Valorization of mulberry (*Rubus glaucus*) by-products: ultrasound-assisted extraction of total anthocyanins

Valorización de subproductos de mora (*Rubus glaucus*): extracción asistida por ultrasonido de antocianinas totales

Luis Eduardo Ordóñez-Santos [1*], Jessica Esparza-Estrada [1], Oswaldo Osorio Mora [2]

Originales: *Recepción:* 01/03/2018 - *Aceptación:* 11/03/2019

Abstract

In this study, the objective was to establish parameters of optimization of the factors such as solvent concentration, extraction time and liquid-to-solid ratio in ultrasound-assisted extraction (UAE) of total anthocyanins from mulberry by-products. The study sample corresponds to mature fruits that did not comply with the size demanded by the market (by-product). Three independent variables including extraction time (6-74 min), liquid-solid ratio (33-66.16 mL/g), and ethanol concentration (63-96%) were investigated. A rotatable central composite design (CCD) and response surface methodology (RSM) was used to investigate the effect of process variables. The optimum conditions of ultrasound-assisted extraction obtained through response surface methodology were as follow: extraction time, 20 min; liquid to solid ratio, 60:1 mL/g, and ethanol concentration, 90%, allowed to obtain a maximum concentration of 259.66 mg/L. To validate the optimized model, the experimental values were compared with the predicted values to check the adequacy of the model. Ultrasound extraction was 13.60% higher than maceration technique and reduces the time from 24h to 20 minutes. The results show that ultrasonic-assisted extraction as a promising agro-industrial process in the recovery of anthocyanins from mulberry by-products.

Keywords
central composite design • response surface methodology • natural colorant • optimization

1 Universidad Nacional de Colombia-Sede Palmira. Facultad de Ingeniería y Administración. Departamento de Ingeniería. Carrera 32 N 12-00. Palmira. Valle del Cauca. Colombia. * leordonezs@unal.edu.co

2 University of Nariño. Faculty of Agro-Industrial Engineering. Department of Industrial Processes. Agro-Alimentary Research and Development Support Group (GAIDA).

Resumen

En este estudio, el objetivo fue establecer los parámetros de optimización de los factores como la concentración de disolvente, el tiempo de extracción y la relación líquido a sólido en la extracción asistida por ultrasonidos (EAU) de antocianinas totales de subproductos de mora. La muestra del estudio corresponde a frutas maduras que no cumplieron con el tamaño exigido por el mercado (subproducto). Se investigaron tres variables independientes, incluido el tiempo de extracción (6-74 min), la relación líquido-sólido (33-66,16 mL/g) y la concentración de etanol (63-96%). Se utilizó un diseño compuesto central rotacional (DCCR) y una metodología de superficie de respuesta (RSM) para investigar el efecto de las variables de proceso. Las condiciones óptimas de extracción asistida por ultrasonido obtenidas a través de la metodología de superficie de respuesta fueron las siguientes: tiempo de extracción, 20 min; relación líquido-sólido, 60:1 mL/g, y concentración de etanol, 90%, permitieron obtener una concentración máxima de 259,66 mg/L. Para validar el modelo optimizado, los valores experimentales se compararon con los valores predichos para verificar la adecuación del modelo. La extracción de ultrasonido fue 13,60% más alta que la técnica de maceración y reduce el tiempo de 24h a 20 minutos. Los resultados muestran que la extracción asistida por ultrasonidos es un prometedor proceso agroindustrial en la recuperación de antocianinas de los subproductos de la mora.

Palabras clave

diseño compuesto central • metodología de superficie de respuesta • colorante natural • optimización

Introduction

The mulberry is of the Rosaceae family, there are between 700 and 750 species distributed in 12 genera, being *Rubus*, the largest species within this family (5, 29). Mulberry is one of the most appreciated fruits in the world market, as this fruit has gained an important position in the food industry, thanks to the presence of anthocyanins (16, 30, 35).

Different research has reported that the health benefits of anthocyanin focus on antioxidant and anti-inflammatory activity, reduced risk of coronary heart disease, stroke, cancer, and aging (1, 16, 30). In addition, the anthocyanins of natural origin are authorized by the European Food Safety Authority (EFSA) and Food and Drug Administration (FDA) to produce colorants in the food industry (6).

The growing interest of the consumer and industry, due to the health benefits and coloring properties of natural anthocyanins, has encouraged the search for new sources of anthocyanins.

Ayala-Zavala *et al.* (2011) reports, that fruit by-products are an important source of natural pigments, as these residues are produced in high volumes and are available at a low cost.

Different fruit by-products are sources of anthocyanins, among them banana (28), mulberry (38), black chokeberry (10), grape (3, 9, 15), coffee exocarp (31) blackberry pomace (13, 27).

Ghafoor *et al* (2009), Ghafoor *et al* (2011), and Tao *et al.* (2015) commented that extraction is an important stage in the processes of obtaining the phenolic compounds; also indicate that the time, temperature, solvent/sample ratio and solvent concentration are important factors to be optimized.

Bioactive compounds in plant matrices can be obtained with conventional techniques such as maceration, Soxhlet and cold compression, also with emerging techniques such as supercritical fluid, extraction by electric pulses, high pressure extraction and ultrasonic assisted extraction (14).

The emerging technology of ultrasound is a valid technique in obtaining bioactive compound on natural matrices due to the high yields, simplicity of management and reduced costs in the extraction of such compounds (7, 8).

Dranca and Oroian (2016) indicate that the ultrasound process exerts a mechanical effect, which breaks plant cell walls, improving mass transfer by increasing the contact surface between the solvent and the plant material.

Ultrasonic assisted extraction (UAE) of anthocyanins were reported in plant materials by-product such as saffron, eggplant, mulberry, grape, blueberry, and jabuticaba (11, 14, 16, 17, 19, 20, 22, 32). Although studies have been carried out for the extraction of anthocyanins from different plant matrices, the scientific literature does not report any investigations on the optimization UAE of anthocyanins from mulberry (*Rubus glaucus*) by-products. In addition, this emerging technology can be an alternative of valorization of the mulberry by-products produced in the commercialization of this fruit, since this residue is an important source of anthocyanins, appreciated by the food, pharmaceutical and cosmetic industry as natural pigments. Therefore, the objective of the present investigation was to establish parameters of optimization of the factors such as solvent concentration, extraction time and liquid-to-solid ratio in UAE of total anthocyanins from mulberry by-products.

Materials and methods

Materials

Fresh mature mulberry (R*ubus glaucus*) fruits were obtained from a commercial crop, located in the rural area of the municipal of Cali, Valle del Cauca.

The study sample corresponds to mature fruits that did not comply with the size demanded by the market (by-product), according to what is established by the Colombian Technical Standard NTC 1406 (1997). The material was left to dry at room temperature for 3 hours, pre-cooled to 3°C and immediately frozen for 12 hours at -80°C.

Finally, the material went through lyophilization (FreeZone 4.5 freeze-dryer). The lyophilization of the samples was conducted at a temperature of less than -55°C with a condenser surface of 13.6 dm^2 and a pressure of 0.12 mbar for 24 hours.

The lyophilized pod was hammer milled (IKA WERKE M20), screened with a 60 mesh sieve (particle size of 246 µm), packed in glass tubes with plastic caps, and covered with aluminum foil.

The samples were kept refrigerated at 4°C until the various analyses were performed. All of the chemicals used were of analytical grade, and they were purchased from were obtained from Merck.

Ultrasound-assisted extraction of the total anthocyanins in mulberry by-products

The extraction was assisted by an ultrasonic cleaning bath using a Branson 2510R-DTH ultrasonic cleaner (Branson Ultrasonics Corp. Danbury, USA), which operates at a frequency of 40 kHz with a power intensity of 130 W and a tank capacity of 2.81 L (internal dimensions: 241.3 x 139.7 x 102.6 mm), equipped with a digital timer and a temperature controller.

The temperature was controlled and maintained at 30 ± 2°C by circulating external water from a thermostatic water bath. The power of the ultrasonic bath during the experiments was 90-110 W, this parameter was determined following the calorimetric method described by Kiani *et al.* (2012). Samples of the powder of mulberry by-products were transferred into a volumetric flask (10 mL), and the extraction procedure was conducted, according to the experimental design conditions under study.

The extractor solution used in the present study corresponds to ethanol: water solution acidified at pH 1 with HCl, as recommended by Rodrigues *et al*. (2015).

The volumetric flask with the solution was immersed into water in the ultrasonic device, and irradiated for the predetermined extraction time.

After the extraction process, the extracts were filtered with Whatman Grade 4 filter paper and filtered through a 0.20 µm nylon syringe filter, and the filtered material was used to quantify the total anthocyanin. Maceration was carried out as a control for comparison with UAE. Samples were extracted at room temperature in the dark for 24 h, using liquid to solid ratio, 60:1 mL/g, and ethanol concentration 90%.

Determination of total anthocyanin concentration

The total anthocyanin content in mulberry by-products extract was determined using the pH differential method, previously described by Lee *et al.* (2005). The extracts were separately mixed with potassium chloride buffer (KCl, 0.025 M, pH 1.0) and sodium acetate buffer (CH3COONa, 0.4 M, pH 4.5) and the absorbance was measured at 520 nm and 700 nm, against a blank consisting of distilled water, using a spectrophotometer Jenway 6320D.

The concentration of anthocyanins in the extract was expressed as cyanidin -3-O-glucoside equivalent according to Equation 1.

$$Total\ anthocyanin\left(\frac{mg}{L}\right) = \frac{AxMWxDFx1000}{\epsilon \text{ x L}} \quad (1)$$

where:

$A = (A520\text{nm} - A700\text{nm})_{pH1.0} - (A520\text{nm} - A700\text{nm})_{pH\ 4.5}$

MW (molecular weight) = 449.2 g/mol for cyanidin-3-glucoside (cyd-3-glu)

DF = dilution factor

L = path length (1 cm)

ε = 26 900 molar extinction coefficient, in L x mol^{-1} x cm^{-1}, for cyd-3-glu

1000 = factor for conversion from g to mg.

Experimental design

Following the recommendation of Bezerra *et al.* (2008), preliminary tests of one factor were carried out, which allowed the identification of the three main factors, with greater effect in the extraction of the total anthocyanins presents in the mulberry by-products.

The effects of extraction time (20, 30, 40, 50, and 60 min), liquid-solid ratio (30:1, 40:1, 50:1, 60:1, and 70:1 mL/g), and ethanol concentration (50, 60, 70, 80, and 90%) were investigated separately on the basis of extraction efficiency.

The optimal levels were selected as center points in the designed experiment. A 2K rotatable central composite design (CCD) with 8 factorial points, 6 axial points and 6 center points was used to obtain the best combination of process variables that optimizes the ultrasound-assisted extraction of the total phenols from the mulberry by-products.

The model selection criteria included coefficient of determination (R2) and Lack or Fit, and analysis of variance (ANOVA) was used to assess the significance of each factor, their quadratic effects and interactions.

The experiments were carried out in triplicate, and the standard deviation was used to express the results.

Table 1, shows the independent variables used in the CCD.

A regression analysis was performed on the results obtained with regard to the implementation of the independent variables, and it was adjusted to an empirical second order polynomial model as shown in general equation 2 for the total anthocyanins.

Design Expert 11 statistical software was used to optimize the extraction process of anthocyanins in the mulberry by-products.

$$Y_i = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 \quad (2)$$

where:
Y_i = response variable
β_0 = constant
β_1, β_2 and β_3 = regression coefficients for the linear effect
β_{11}, β_{22} and β_{33} = coefficients for the quadratic effects
β_{12}, β_{13}, and β_{23} = coefficients for interactions X_1, X_2 and X_3 = independent variables (time extraction, ethanol concentration, and liquid-to-solid ratio, respectively).

Results and discussion

Effect of independent variables on ultrasound extraction of anthocyanin

Figure 1(A) (page 372), shows the effect of extraction time on the extraction of total anthocyanins. The yield significantly increased from 212.85 to 254.88 mg/L as the extraction time increased from 20 to 40 min, then began to decrease as the extraction time increased from 40 to 60 min.

Table 1. Independent variables and their levels used for in a central composite rotatable design.

Tabla 1. Variables independientes y sus niveles utilizados en un diseño central compuesto rotacional.

Independent variables	Coded level				
	-α (-1.68179)	-1	0	1	+α (+1.68179)
	Natural levels				
Extraction time (X_1)	6	20	40	60	74
Ethanol concentration (X_2)	63	70	80	90	96
Liquid-to-solid ratio (X_3)	33	40	50	60	66.16

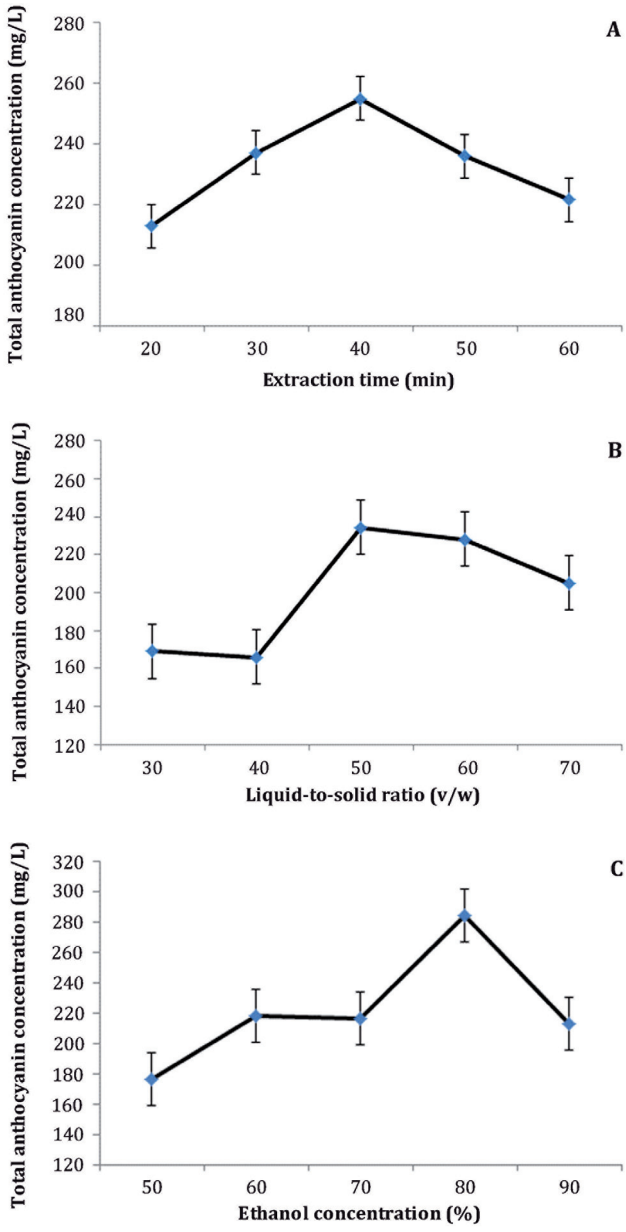

Figure 1. The effects of extraction parameters on total anthocyanin concentration. (A) Effect of time on the extraction, (B) Effect of liquid-to-solid ratio on the extraction, (C) Effect of ethanol concentration on the extraction.

Figura 1. Los efectos de los parámetros de extracción en la concentración total de antocianinas. (A) Efecto del tiempo en la extracción, (B) Efecto de la relación líquido-sólido en la extracción, (C) Efecto de la concentración de etanol en la extracción.

According to the results, 40 min was selected as the central point for this treatment. Tao and Da-Wen (2015) reports that the extraction efficiency depends directly on duration of ultrasound-assisted extraction (UAE).

Explanation for this increase is due to the action of the cavitation phenomena that affect the cell wall, allowing the diffusion of the pigment to the solvent (37). Reduction in the extraction of total anthocyanins could be explained that, within a certain time the internal and external pressure of the cell reached the equilibrium, with the extraction time prolonged, generated the reactions of oxidation and polymerization, responsible for the degradation of anthocyanins (23, 37).

The extraction of total anthocyanins by different liquid-to-solid ratio to material is shown in figure 1B (page 372).

The results indicate a greater extraction of total anthocyanins from 165.98 to 234.20 mg/L, with the liquid-to-solid ratio increasing from 40:1 to 50:1 mL/g. According to the results, 50:1 liquid-to-solid ratio is recommended as the center points in the optimization experiment. Zou *et al.* (2011) reports that liquid-to-solid ratio is other independent variables to be studied, in order to avoid solvent losses or lower yields of the compound of interest. This result was consistent with the principle of mass transfer, where the concentration gradient between the material and the bulky solvent is the driving force of mass transfer (34). However, increasing the solvent does not increase the extraction yield of the total anthocyanins as shown in figure 1B (page 372). Lou *et al.* (2010) reports that the mass transfer is more restricted to the solid interior, therefore, larger amount of solvent would not change the driving force.

Other possible explanations for the reduction in the extraction of total anthocyanins are the extractions of impurities such as polysaccharides and proteins were dissolved out, affecting the dissolution of total anthocyanins (37). As shown in figure 1C (page 372), the extraction of total anthocyanins were increased to increase the ethanol concentration from 50 to 80%, and decreased when the ethanol concentration was higher than 80%.

According to the results, ethanol solution 80% is recommended as the center points in the optimization experiment. The increase and decrease of the extraction of total anthocyanins in the samples can be explained by the polarity between the solvent and the solute, as previously explained by Yang *et al*. (2010) authors, indicate that as there are similar polarities between the solvent and solute, the bioactive compound of interest is easily dissolved from plant cells, and Roselló-Soto *et al.* (2015) reports that polyphenols are easily solubilized in hydroalcoholic mixtures (polar protic mediums), where fractions can be recovered based on polarity by varying alcohol concentration.

Solvent type is an important criterion for ultrasonic assisted extraction, since the physical properties of solvent (polarity, viscosity, surface tension, density, diffusivity and vapor pressure) are correlated with its molecular affinity with the components of interest and its diffusion into biological matrix (17). Yang *et al.* (2010) and Chemat *et al.* (2012) reports that ethanol is one of the most used solvents in the extraction of phenolic compounds in the plant matrix, it is a bio-solvent, completely biodegradable, non-toxic, and economical.

Response surface optimization

Table 1 (page 371), presents the experiment design and corresponding response data for the total anthocyanins extraction.

The range of total anthocyanin extracted mulberry by-products, experimental values between 104.64 - 262.17 mg/L (table 2).

Rodrigues *et al.* (2015) obtained values of total anthocyanin in jabuticaba peel (373.72 mg/L) that exceed those recorded in the present study; extraction conditions used by researchers were 150W ultrasonic power, ethanol concentration of 38% on 30°C, with an extraction time of 60 min and solvent to ratio 1:20 w/v. By contrast, our results exceed the values reported by Demirdöven *et al.* (2015) in red cabbage, where the concentration of total anthocyanins were 28.65 to 58.67 mg/L, which was obtained using 150W ultrasonic power, ethanol concentration of 42.39% on 40°C, with an extraction time of 75 min and solid–liquid ratio 1:3 w/v.

Zhao *et al.* (2011) commented that these differences in total anthocyanins concentration are due to genetic differences between species, which affect the type of structures and solubility of anthocyanins in the plant matrix.

Table 2. Central composite rotatable design matrix and response values for total anthocyanin.

Tabla2. Matriz del diseño central compuesto rotacional y valores de respuesta para antocianina total.

Experimental number	Extraction conditions			Analytical results total anthocyanin (mg/L)
	X_1, Extraction time (min)	X_2, Ethanol concentration (%)	X_3, Liquid-to-solid ratio (mL/g)	
1	40(0)	80(0)	50(0)	127.33 ± 15.94
2	20(-1)	90(1)	60(1)	262.17 ±13.60
3	6(-1.68179)	80(0)	50(0)	248.26±14.97
4	60(1)	70(-1)	40(-1)	219.31 ± 5.84
5	40(0)	80(0)	50(0)	138.60 ± 5.47
6	74(+1.68179)	80(0)	50(0)	230.72 ± 25.05
7	60(1)	90(1)	60(1)	239.46 ± 25.92
8	40(0)	80(0)	66.16(+1.68179)	222.50 ± 33.10
9	20(-1)	70(-1)	40(-1)	242.47 ± 38.76
10	40(0)	80(0)	50(0)	113.83 ± 19.12
11	40(0)	96(+1.68179)	50(0)	208.74 ± 19.26
12	40(0)	63(-1.68179)	50(0)	153.91 ± 20.70
13	40(0)	80(0)	33(-1.68179)	234.38 ± 11.77
14	60(1)	70(-1)	60(1)	205.06 ± 25.94
15	40(0)	80(0)	50(0)	107.98 ±6.75
16	40(0)	80(0)	50(0)	104.64 ± 5.43
17	20(-1)	90(1)	40(-1)	212.63 ± 8.24
18	40(0)	30(0)	050(0)	109.40 ± 8.24
19	20(-1)	70(-1)	60(1)	239.13 ± 15.30
20	60(1)	90(1)	40(-1)	192.82 ± 4.74

The analyses of the ANOVA of the experiment are presented in table 3. The model allows monitoring the optimization of the extraction stage, since it presented a level of significance ($p < 0.05$).

The interaction ethanol concentration and liquid-to-solid ratio, and quadratic effect of factors statistically influenced on extraction of total anthocyanins (table 3).

Lack of fit was not significant ($p > 0.05$) in the proposed mathematical model guarantees that it is possible to predict the variations within the extraction process (table 3).

The experimental values showed a good fit with the empirical regression equation $Y = 116.83 - 9.46X_1 + 6.83X_2 + 4.29X_3 + 1.84 X_1X_2 - 1.73X_1X_3 + 14.22X_2X_3 + 44.20X^2_1 + 23.64X^2_2 + 40.30 X^2_3$, as they presented the value of the coefficient determination r^2 was 0.9550 and $r^2_{adj} = 0.9144$ (table 3). Therefore, the model adequately represents the real relationship between the chosen parameters.

Response surface and contour plots

The response surface is plotted to evaluate the interaction of the independent variables and to estimate the optimal level of each variable to obtain a maximum extraction of total anthocyanins.

Figure 2A (page 376), the response surface generated by the effect of ethanol extraction and extraction time of total anthocyanin extraction is presented. As was shown in figure 2A (page 376), a positive quadratic effect of ethanol concentration and extraction time on extraction of total anthocyanins were illustrated as a parabolic shaped surface plot where initially reached its minimum at 80% and 40 minutes followed by a marked increase.

Table 3. Analysis of variance (ANOVA) for the fitted quadratic polynomial model for optimization of extraction parameters.

Tabla 3. Análisis de varianza (ANAVA) para el modelo polinomial cuadrático ajustado en la optimización de los parámetros de extracción.

Source	Sum of squares	Degree of freedom	Mean squares	F value	p > F	Significance[a]
Model	54463.74	9	6051.53	23.54	< 0.0001	***
Extraction time (X_1)	1222.98	1	1222.98	4.76	0.0541	NS
Ethanol concentration (X_2)	637.71	1	637.71	2.48	0.1463	NS
Liquid-to-solid ratio (X_3)	251.51	1	251.51	0.98	0.3459	NS
$X_1 X_2$	26.99	1	26.99	0.11	0.7526	NS
$X_1 X_3$	23.82	1	23.82	0.093	0.7670	NS
$X_2 X_3$	1618.10	1	1618.10	6.30	0.0310	*
X_1^2	28157.51	1	28157.51	109.55	< 0.0001	***
X_2^2	8051.68	1	8051.68	31.33	0.0002	***
X_3^2	23401.34	1	23401.34	91.04	< 0.0001	***
Residual	2570.37	10	257.04			
Lack of Fit	1695.09	5	339.02	1.94	0.2428	NS
Pure error	875.28	5	175.06			
Cor total	57034.10	19				

$r^2 = 0.9550$. $r^2_{Adj} = 0.9144$, [a]*($p < 0.05$); **($p < 0.01$) and ***($p < 0.001$), and NS = Not significant. / No significativa.

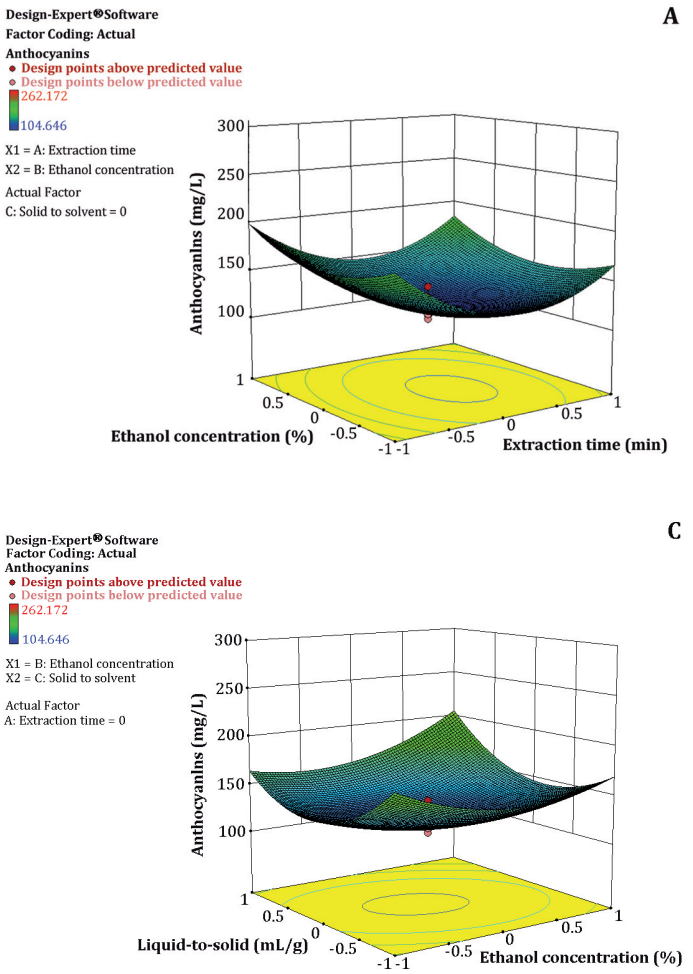

Figure 2. Response surface plots showing the interaction of the independent variables. (A) Effect of ethanol concentration and extraction time on extraction of total anthocyanin. (B) Effect of liquid-to-solid and extraction time on extraction of total anthocyanin. (C) Effect of liquid-to-solid and ethanol concentration on extraction of total anthocyanin.

Figura 2. Diagramas de superficies de respuesta que muestran la interacción de las variables independientes. (A) Efecto de la concentración de etanol y el tiempo de extracción en la extracción de antocianina total. (B) Efecto de líquido-sólido y tiempo de extracción en la extracción de antocianina total. (C) Efecto de la concentración de líquido-sólido y etanol en la extracción de antocianina total.

Figure 2B (page 376), a significant effect in liquid to solid ratio and extraction time to total anthocyanin extraction is observed, a positive quadratic effect of liquid to solid ratio and extraction time on extraction of total anthocyanins were illustrated as a parabolic shaped surface plot where initially reached its minimum at 50 and 40 minutes followed by a marked increase.

Figure 2C (page 376), confirm the effect of ethanol concentration and solid to solvent ratio of the level of total anthocyanins extraction.

The incidence of the quadratic effect of liquid to solid ratio and ethanol concentration also is observed (figure 2C, page 376). Zou *et al.* (2011) and Demirdöven *et al.* (2015) observed that the liquid-solid ratio, solvent concentration and extraction time have a quadratic effect positive on the extraction of total anthocyanins from mulberry and red cabbage.

The parabolic form of the response surface of the total anthocyanins concentration may be associated with differences in pigment solubility in the extraction solvent, Teng *et al.* (2014) report that solubility is affected by the molecular structures of anthocyanin, as cyanidins include two hydroxyl groups and one hydrogen group at different bound positions, resulting in hydrophilism and dissolvability.

Optimum conditions for maximum extraction

The optimal condition obtained using response surface methodology (RSM) corresponded to a extraction time, 20 min; liquid to solid ratio, 60:1 mL/g, and ethanol concentration, 90%, allowed to obtain a maximum concentration of 259.66 mg/L.

The values of optimization extraction were validated experimentally obtaining total anthocyanins concentration of 265.81 ± 22.33 mg/L, which showed no significant difference ($p > 0.05$) with the value recorded in the optimization.

Moreover, ultrasound extraction was compared with maceration techniques, considered conventional methods of extraction of anthocyanin. Total anthocyanin maceration extraction was 230.44 ± 8.60 mg/L. These results show that the ultrasound technique is effective for extraction of total anthocyanin in the mulberry by-product, compared to extraction with maceration; ultrasound exceeds performance by 13% and reduces the time from 24h to 20 min.

The efficient extraction of total anthocyanin in mulberry by-products by ultrasound is the result of the mechanical fracture of the cell wall, which allows the solvent to enter the cell interior, increasing the mass transfer (14). Ghafoor *et al.* (2009) reports that the efficiency of ultrasonic extraction has its explanation in that ultrasound simultaneously increased the process of hydration and fragmentation process facilitating the mass transfer of solutes to the extraction solvent. Author also comments that mass transfer is positively affected by changes in the diffusion coefficients induced by the liquid-solid ratio, solvent concentration and extraction time.

Conclusions

In the present work, ultrasonic-assisted extraction process for maximum total anthocyanins from mulberry by-products was investigated by RSM.

The response surface methodology using the Composite Central Design allows the optimization of the extraction conditions for important valorization of

the total anthocyanins from mulberry by-product. The optimum conditions of UEA obtained through RSM were as follow: extraction time, 20 min; liquid to solid ratio, 60:1 mL/g, and ethanol concentration, 90%, allowed to obtain a maximum concentration of 259.66 mg/L.

The extraction time, the liquid-solid ratio and the ethanol concentration showed an important quadratic effect on the extraction yield of total anthocyanins. The results show that ultrasonic-assisted extraction as a promising agro-industrial process in the recovery of anthocyanins from mulberry by-products.

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Acknowledgements

This work was supported by National University of Colombia, Palmira, Valle del Cauca, Colombia.