pm 102.0_{bgi}$
		60	$265.3 \pm 21.3_{ejln}$	$1047.8 \pm 149.6_{bgi}$
1.0 mm	Distilled water	30	$321.5 \pm 19.9_{bd}$	$1491.1 \pm 75.9_{ce}$
		45	$350.5 \pm 24.7_{bc}$	$1582.2 \pm 175.4_{de}$
		60	$364.6 \pm 8.4_{c}$	$1628.8 \pm 139.0_{de}$
	Citric acid (2%)	30	$282.4 \pm 12.5_{ei}$	$1224.0 \pm 32.8_{bgi}$
		45	$294.1 \pm 9.5_{dfgij}$	$1266.7 \pm 52.0_{chi}$
		60	$297.9 \pm 8.0_{dfl}$	$1252.1 \pm 111.0_{chi}$
	Ethanol (50%)	30	$284.2 \pm 13.5_{ejlm}$	$1247.7 \pm 83.8_{chi}$
		45	$253.2 \pm 9.3_{en}$	$1064.7 \pm 89.1_{bgfi}$
		60	$238.1 \pm 15.8_{hkn}$	$1069.0 \pm 96.0_{bgfi}$

Values represent the mean ± standard deviation (n = 6). Values bearing different letters in the same column are significantly different ($p \leq 0.05$). [a]TMA: total monomeric anthocyanins, expressed as mg equivalents of cyanidin-3-glucoside. [b] TP: total phenols, expressed as mg equivalents of Gallic acid.

3.4.1 Total Anthocyanins Content (TAC)

The highest concentration of anthocyanins was obtained using 50% ethanol and an average particle size of 0.5 mm. The extraction time was not statistically different ($p > 0.05$). A negative correlation ($R^2 = 0.9904$) was observed between concentration of anthocyanins and the extraction time; as time of extraction increased, the anthocyanins content decreased showing overall averages of 318.9 ± 64.0, 313.4 ± 70.3, and 309.5 ± 70.8 mg/100 g of calyces for extraction times of 30, 45, and 60 min, respectively. This tendency was observed in most of the treatments except for the extracts with 50 % ethanol and distilled water using an APS of 1.0 mm.The anthocyanins degradation by temperature may be due to interaction of anthocyanins with sugars and proteins, originating by the Maillard reaction (Tonon, Brabet, & Hubinger, 2010). The presence of sugars or reaction products (such as furfural and hydroxy-methyl-furfural), by the Maillard reaction, can accelerate the degradation of anthocyanins; they can be condensed to form browning pigments. This reaction is highly dependent on the temperature and the presence of oxygen (Von Elbe & Schwartz, 1996). Coloring of Roselle calyces extracts is mainly due to anthocyanins, responsible for the red color and much of their antioxidant capacity (Tsai, Mcintosh, Pearce, Camden, & Jordan, 2002; Tsai & Huang, 2004). It has been reported higher antioxidant capacity in red than in white Roselle calyces (Christian & Jackson, 2009). Thus, an intense red color of extracts suggests a higher content of these compounds. Extracts that had the highest anthocyanins content were obtained with 50% ethanol, using an APS of 0.5 mm, which showed a darker red coloration. A positive correlation was observed between anthocyanins and purity for alcoholic extracts obtained with an APS of 0.5 mm (Figure 3).

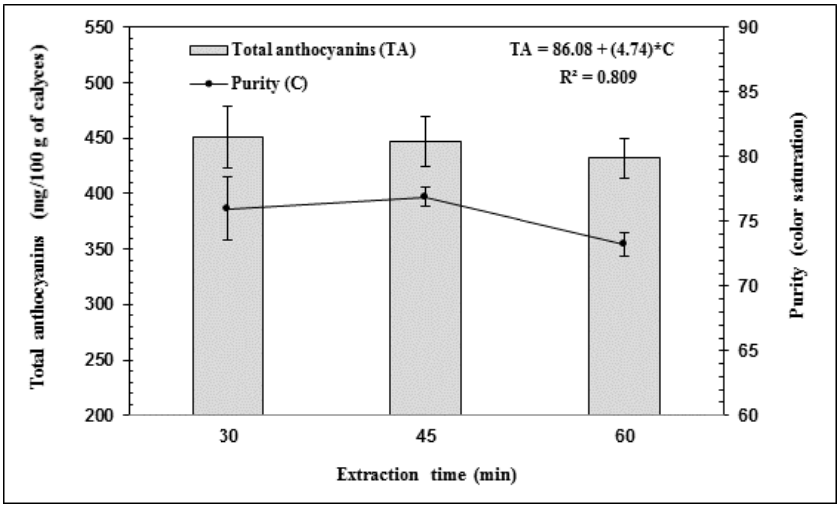

Figure 3. Relationship between anthocyanins concentration and purity of alcoholic extracts obtained at 50°C using an average particle size of 0.5 mm. Values represent the mean ± standard deviation (n = 3)

3.4.2 Total Phenolics

The total phenols content showed similar behavior as the total anthocyanins content (Table 4) regarding to the independent variables. The average particle size, solvent type, and extraction time showed a statistically significant difference ($p \leq 0.05$). In general, in Table 4 is shownthat the alcoholic extracts have higher phenols content in comparison to the aqueous and acid extracts. The extraction time of 30 min showed better performance in the amount of extraction of total phenols than 45 and 60 min; however, no correlation (R^2 = 0.625) was observed within the total phenols content. Additionally, a positive correlation was observed between phenols and anthocyanins content (Figure 4).

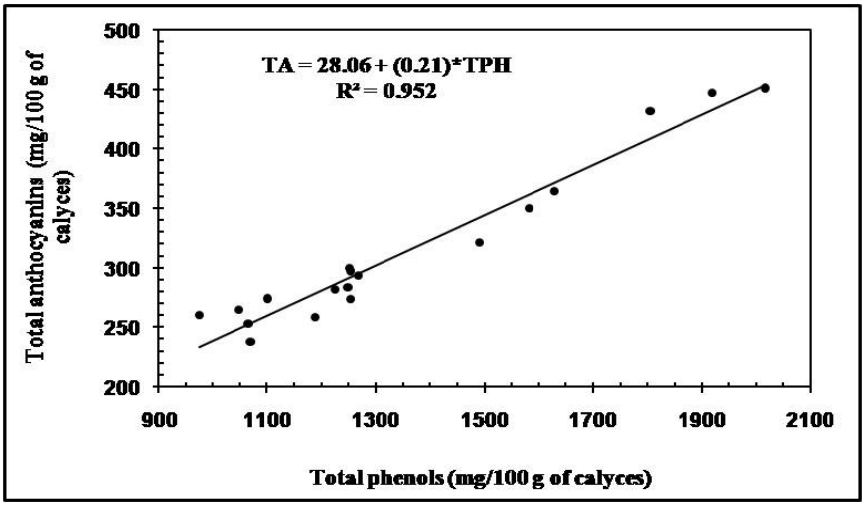

Figure 4. Correlation between the concentration of anthocyanins (TA) and total phenols (TPH). Values represent the mean of six analyses

Abou-Arab et al. (2011) reported a concentration of anthocyanins (as cyanidin-3-glycoside) and total phenols (as Gallic acid) of 1063 ± 3.0 and 4172 ± 200 mg/100 g of calyces, respectively, using a 2% citric acid solution as the extracting agent. When using distilled water they reported 545.39 ± 2.0 and 3839 ± 200 mg/100 g of calyces of anthocyanins and phenols, respectively. Similar results were reported by Chumsri et al. (2008); they used a ratio of Roselle calyces:water of 1:10 at 50 °C for 30 min. The contents of anthocyanins and total phenols were 502.33 ± 0.52 and 4300 ± 97 mg/100 g calyces, respectively.

Mohd-Esa, Shin-Hern, Ismail, and Lye-Yee (2010) reported a total phenols content of 185.0 ± 10 mg Gallic

acid/100 g of calyces for water extracts as well as 291.0 ± 7 mg Gallic acid/100 g calyces for 80% ethanol (v/v) extracts. The extracts were obtained by stirring; they mixed one gram of Roselle in one L of solvent for 2 hours at room temperature. Salazar-González et al. (2012), who used the same Roselle variety, as the one used in this study, reported an anthocyanins concentration of 209 ± 21 mg/100 g of Roselle and a total phenolics content of 2415 ± 96 mg/100 g of Roselle in 50:50 % (v/v) ethanol:water extracts. Differences in results from this work and other studies are mainly due to the variety of Roselle, as well as sample:solvent ratio, extracting method, and type of solvent. It is noteworthy that in the reportscited above, researchersdo not mention the average particle size; they only allude the use a ground Roselle calyces.

Cissé et al. (2012) evaluated the solid-liquid extraction process for the production of anthocyanins from Roselle. They reported that the calyces: solvent ratio and particle size were the main factors affecting the efficiency of extraction of anthocyanins. The maximum yield (88%) of anthocyanins was achieved using a Roselle calyces:solvent ratio of 1:25, stirring for 10 hours at 25 °C. However, using an average particle size of 0.15 mm, the extraction time was reduced to approximately 10 min to obtain a yield 63%.

According to the results obtained in this study, the average particle size is an important variable to be considered in the extraction of anthocyanins and total phenols. The extraction time and type of solvent significantly influenced ($p \leq 0.05$) the response variables. Better quantities of phenolics were obtained using a 50:50% ethanol-water ratioand an ET of 30 min. Jokić et al. (2010) evaluated the influence of water and ethanol at different concentrations (50, 60, 70, 80%), temperatures (25, 40, 50, 60, 70, 80 °C), and extraction times (5, 10, 15, 20, 30, 40, 60, 90, 120 min) on the extraction of total phenols of soybeans, Ika variety, using an average particle size of 0.459 mm and a liquid-solid ratio of 20 mL/g. The total phenols concentration increased as temperature increased. Grater total phenols concentration was obtained using 50% ethanol at 80 °C for 120 min of extraction.

Dent et al. (2013) conducted a study for evaluating the effect of type of solvent (30, 50, and 70% ethanol:water, acetone-water solutions and distilled water), temperature (60 and 90 °C), and time (30, 60, and 90 min) on the extraction of phenolic compounds from sage (*Salvia officinalis* L). They reported that 30% ethanol or acetone were the most efficient extracting agents of phenolic compounds for sage calyces at 60 °C for 30 min. As noted, the conditions for extraction of bioactive compounds (mainly anthocyanins and phenolic compounds) largely depend on solvent type, sample type, and particle size. An important aspect to note is that the binary solvent systems are more efficient for the extraction of these compounds than mono-solvent systems, very probably due to their relative polarity.

4. Conclusions

This work demonstrates the importance of the average particle size, type of solvent and extraction time on the extraction of bioactive compounds such as anthocyanins and total phenols of Roselle calyces. The extraction at 50 °C in the range of 30 to 45 min did not significantly reduce the content of anthocyanins and total phenols. The use of 50% ethanol:water and an average particle size of 0.5 mm significantly improved yields of bioactive compounds from Roselle. Roselle extracts obtained with these conditions represent an important source of these bioactive compounds, which could be used as ingredients in the food industry.

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