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Kinetics of solid-liquid extraction of anthocyanins obtained from *Hibiscus rosa-sinensis*

Cinética de la extracción sólido-líquido de antocianinas obtenidas a partir de *Hibiscus rosa-sinensis*

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Abstract

This study analyzes the solid-liquid extraction kinetic of anthocyanins from *Hibiscus rosa-sinensis*. The effects of temperature, solvent polarity using water (H_2O)-ethanol (EtaOH) solutions, and solvent-to-solid ratio on the mass transfer rate and the anthocyanin extraction yield were evaluated. The experimental data were fitted to Spiro and Siddique model with a good accuracy ($r^2 \geq 95.68\%$) and the specific extraction rate constant was perfectly related to the apparent diffusivity using the lumped parameter model. The apparent diffusivity was more sensitive to temperature changes as the solvent polarity decreased. The major percentage of the anthocyanin extraction yield was 63.81±1.33, 68.95 ±1.53, and 83.09±3.14 when the used solvent was H_2O, H_2O-EtaOH (90:10% v / v), and H_2O-EtaOH (80:20% v / v), respectively, at highest conditions of the temperature (60 ºC) and the solvent-to-solid ratio (80/1 mL/g). Finally, the anthocyanin extraction yield was successfully related to experimental extraction conditions using response surface methodology.

Keywords: Extraction kinetics, diffusivity, anthocyanins, *Hibiscus rosa-sinensis*.

Resumen

En este estudio se analiza la cinética de extracción sólido-líquido de antocianinas de *Hibiscus rosa-sinensis*. Los efectos de la temperatura, la polaridad del solvente usando soluciones de agua (H_2O) y etanol (EtaOH) y relación solvente-sólido sobre el rendimiento de la extracción de antocianinas fueron evaluados. Los datos experimentales se ajustaron al modelo de Spiro y Siddique ($r^2 \geq 95.68\%$) y la constante específica de rapidez de extracción se relacionó con la difusividad aparente mediante el modelo de parámetros agrupados. La difusividad aparente fue más sensible a los cambios de temperatura a medida que la polaridad del solvente disminuyó. Los rendimientos mayores de extracción de antocianinas fueron 63.81±1.33, 68.95±1.53 y 83.09±3.14 para el H_2O, H_2O-EtaOH (90:10% v / v) y H_2O-EtaOH (80:20% v / v), respectivamente, a las condiciones más altas de temperatura (60 ºC) y relación solvente-sólido (80/1 mL/g). Finalmente, el rendimiento de extracción de antocianinas se relacionó con éxito con las condiciones experimentales de extracción usando la metodología de superficie de respuesta.

Palabras clave: Cinética de extracción, difusividad, antocianinas, *Hibiscus rosa-sinensis*.

1 Introduction

Recently, the use of natural colorants in the food industry is increased due to questioning synthetic colorants to toxic issues and increasing consumer consciousness towards to diet and nutrition as tools to promote human health (Chou *et al*., 2007). To this, anthocyanins are water-soluble vegetable colorants with enormous potential for obtaining functional foods and nutraceuticals, due to their antioxidant activity properties to the prevention of neuronal and cardiovascular illnesses, cancer and diabetes, among others (Castañeda-Ovando *et al*., 2009).

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Anthocyanins are pigment based on the vacuoles of plant cells, and they are responsible for the shiny orange, red, pink, violet, purple or blue colors displayed by many leaves, flowers, and fruits. Chemically, anthocyanins belong to the flavonoids group, and they naturally occur as glycosides of flavylium or 2-phenylbenzopyrylium salts and are most commonly based on six anthocyanidins: pelargonidin, cyanidin, peonidin, delphinidin, petunidin, and malvidin. The sugar moieties vary but are common, glucose, rhamnose, galactose or arabinose (Mateus and Freitas, 2009).

Hibiscus rosa-sinensis, commonly referred as Chinese hibiscus, China rose and shoe flower, is a glabrous shrub belonging to family Malvaceae, and it is widely cultivated as an ornamental plant in the tropical and subtropical regions (Cabarrubias *et al*., 2017). Traditionally, the leaves, flowers, and roots of *Hibiscus rosa-sinensis* are used in the treatment of a variety of ailments such curing skin diseases, fatigue, hair growth, healing of ulcers, constipation, diarrhea, epilepsy, diabetes, among other (Mondal *et al*., 2016). Infusion of the petals is given as refrigerant and demulcent; meanwhile, leaves are used as a laxative, while the root is used in a cough (Upadhyay and Upadhyay, 2011). The intake of *Hibiscus rosa-sinensis* prevented the oxidative stress and the biochemical changes related to cerebral ischemic reperfusion injury associated with cerebrovascular insufficiency states and dementia (Nade *et al*., 2010).

The flowers of *Hibiscus rosa-sinensis* possessed abundant phenolic (42.38±2.66 mg gallic acid equivalent (GAE) per gram) content and exhibited excellent antioxidant and antihaemolytic activities (Purushothaman *et al*., 2016). Aqueous extracts of *Hibiscus rosa-sinensis* flowers contain compounds that inhibit melanoma cell growth in a dose-dependent manner at concentrations that did not affect the growth of nontransformed cells (Goldberg *et al*., 2017). The hypoglycemic, hypocholesterolemic and hypotriglyceridemic action of ethanolic extract of *Hibiscus rosa-sinensis* flowers in a well-characterized and validated animal model of diabetes (Sachdewa and Khemani, 2003). The ethanolic extract of the flower of *H. rosa-sinensis* can also be used as an anti-solar agent due to the ability to absorb ultraviolet radiation (Nevade *et al*., 2011). Therefore, the *Hibiscus rosa-sinensis* flowers have great potential for application in the development of nutraceuticals and functional food or ingredients, which they are currently in demand for the health benefits associated with their use.

The extraction solid-liquid is a traditional method used to obtain diverse active compounds, including flavonoids, from many fruits, plants, cereal grains, among others vegetable resources. The most common solvents used in the anthocyanin extractions, due to the polar character, are aqueous mixtures of ethanol, methanol or acetone (Castañeda-Ovando *et al*., 2009). Recently, microwave assisted extraction has been proposed to extract anthocyanins, due to microwave energy heat solvents rapidly and efficiently, and internal superheating causes cell disruption facilitating the extraction process (Liazid *et al*., 2007; Jain *et al*., 2009; Armenta *et al*., 2008).

The microwave assisted extraction was used to extract anthocyanins and phenolic compounds from cherry Marasca (Garofulić *et al*., 2013), sweet cherries (Grigoras *et al*., 2012), *Delonix regia* tree flowers (Adjé *et al*., 2010). Another example of green extraction processes is ultrasound-assisted extraction, which show an important positive effect on the reduction of time and energy during the extractions of the bioactive compounds such as polyphenols and β-carotene from various vegetal sources (D' Alessandro *et al*., 2013; Chemat *et al*., 2011; Sun *et al*., 2011; Mercado-Mercado *et al*., 2019). Modern techniques require special equipment and energy consumption, which will inevitably increase the process risk and cost, so the solid-liquid has been a common practice to extract bioactive compounds from vegetable resources.

The anthocyanin investigations have focused on a study of diverse purification techniques as aforementioned. However, engineering analysis of the extraction process of active substances is not sufficient. For a process design, the determination of mass transfer rate during the solid-liquid extraction is essential, and it may be increased by increasing surface area due to the reduction of the particle size, or by increasing the diffusion coefficient, due to increase temperature or modified pH in the extraction medium (Türker and Erdoğdu, 2006; Cissé *et al*., 2012). Nowadays, this information and analysis of the extraction process to obtain anthocyanins from *Hibiscus rosa-sinensis* are not available. Therefore, the aims of this study were to evaluate the effect of temperature, solvent, solvent-to-solid ratio on the aqueous anthocyanins extraction from *Hibiscus rosa-sinensis*, and calculated the mass transfer parameters of kinetic extraction using lumped parameter model.

2 Materials and methods

2.1 Biological material and reagent

Double Red Flowers of *Hibiscus rosa-sinensis* L. (Malvaceae) were collected in the locality of Xalisco, Nayarit, México, between august and november 2018, in the morning. The calyx and anther parts were separated. The separated petals were dried at 50 °C for 24 h using a forced convection oven (TE-H61DM, Terlab). The dried petals were manual grinding, and the particles were sieves and collected between 250 and 500 μm, the moisture content of the powders was determined using a thermobalance MA. 50.1R, Radwag.

Analytical grade ethanol, potassium chloride and sodium acetate were acquired from Sigma-Aldrich, S.A. de C.V. The water type II used for all the experiments was processed in the Elix® Essential 3 Water Purification System.

2.2 Extraction

The anthocyanins of the *H. rosa-sinensis* were obtained by the solid-liquid extraction method, using a batch reactor under constant agitation with a magnetic stir bar and temperature controller (±1 °C). The anthocyanin extraction yields are defined regarding the initial concentration of anthocyanin in the solid. The kinetic extraction of anthocyanins was followed quantitatively by spectrometric measurements at specific intervals time during 120 min. The anthocyanin total content in the dried powders of *H. rosa-sinensis* was determinate by a sequential solid-liquid extraction until exhaustion, with a contact time of 30 min and change of solvent in each contact.

The anthocyanins of *H. rosa-sinensis* were determined by the pH-differential method (Lee, 2005). The sample adsorption was measured at pH 1.0 and pH 4.5 in a wavelength of 510 nm and 700 nm, using a Microplate Reader Synergy HTX (Biotek, northern Vermont, USA.). The total monomeric anthocyanin content, CA (mg / L), was calculated with the equation:

$$C_A = \frac{AxMWxDFx10^3}{\varepsilon xB} \tag{1}$$

where A is the combined absorbance, MW is the molecular weight of cyanidin-3-glucoside (449.2 g/mol), DF is the dilution factor, ε is the molar absorbance of the cyanidin-3-glucoside (26900 L cm^{-1} mol^{-1}), B is the cell pathlength (0.65 cm). The combined absorbance is defined as:

$$A = (A_{520} - A_{700})_{pH1} - (A_{510} - A_{700})_{pH4.5} \tag{2}$$

In order to monitor and compare the different extraction kinetics, the extraction yield of anthocyanins, RA, was calculated from equation:

$$R_A = \frac{m_{A/L}}{m_{A_0/S}} = \frac{C_A V_L}{X_{S_0} m_S} \tag{3}$$

where $m_{A/L}$ is the mass of anthocyanins (mg) in the extract (kg) at the time (t), $m_{A_0/S}$ is the initial mass of anthocyanins in the dried powers of *H. rosa-sinensis*, C_A is the anthocyanins concentration (mg / L) in the liquid phase, X_{S_o} is the is the initial anthocyanins concentration (mg / g) in the solid phase, V_L is the liquid phase volume (L), and m_S is mass (g) of the dried solid phase.

2.3 Mathematical background of the extraction kinetics

Generally, the extraction kinetic of active compounds from plant tissues is derived using a lumped parameter model, where the rate of mass transfer is proportional to the difference between the average concentrations in the solid and in the aqueous phase, corrected by a partition coefficient (Spiro and Siddique, 1981). In this study, this model was used to analyze the extraction of anthocyanins from *H. rosa-sinensis* using the following equation:

$$\frac{d(C_{eq} - C_A)}{dt} = -k_{obs}(C_{eq} - C_A) \tag{4}$$

where C_A (mg / L) is the anthocyanin concentration in the aqueous phase at time t (min), C_{eq} is the anthocyanins concentration in the aqueous phase at equilibrium, and k_{obs} is the specific extraction rate constant (min-1). This model allows a calculation to be made of effective diffusion coefficient or effective diffusivity of the solute in the solid from observed a specific extraction rate constant. For a geometry of a sphere, the Price and Spiro equation (1985) that relates k_{obs} to effective diffusivity into the solid is defined as:

$$k_{obs} = \frac{12 D_{eff}}{R^2}\left(1 + \frac{m_s}{\kappa V_l}\right) \tag{5}$$

with

$$\kappa = \frac{C_{eq}}{X_S} \tag{6}$$

where R is the radius of the sphere, m_s is the dried mass of the solid phase, V_l is the volume (L) of the liquid phase, κ is the partition coefficient, C_{eq} is the equilibrium composition in the liquid phase and X_S is the composition of the solute in the solid phase. The term $m_s/\kappa V_l$ was calculated as a ratio of anthocyanin content into solid phase to anthocyanin content in the liquid phase at equilibrium.

The temperature dependence of effective diffusivity was evaluated using the Arrhenius equation:

$$D_{eff} = A\exp\left(\frac{-\Delta E_a}{RT}\right) \tag{7}$$

where A is the pre-exponential factor, ΔE_a is the activation energy, T is the absolute temperature and R is the universal gas constant.

2.4 Statistical analysis

In order to determinate the effects of temperature, solvent and solid-to-solvent ratio on the anthocyanin extraction yield, a quadratic factorial design involving 3^3 experiments was created. The order of the experiments was randomized. The levels of the analyzed variables were: temperature with values of 25, 40 and 60 °C; solvent polarity using water (H_2O)-ethanol (EtaOH) solvent with 100, 90:10 and 80:20 (H_2O:EtaOH% v/v); and solvent-to-solid ratio with values of 40/1, 60/1 and 80/1 (mL/g). All experimental measurements were made for triplicate and data was expressed as the mean± standard deviation. An analysis of variance (ANOVA) and Tukey's test with 95% confidence ($\alpha \leq 0.05$) were performed on the kinetic parameters of extraction using the software Minitab 17.0 (Minitab Inc., State College, PA, USA). Finally, the end extraction yield of anthocyanins was fitted to a second-order model in order to correlate the response variable to the independent variables.

3 Results and discussion

3.1 Extraction kinetics of anthocyanins

The chemical analyses indicated that the petal powders of *H. rosa-sinensis* contain a 22.57 ± 0.83% moisture and 56.03 ± 3.51 mg / g ds of the total monomeric anthocyanins (mg cyanidin-3-glucoside/g ds). Anthocyanin cyanidin was identified in *H. rosa-sinensis* flowers, with concentration ranging from 1 to 77 mg/g. The highest concentrations of cyanidin were found in the very dark magenta-red flowers from BOSTx® 'Razberri Rhapsody' and the lowest levels of cyanidin were found in cultivars with pink, white, yellow, or purple flowers (Puckhaber *et al*., 2002). This differences may be due to several factors such as the genetics, type of cultivar, flower maturation, extraction procedures, among others.

Figure 1 shows the percentage of extraction yield of the anthocyanin from *H. rosa-sinensis*, defined as the percentage ratio between the mass of anthocyanins present in the liquid phase at the instant time and the initial mass of anthocyanins present in the solid phase, using water as a solvent. The extraction yield is improved by increasing the extraction temperature and tend to equilibrium (asymptotical curve when time increases) into the experimental contact time range. This behavior is classical for the extraction of the bioactive compound from vegetable resources. Similar results were obtained during aqueous extraction of anthocyanins from *Hibiscus sabdariffa* (Cissé *et al*., 2012), ultrasound-assisted extraction of anthocyanins from *Aronia melanocarpa* (D'Alessandro *et al*., 2014), with a semi-batch extraction of anthocyanins from red grape pomace (Mantell *et al*., 2002), and extraction of antioxidant phenolic compounds from compressional-puffing pretreated *Pinus morrisonicola* (Pai-Shih *et al*., 2017).

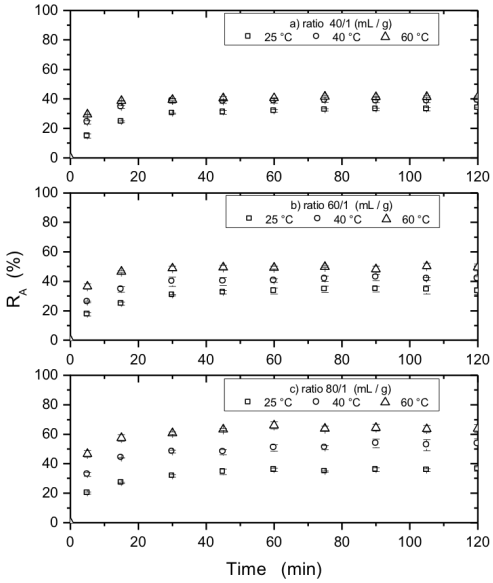

Fig. 1. Extraction yields of anthocyanins obtained from of *H. rosa-sinensis* vs time using H_2O and different temperatures, with a solvent-to-solid ratio: a) 40 mL/g; b) 60 mL/g; c) 80 mL/g.

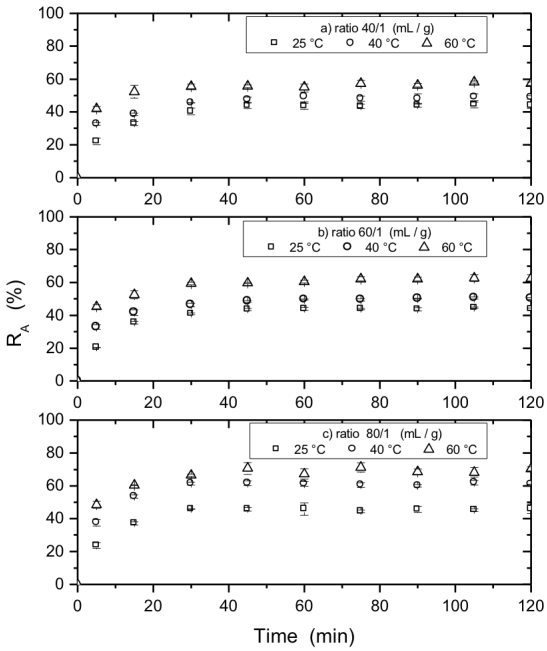

Fig. 2. Extraction yields of anthocyanins obtained from of *H. rosa-sinensis* vs time using H_2O-EtaOH (90:10) and different temperatures, with a solvent-to-solid ratio: a) 40 mL/g; 60 mL/g; c) 80 mL/g.

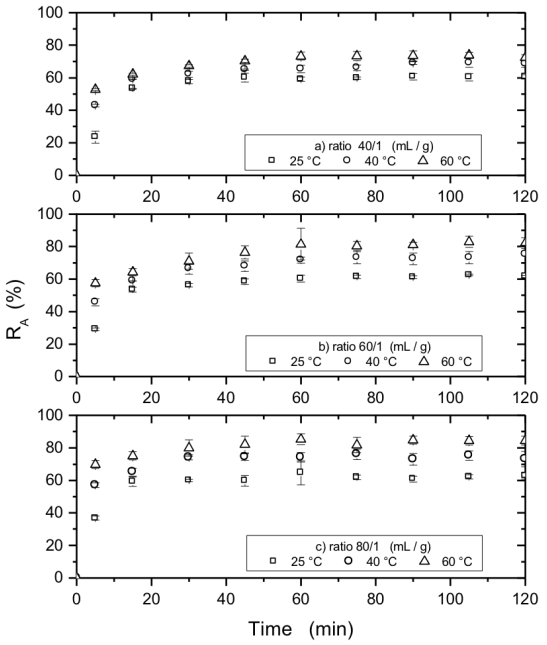

Fig. 3. Extraction yields of anthocyanins obtained from of *H. rosa-sinensis* vs time using H_2O-EtaOH (80:20) and different temperatures, with a solvent-to-solid ratio: a) 40 mL/g; b) 60 mL/g; c) 80 mL/g.

For the solvent-to-solid ratio of 40/1 (mL/g), the extraction kinetic reached equilibrium very fast regardless the extraction temperature, with extraction yield values less than 50%, indicating unfavorable conditions, this could be due to this ratio tend to minimum values of solvent to be used. Changes in the polarity of the solvent affect the anthocyanin extraction yields (Figure 2 and Figure 3); the decrease in eluotropic strength of solvent increased the extraction yield of the anthocyanins. The highest extraction efficiencies were obtained with the H_2O-EtaOH (80:20). These results are in agreement with previous reports in which solvents with a very high polarity, such as water, or very little eluotropic strength, such as chloroform or hexane, do not give good results for the extraction of phenolic compounds present in plants (Pace *et al*., 2014). This allows inferring that the profile of anthocyanins extracted from *H. rosa-sinensis* may be moderately polar, such as phenolic derivatives, hydroxycinnamic derivatives, flavonols, flavan 3-ol monomers, flavanones, and flavones, among others (An-Na *et al*., 2014). However, the use of alcohols, such as methanol or ethanol, can lead to the extraction of other polar components such as alkaloids with undesirable properties, especially if the objective is to obtain anthocyanin concentrates with antioxidant capacity. The increasing of solvent-to-solid ratio increased the extraction yields for all conditions of extraction temperature and solvent polarities (Figure 1, 2 and 3). The addition of solvent ensures the formation of a homogeneous mixture, improving the hydration and swelling of the solid. Consequently, the concentration gradient increases into interstices of the structural matrix of plant cells, which in turn enhancing the mass transfer rate. Similar behavior was exhibited during the extraction of polyphenols and anthocyanins from saffron bio-residues (Da Porto and Natolino, 2018) and extraction of polyphenols from chicory grounds (Pradal *et al*., 2016).

3.2 Analysis of kinetic parameters

Table 1 reports the values of the kinetic parameters of the Spiro and Siddique model fitted to the experimental data of anthocyanin extraction with a good accuracy ($r^2 \geq 0.9568$, 0.9722, 0.9719 using H_2O, H_2O-EtaOH (90:10), and H_2O-EtaOH (80:20) as a solvent, respectively). As can be seen, the specific extraction rate constants (k_{obs}) increased with increasing the extraction temperature regardless of the type of solvent or solvent-to-solid ratio, due to high

temperature reduces the surface tension of solvent and enhanced molecular fluctuations (Pai-Shih *et al*., 2017).

In general, the solvent-to-solid ratio did not affect the k_{obs} values at the same temperature regardless the used solvent, excepting when the conditions were H_2O-EtaOH (80:20) with ratio of 80/1 (mL/g) at 40 and 60 °C. In these extraction conditions, the major ethanol content in the liquid phase combined with the temperature could be damage the vegetal matrix, which in turn modified the initial diffusion during the extraction. It well knows that ethanol alters the cell membrane structures both chemically and biophysically, acting primarily within the phospholipid bilayer of biological membranes (Boussetta *et al*., 2012). Increasing the ethanol content in the solvent favored the equilibrium, which increased the C_{eq} values and the extraction rate and reduced the extraction time at the same solvent-to-solid ratio (Table 1). These results confirmed that the variation of polarity affects the extraction efficiency, because of a solubility enhancement of anthocyanins when the dielectric constant of the solvent was diminished by the addition of ethanol.

Table 1. Kinetic parameters of the Spiro-Siddique model for anthocyanin extractions from *H. rosa-sinensis*.

Solvent	**Solvent-to-solid ratio (mL/g)**	**Temperature (°C)**	k_{obs} **(min^{-1})**	C_{eq} **(mg/L)**
H_2O	40/1	25	$0.1031\pm0.0068^{a,1}$	$457.99\pm10.34^{a,1}$
		40	$0.1861\pm0.0073^{c,5}$	$543.78\pm13.80^{d,4}$
		60	$0.2526\pm0.0038^{d,9}$	$576.37\pm05.57^{g,7}$
	60/1	25	$0.1136\pm0.0143^{a,b,2}$	$315.05\pm17.60^{b,10}$
		40	$0.1804\pm0.0115^{c,6}$	$384.70\pm18.46^{e,13}$
		60	$0.2675\pm0.0196^{d,10}$	$463.24\pm09.73^{h,16}$
	80/1	25	$0.1335\pm0.0098^{b,3}$	$246.63\pm06.74^{c,19}$
		40	$0.1877\pm0.0129^{c,7}$	$358.88\pm06.64^{f,22}$
		60	$0.2571\pm0.0149^{d,11}$	$446.99\pm09.30^{i,25}$
H_2O-EtaOH (90:10)	40/1	25	$0.1152\pm0.0111^{A,1}$	$614.50\pm23.66^{A,2}$
		40	$0.2011\pm0.0188^{B,5}$	$670.02\pm24.99^{D,5}$
		60	$0.2659\pm0.0204^{C,9}$	$794.28\pm08.18^{G,8}$
	60/1	25	$0.1170\pm0.0075^{A,2}$	$411.91\pm04.46^{B,11}$
		40	$0.1997\pm0.0257^{B,6}$	$460.48\pm04.41^{E,14}$
		60	$0.2569\pm0.0081^{C,10}$	$570.34\pm07.79^{H,17}$
	80/1	25	$0.1342\pm0.0053^{A,3}$	$321.72\pm08.88^{C,20}$
		40	$0.1791\pm0.0127^{B,7}$	$430.12\pm04.09^{F,23}$
		60	$0.2315\pm0.0155^{C,11}$	$482.96\pm10.70^{I,26}$
H_2O-EtaOH (80:20)	40/1	25	$0.1170\pm0.0077^{a,1}$	$851.65\pm04.39^{a,3}$
		40	$0.1950\pm0.0113^{c,5}$	$935.57\pm21.37^{b,6}$
		60	$0.2507\pm0.0188^{e,9}$	$1006.66\pm22.14^{g,9}$
	60/1	25	$0.1317\pm0.0065^{a,2}$	$571.16\pm06.42^{b,12}$
		40	$0.1785\pm0.0076^{c,6}$	$668.19\pm29.17^{e,15}$
		60	$0.2361\pm0.0122^{e,10}$	$736.22\pm22.74^{b,18}$
	80/1	25	$0.1834\pm0.0010^{b,4}$	$437.78\pm08.44^{c,21}$
		40	$0.2855\pm0.0087^{b,8}$	$519.16\pm15.14^{f,24}$
		60	$0.3693\pm0.0089^{f,12}$	$581.97\pm22.05^{i,27}$

[a,A,α] Means followed by the same lowercase letters, capital letters and script letters in the same column are not significantly different ($\alpha \leq 0.05$) for the effect of solvent-to-solid ratio at the same temperature, when the solvent was H_2O, H_2O-EtaOH (90:10), H_2O-EtaOH (80:20), respectively.

[1] Means followed by the same number in the same column are not significantly different ($\alpha \leq 0.05$) for effect of solvent at the same solvent-to-solid ratio and temperature.

The adjusted coefficient of determination for experimental data were: $r^2 \geq 0.9568, 0.9722, 0.9719$ using H_2O, H_2O-EtaOH (90:10), and H_2O-EtaOH (80:20) as a solvent, respectively

Table 2. Effective diffusivity and activation energies for anthocyanin extractions from *Hibiscus rosa-sinensis*.

Solvent	**Solvent-to-solid ratio (mL/g)**	**Temperature (°C)**	$D_{eff}x10^{09}$ **m^2/s**	ΔE_a **kJ/mol**
H_2O	40/1	25	1.645±0.097^{a,1}	26.25
		40	3.529±0.222^{c,4}	
		60	5.075±0.079^{e,7}	
	60/1	25	1.862±0.134^{a,10}	29.25
		40	3.624±0.156^{c,13}	
		60	6.480±0.522^{f,16}	
	80/1	25	2.293±0.125^{b,19}	29.28
		40	4.696±0.309^{d,22}	
		60	8.016±0.619^{g,24}	
H20-EtaOH (90:10)	40/1	25	2.466±0.247^{A,2}	25.61
		40	4.686±0.301^{C,5}	
		60	7.365±0.636^{D,8}	
	60/1	25	2.518±0.135^{A,11}	26.06
		40	4.805±0.577^{C,14}	
		60	7.659±0.229^{D,17}	
	80/1	25	3.009±0.144^{B,20}	22.22
		40	5.372±0.431^{C,22}	
		60	7.795±0.555^{D,24}	
H_2O-EtaOH (80:20)	40/1	25	3.475±0.233^{a,3}	21.64
		40	6.354±0.264^{c,6}	
		60	8.790±0.480^{e,9}	
	60/1	25	3.932±0.208^{a,12}	19.64
		40	6.228±0.271^{c,15}	
		60	9.083±0.320^{e,18}	
	80/1	25	5.593±0.190^{b,21}	22.99
		40	10.331±0.296^{b,23}	
		60	14.977±0.509^{f,25}	

$^{a,A\,a}$ Means followed by the same lowercase letters, capital letters and script letters in the same column are not significantly different ($\alpha \leq 0.05$) for the effect of solvent-to-solid ratio at the same temperature, when the solvent was H_2O, H_2O-EtaOH (90:10), H_2O-EtaOH (80:20), respectively.

^{1} Means followed by the same number in the same column are not significantly different ($\alpha \leq 0.05$) for effect of solvent at the same solvent-to-solid ratio and temperature.

Similar results were reported for phenolic compounds extracted from grape (Spigno *et al*., 2007), *Quercus coccifera* L. and *Juniperus phoenica* L. (Hayouni *et al*., 2007), anthocyanins and polyphenols extracted from *Oryza sativa* L. (Pedro *et al*., 2016), among others. The effective diffusivity of anthocyanins from *H. rosa-sinensis* calculated with equation (5) at different extract conditions is depicted in Table 2. For each solvent, the solvent-to-solid ratio with values 40/1 and 60/1 did not significantly affect diffusivity, with a slight increase in their average values when increasing the ethanol concentration of the aqueous phase.

To these extraction conditions, the mass transfer of anthocyanins from solid phase to liquid phase have also been affected by driven forces, due to modifications on solubility and solute-solvent interactions (Cacace and Mazza, 2003). In the case of solvent-to-solid ratio of 80/1, the increase in the effective diffusivity values, with a significant variation compared with the other ratios at the same temperature, corroborated the aforementioned cellular rupture. The modification of activity coefficients and solubility in the liquid phase (ethanol modified the equilibrium in the systems) and the decrease in the diffusive internal resistance allowed the increase the

extraction yields of anthocyanins to a maximum at the highest solvent-to-solid ratio.

The effective diffusivity exhibited a positive trend with temperature. The increase in diffusivity when increasing temperature may be due to an increase of internal energy of the molecules and thus their mobility, and a reduction of the dynamic coefficient. This behavior increases the extraction yields of anthocyanins. The values of D_{eff} reported in Table 2 were in agreement with those obtained during the extraction of anthocyanins from *Hibiscus sabdariffa* (Cissé *et al*., 2012), vanilla extraction (Castillo-Santos *et al*., 2017), and phenolic compound extraction from grape (Bucić-Kojić *et al*., 2013). The temperature dependence of effective diffusivity can be determined using the Arrhenius model (equation 7) and the average values of D_{eff}. The activation energies for anthocyanin diffusion are summarized in Table 2, which magnitude values were H_2O > H_2O-EtaOH (90:10) > H_2O-EtaOH (80:20), which implies that effective diffusivity tends to be more temperature sensitive as the solvent polarity decreases.

Table 3. Analysis of variance and equation of regression for extraction yield of anthocyanin obtained from *Hibiscus rosa-sinensis*.

Solvent / Model	Source	DF	F-Value	P-Value
	Model	5	239.55	0.000
	Linear	2	528.09	0.000
H_2O	X_1	1	371.39	0.000
$\%Ra_{eq} = 45.96 - 0.976X_1 + 0.300X_2 + 0.00578X_1^2 - 0.0077X_2^2 + 0.01431\,X_1X_2$	X_2	1	684.80	0.000
	Square	2	15.60	0.000
$r^2 = 98.28\%$ $r^2_{adj} = 97.87\%$ $r^2_{pred} = 97.26\%$	X_1^2	1	15.66	0.001
	X_2^2	1	15.64	0.001
	2-way int $X_1\,X_2$	1	148.21	0.000
	Lack of fit	3	3.44	0.039
	Model	5	82.11	0.000
	Linear	2	195.11	0.000
H_2O-EtaOH (90:10)	X_1	1	88.13	0.000
	X_2	1	302.09	0.000
$\%Ra_{eq} = 53.58 - 0.836X_1 + 0.285X_2 + 0.00655X_1^2 - 0.00223X_2^2 + 0.00679\,X_1X_2$	Square	2	4.75	0.020
	$X_1^2$1	8.92	0.007	
	X_2^2	1	0.58	0.456
$r^2 = 95.13\%$ $r^2_{adj} = 93.97\%$ $r^2_{pred} = 92.06\%$	2-way int $X_1\,X_2$	1	14.79	0.001
	Lack of fit	3	18.71	0.000
	Model	5	79.30	0.000
	Linear	2	189.91	0.000
H_2O-EtaOH (80:20)	X_1	1	57.69	0.000
	X_2	1	322.13	0.000
$\%Ra_{eq} = 39.47 + 0.086X_1 + 0.716X_2 - 0.00163X_1^2 - 0.00761X_2^2 + 0.00668\,X_1X_2$	Square	2	4.45	0.025
	$X_1^2$1	0.67	0.421	
	X_2^2	1	8.22	0.009
$r^2 = 94.97\%$ $r^2_{adj} = 93.77\%$ $r^2_{pred} = 91.69\%$	2-way int $X_1\,X_2$	1	17.48	0.000
	Lack of fit	3	0.52	0.671

The ΔE_a can be associated with the energetic barrier to be overcome in order to process begins, meaning that the extraction of anthocyanins is facilitated when using an ethanolic aqueous solution as a solvent. The ΔE_a values reported in Table 2, in range from 19.64 to 29.28 kJ mol^{-1}, are similar to values of 36.80 and 33.52 kJ mol^{-1} for aqueous extraction of total phenolic and flavonoid compounds from *Salvia fruticosa* leaves (Torun *et al*., 2014), 23.0 kJ mol^{-1} for total phenol extraction from grape marc (Sant' Anna *et al*., 2012), 22.87 kJ mol^{-1} for the extraction of the total phenolic compounds from *Azadirachta indica* leaves (Shewale and Rathod, 2018), among others. The ΔE_a values decreased as the eluotropic strength of the solvent diminished, confirming that the extraction of anthocyanins is favored when using an ethanolic aqueous solution.

3.3 Data analysis using RSM

Response surface methodology (RSM) is a statistical method to optimize process when many factors and interactions affected desired variables, performing a minimum of experiments and time (Xi and Yan, 2017). The extraction yield response ($Y = RA_{end}$) of anthocyanins at the end of the extraction was fitted to the second quadratic model:

$$Y = a + bX_1 + cX_2 + dX_1^2 + eX_1^2 + fX_1X_2 \qquad (8)$$

where X_1 is the solvent-to-solid ratio and X_2 is the extraction temperature (°C). Table 3 shows the parametric values, the variability and the analysis of variance of the second quadratic model. When H_2O was used as a solvent, the coefficient of determination (r^2) was 98.28%, the predicted r^2_{pred} was 97.26%, and the adjusted r^2_{adj} was 97.87%, showing the model to be successful to predict the extraction anthocyanin yield from sour *H. rosa-sinensis*. The p-values of the parametric coefficients indicated that temperature and solvent-to-solid ratio, their square effect, and their interaction were a significant effect ($\alpha < 0.05$) on the extraction of anthocyanins. The parametric coefficient values also revealed that the linear effects and the interactions between solvent-to-solid ratio and temperature prevailed over the quadratic effects, with negative contributions regarding the solvent-to-solid ratio. However, the values and sign of the parametric coefficients cannot be associated with physical or chemical aspects because there are empirical (Ranic *et al*., 2014).

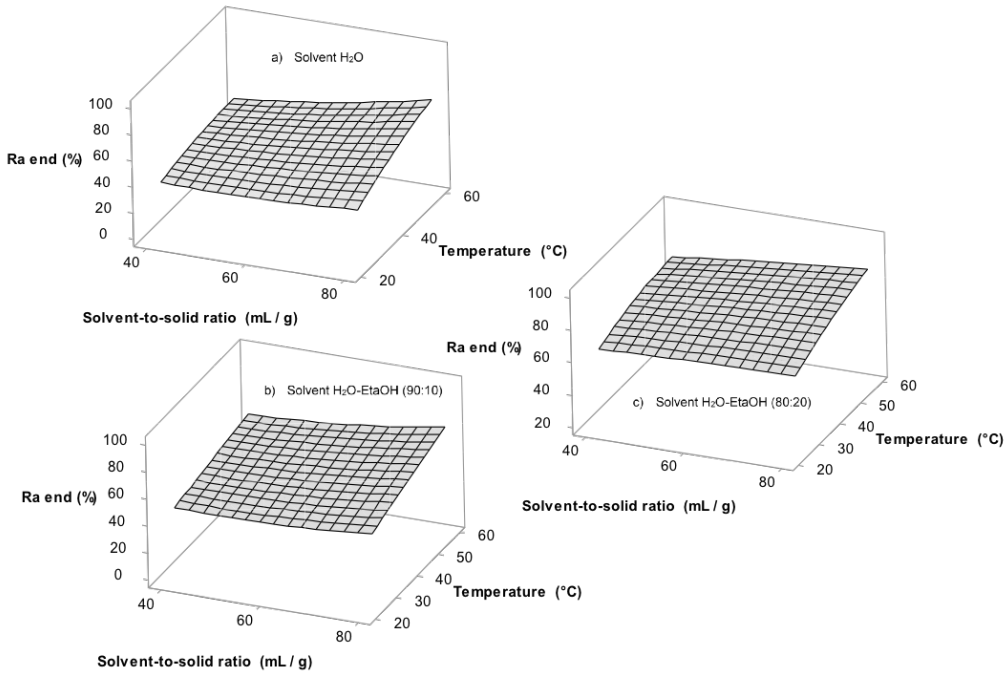

Fig. 4. Response surface plots for the effects of solvent-to-solid ratio and temperature on the extraction yield of *H. rosa-sinensis* anthocyanins using solvent: a) H_2O; b) H_2O-EtaOH (90:10); c) H_2O-EtaOH (80:20).

The quadratic effects and the quality of the proposed model decreased when the eluotropic strength decrease (Table 3). The model explains the 95.13% and 94.97% of the variability of the results for H_2O-EatOH (90:10) and H_2O-EtaOH (80:20), respectively. Negative and positive effects in the coefficients were observed using for the RSM analysis during the anthocyanins extractions from P*runus avium* cherries, phenolic compound extractions from sour cherry (*Prunus cerasus* L.) pomace, and another antioxidant compound extractions from sweet and traditional cherries (Blackhall *et al*., 2018; Yilmaz *et al*., 2015; Usenik *et al*., 2008; Serra *et al*., 2011). The major percentage of the extraction yield was 63.81 ± 1.33, 68.95 ± 1.53, and 83.09 ± 3.14 when the used solvent was H_2O, H_2O-EtaOH (90:10), and H_2O-EtaOH (80:20), respectively. These values corresponded to the highest experimental conditions of solvent-to-solid ratio and temperature, 80 mL/g and 60 °C. The differences of the optimal solvent-to-solid ratio for anthocyanin extraction depend on several factors such as the source and stage of maturation of the material, material processed prior to extractions, among others.

Figure 4 shows the response surface plots of extraction yield of anthocyanins as function of temperature and solvent-to-solid ratio. As seen, not plots of the extraction yield displayed a maximum or minimum behavior within the analyzed variable range. The extraction yield had a greater dependence respect to solvent-to-solid ratio when the solvent was H_2O and the temperature was kept to the optimal level (Fig. 4a). This functionality was less noticeable with the increase of ethanol content until being almost independent of said variable at lower temperatures (Fig. 4b and 4c).

While the temperature affected always the extraction yield of anthocyanins at any values of solvent-to-solid ratio, increasing its values at a higher temperature. This behavior is consistent with the diffusion process where the driving force during the solute extraction from solid phase is the concentration gradients, which was greater at a higher solvent-to-solid ratio, resulting in a higher contact between solute and solvent and hence, the mass transfer rate was increased. This phenomenon was enhanced by the temperature due to the increase of diffusivity. The linear link between temperature and solvent-to-solid ratio with anthocyanin extraction yield was also reported for the extraction of anthocyanins using calyces of *H. sabdariffa* (Cissé *et al*., 2012), grape pomance (Pinelo et at., 2005), milled berries (Cacace and Mazza, 2003), and solid-liquid extraction of andrographolide from plants (Wongkittipong et at., 2004).

Conclusions

In this study, it has been found that double red flowers of *Hibiscus rosa-sinensis* L. are good sources of natural colorants with several potential applications in the food, pharmaceutical, and cosmetic industries, due to high total monomeric anthocyanin content (56.03 ± 3.51 cyanidin-3-glucoside/g ds). For experimental data, the equations prescribed by Spiro and Siddique (1981) and Price and Spiro (1985) were appropriate ($r^2 \geq 0.9568$) to calculate and relate the specific extraction rate constant (k_{obs}) and the apparent diffusivity (D_{eff}). In general, the solvent-to-solid ratio did not affect the k_{obs} values at the same temperature regardless of the used solvent. However, the equilibrium was favored with solvent polarity diminished. This fact increased the D_{eff} values due to higher values of the partition coefficient. The activation energy values, in range from 19.64 to 29.28 kJ/mol, decreased as the eluotropic strength of the solvent diminished, confirming that the extraction of anthocyanins is favored when using an ethanolic aqueous solution. The RMS showed that the solvent-to-solid ratio affected significantly ($\alpha = 0.05$) the extraction yield of anthocyanins from at higher temperature when H_2O was used as a solvent. This functionality decreased with the increase of ethanol content in the solvent. However, the H_2O-EtaOH (80:20) gave a significant increase in anthocyanin yield, which seemingly ascribed to enhanced mass transfer resulted from disruption of samples cells. This information can be used to choose the appropriate extraction conditions and to scale up the extraction process to industrial level.

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