



PRELIMINARY PURIFICATION OF ANTHOCYANINS FROM BLUE CORN BY ADSORPTION AND ELECTROPHORESIS

PURIFICACIÓN PRELIMINAR DE ANTOCIANINAS DE MAÍZ AZUL POR ADSORCIÓN Y ELECTROFORESIS

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Abstract

Anthocyanins pigments are natural antioxidants of interest in food, pharmaceutical and cosmetic industries. In this study a process for preliminary purification of anthocyanins from blue corn (*Zea mays*) was implemented. The anthocyanin purification advancement was analysed in terms of Total Organic Carbon (TOC), Total Anthocyanin Content (TAC), and reverse phase High Performance Liquid Chromatography (HPLC). Microfiltration and ultrafiltration steps were used as pre-treatment of the extracts. Purification of anthocyanins was performed by adsorption and electrophoresis. The adsorption was achieved using a (SP-207) styrene-divinylbenzene resin, the results were correlated using Freundlich and Langmuir models. The best condition for purification of anthocyanins in electrophoresis was at pH 3 and 220 V. The purification process was improved using electrophoresis resulting in a decreasing on TOC from 59.52±0.53 mg to 3.966±0.257 mg per milligram of anthocyanins. The process implemented was successfully used in red cabbage (*Brassica oleracea var. Capitata*) and blueberries (*Vaccinium corymbosum*). In conclusion, proposed process can be useful as a preliminary purification process of anthocyanins from different sources.

Keywords: anthocyanins purification, electrophoresis, adsorption, ultrafiltration, blue corn.

Resumen

Las antocianinas son pigmentos naturales y antioxidantes de interés en la industria alimentaria, farmacéutica y cosmética. En el presente trabajo se implementó un proceso de purificación preliminar de antocianinas de maíz azul (*Zea mays*). El avance del proceso de purificación fue analizado en términos del contenido total de antocianinas (TAC), carbono orgánico total (TOC) y Cromatografía Líquida de Alta Resolución (HPLC) fase reversa. Para el pre-tratamiento de los extractos se usó microfiltración y ultrafiltración. La purificación de antocianinas se realizó mediante adsorción y electroforesis. La adsorción se realizó usando una resina polimérica estireno-divinilbenceno (SP-207), los resultados fueron correlacionados usando los modelos de Freundlich y Langmuir. Las mejores condiciones de purificación de antocianinas en electroforesis fueron a pH 3 y 220 V. El proceso de purificación fue mejorado usando electroforesis resultando una disminución de TOC de 59.52±0.53 mg a 3.966±0.257 mg por miligramo de antocianinas. El proceso implementado fue exitosamente usado en col morada (*Brassica oleracea var. Capitata*) y arándanos (*Vaccinium corymbosum*). En conclusión, el proceso propuesto puede ser utilizado como un proceso de purificación preliminar de antocianinas en diferentes fuentes.

Palabras clave: purificación de antocianinas, electroforesis, adsorción, ultrafiltración, maíz azul.

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1 Introduction

Anthocyanins are hydrosoluble flavonoids present in foods with colour from pink to purple (Morales-Delgado *et al.*, 2014). Many potential health benefits have been found in anthocyanins extracts. In recent years, increasing attention has been paid to black, blue and purple cereal grains due to their high levels of anthocyanins as dietary antioxidants (Lee *et al.*, 2010).

In Mexico, blue corn is employed for preparing traditional dishes and its colour coming from anthocyanins present in pericarp and aleuronic layers, these therapeutic substances promote alternative practice that have not been properly exploited (Salinas Moreno *et al.*, 2013; Fernández Suárez *et al.*, 2013). Specifically, cyanidin-3-glucoside, pelargonidin-3-glucoside, peonidin-3-glucoside and their malonate counterparts have been found in blue corn (Abd-Aal *et al.*, 2006; González-Manzano *et al.*, 2008). Challenge to process these natural pigments is their stability against pH, temperature, light, oxygen and extract composition (Moldovan *et al.*, 2012; Reyes and Cisneros-Zevallos, 2007).

High quantity of blue corn anthocyanins are lost in the Nixtamalization industry, this is a thermal and alkaline common process used for softening kernels and remove pericarp (Escalante-Aburto *et al.*, 2013). In order to benefit from the high contents of the anthocyanins, it is necessary to establish a processing for their isolation. New approaches, in anthocyanins recovery involving process integration and intensification to improve the purity, yield and throughput of products since primary recovery until purification strategy, in the same line adsorbents should be explored (González-Váldez *et al.*, 2014; Sánchez-Rangel *et al.*, 2014).

Membrane processes become crucial for recovering thermolabile bioactive compounds from different aqueous systems in food processing industry (Cassano *et al.*, 2013, Conidi *et al.*, 2012; Conidi *et al.*, 2014); no involve phase change or chemical agents and energy expense is very low, becoming suitable for anthocyanins (Cassano *et al.*, 2011; Cissé *et al.*, 2011). Therefore, the aim of this work was to evaluate the integration of different separation technologies appropriate for anthocyanins purification. Recent process integration for purification red cabbage anthocyanins was reported by Chandrasekhar and Raghavarao (2015), they used membrane technology (ultrafiltration, osmotic distillation and forward osmosis), aqueous two-phase extraction (ATPE) and thermal evaporation, both may cause the degradation

of the targeted compounds due to high temperature and long extraction times (Calvarano *et al.*, 1996; Xu *et al.*, 2008).

In this study, a microfiltration technology was used in order to clarify blue corn extract, followed by two ultrafiltration steps. In purification stages, the extract was adsorbed using SP-207 resin. Finally, electrophoresis process was implemented as a final purification step. The purification process was analysed in terms of total suspended solids (TSS), Total Organic Carbon (TOC), Total Anthocyanin Content (TAC) and HPLC.

2 Materials and methods

2.1 Preparing extracts

Blue corn was obtained from Otumba, State of Mexico. Blue corn was washed and dried. Product was ground in mills in order to obtain flour. Blue corn powder was treated with acidified water solution (0.1 M phosphoric acid and 0.01 M HCl) ratio 1:10 w/v. The solution was agitated at 90 rpm, at 25 °C by 8 hours. Crude extract (CE) was then stored at -20°C until used.

Red cabbage and blueberries extracts were prepared with acidified water (0.1 M phosphoric acid) ratio 1:5 w/v. The solution was milled in blender and agitated at 90 rpm, at 25 °C by 2 hours. Crude extract (CE) was then stored at -20°C until used.

2.2 Separation process

The purification process to obtain preliminary purified pigment consisted of following steps described in Figure 1.

2.2.1. Pre-clarification

In order to remove suspended solids, the extract was centrifuged in a Heras Megafuge (40Thermo Scientific) at 2500 rpm, 25 °C during 10 min. Supernatant was filtered with conventional Whatman® qualitative 601 grade filtering membranes.

2.2.2. Clarification

2.2.2.1. Microfiltration

The supernatant from pre-clarification process was submitted to Microfiltration (MF) step using

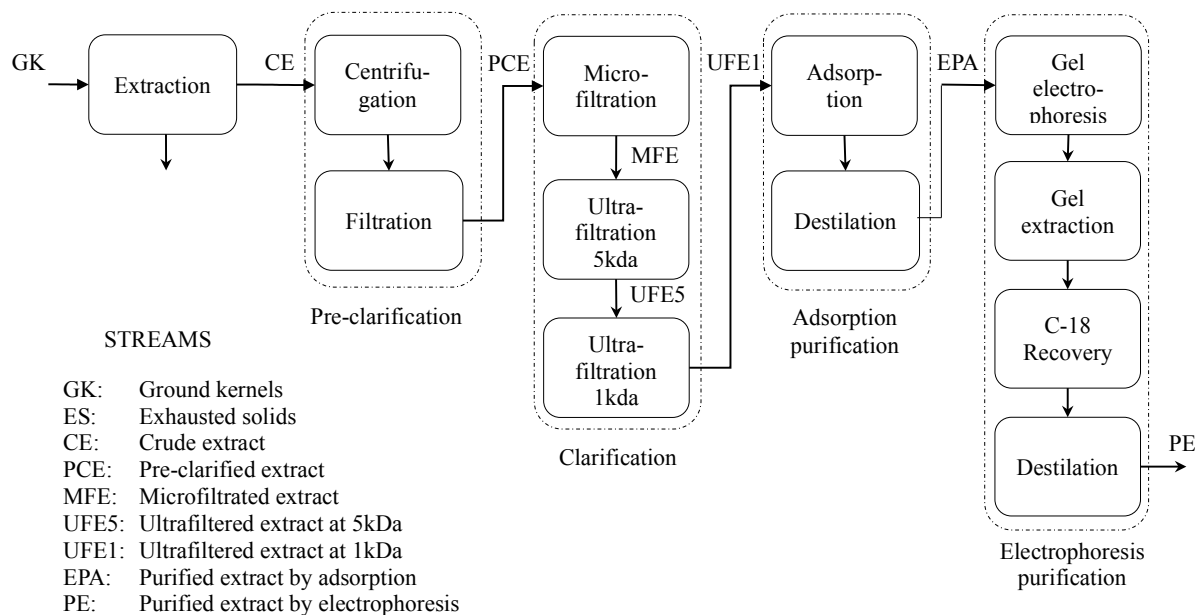


Fig. 1. Separation process for anthocyanin purification

Medivators FiberFlow™ hollow fiber module with polysulfone membrane (pore size 0.2 μm, area 0.55 m²) at transmembrane pressure (TMP) of 34.3 KPa and axial flow (Q_f) of 6 L min⁻¹.

2.2.2.2. Ultrafiltration

For ultrafiltration (UF) step a Millipore Prep/Scale™ system was used. Different wound modules of regenerated cellulose material membrane were adapted to the system. Membranes with molecular weight cut off (MWCO) of 5 kDa (0.25 m², CDUF 002 LC model) and 1 kDa (0.09 m², CDUF 001 LA model) were used. The operation conditions were: TMP (103.4 KPa), Q_f (6 L min⁻¹) and TMP (206.8 KPa), Q_f (6 L min⁻¹), respectively.

2.2.2.3. Rejection and total losses of MF and UF membranes.

The rejection (R) and total percentage losses (TL) in microfiltration and ultrafiltration membranes towards specific component were determined as:

$$R = \left(1 - \frac{C_p}{C_f}\right) \times 100\% \quad (1)$$

$$TL = \left(1 - \frac{V_R \times C_p}{V_f \times C_f}\right) \times 100\% \quad (2)$$

where C_p and C_f are the concentration of a specific component in permeate and feed, respectively, and

V_f and V_R are the feed and retentate volumes, respectively.

2.3 Purification process

2.3.1. Adsorption and desorption experiments

Adsorption capacity (q_e : mg of blue corn anthocyanins adsorbed per gram of adsorbent), desorption capacity (q_d : mg of desorbed anthocyanin per gram of resin) and desorption ratio (D : mg desorbed per mg adsorbed) were evaluated. Adsorption was carried out using 0.2 grams (M : Mass of adsorbent) of activated SP-207 resin contacted with 40 mL of 1kDa cut membrane ultrafiltrated extract (UFE1) of blue corn (V_i : volume of initial sample) in 50 mL conical flasks horizontally set in a Thermo shaker (MaxQ6000) at 110 rpm, 25°C for 6 hours.

When equilibrium was achieved, anthocyanins were desorbed from resin with 40 mL ethanol as eluent (V_d : volume of eluent). The following equations were used for this study:

$$q_e = \frac{(C_o - C_e)V_i}{M} \quad (3)$$

$$q_d = \frac{C_d V_d}{M} \quad (4)$$

$$D = \frac{q_d}{q_e} \times 100\% \quad (5)$$

Where, q_e : Adsorption capacity, C_o : Initial concentration, C_e : Equilibrium concentration, V_i :

Volume of initial sample, M : Mass of absorbent, q_d : Desorption capacity, C_d : Eluent concentration, V_d : Volume of eluent and D : desorption ratio.

In order to describe the adsorption capacity behaviour at different concentrations of the samples, the equilibrium experimental data were fitted to isotherm equations described by Al Duri (1996).

Langmuir equation as:

$$q_e = \frac{q_m C_e}{K_L + C_e} \quad (6)$$

Linearly expressed as:

$$\frac{C_e}{q_e} = \frac{K_L}{q_m} + \frac{C_e}{q_m} \quad (7)$$

Freundlich equation as:

$$q_e = K_F C_e^{1/n} \quad (8)$$

Linear expression:

$$\log q_e = \log K_F + \frac{1}{n} \log C_e \quad (9)$$

where, q_m : Langmuir empirical constant, K_L : Langmuir adsorption equilibrium constant, K_F : Freundlich constant, $1/n$: Empirical Freundlich constant, plotting the equation on a log-log scale, K_F and $1/n$ can be calculated from intercept and slope values.

2.3.2. Procedure for dynamic adsorption and desorption tests

Dynamic adsorption and desorption experiments in column were performed with a Büchi flash chromatography system equipped with C-615 control of pump, C-605 piston pump and C-635 spectrophotometer with a continuous flow cell. A polypropylene column (15 cm length x 1.2 cm diameter and 17 mL effective volume column), loaded with 5.4 g SP-207 resin and fed with UFE1 flowed through the column at 7 mL min^{-1} , the concentration of anthocyanins was monitored at 520 nm, and the values were taken manually; being C_o concentration extract and C_l concentration in leaking column while adsorption is performed. After reaching adsorptive saturation, the adsorbent in the column was washed thoroughly using 1 liter of 0.01 M HCl solution at 25 mL min^{-1} and then eluted using ethanol (100%), the concentration of anthocyanins in the desorption solution was also determined by spectrophotometry to obtain the desorption curve. The dynamic adsorption and desorption trials were performed in triplicated.

2.3.3. Electrophoresis

The electrophoresis of anthocyanins was performed in horizontal chamber (11 cm width and 18 cm length) using 5% agarose gel in buffer solution at pH 3 (0.00125 M potassium hydrogen phthalate and 0.0005 M HCl in deionized water). Each well was filled with 250 μL EPA, the sample was processed at 16.3 V cm^{-1} for 60 min, the initial buffer temperature was 8°C and was refilled each 20 min, the chamber was located in a cool bath at 8°C . When the process finished, the gel containing anthocyanins was cut and rinsed with deionized water. The anthocyanins were extracted using methanol. Methanol was removed from the extract by rotary evaporation, and then solutes were dissolved in water and adsorbed in preparative column (15 cm length and 1.2 cm diameter) packed with Fluka Analytical C-18 resin. Finally, the column was rinsed with acidified water (0.01M HCl) and anthocyanins were eluted with 50% methanol solution; the methanol was separated by distillation. Assays were performed three times.

2.4 Analytical methods

2.4.1. Total anthocyanin content (TAC)

TAC in samples was determined by pH-differential method (Moldovan *et al.*, 2012) using Cole Palmer S2100 UV spectrophotometer. The results were expressed as milligram of cyanidin-3-glycoside per milliliter (mg L^{-1}).

2.4.2. Total soluble solids (TSS)

TSS were determined drying 20 mL of homogeneous sample at $105 \pm 2^\circ\text{C}$ during 24 h. Dried matter was weighted in an analytical balance (Explore® OhausEX224) and results were expressed as g of sample per milliliter (mg mL^{-1}).

2.4.3. Total organic carbon (TOC)

TOC was determined in microfiltered samples (0.45 μm) using Shimadzu equipment (Model TOC-5000) expressed as mg of organic carbon per milliliter (mg mL^{-1}).

2.4.4. High performance liquid chromatography (HPLC)

A Perkin Elmer Series 200 HPLC equipped with UV-VIS detector was used. TotalChromTM Chromatography software was used to analyse the chromatograms. A symmetric, reverse phase column loaded with 5 μm C18 (4.6 x 250 mm, Nucleosil 100) was used. Phosphoric acid 1% / acetic acid 5% in water and acetonitrile 100% were used as solvents in phase A and B, respectively. The elution program consisted of two linear gradients: the first from 0 to 18% in phase B during 51 min and the second one from 30 to 80% in phase B during 10 min. A 5 μL sample was analysed at flow rate of 1 mL min^{-1} . The chromatograms were carried out at 520 nm. The anthocyanins patrons were pelargonidin 3-glucoside and cyanidin 3-glucoside.

3 Results and discussion

3.1 Effect of clarification on the adsorption

Microfiltrated extract (MFE) was directly purified by adsorption using a packed column of SP-207 resin, an incomplete desorption was observed during the elution. In subsequent process, the q_e and q_d showed lower values; impurities may have affected the desorption process. Corn kernels contain about 72 % starch and 9-10 % protein (Dickerson, 2003), these compounds may have interacted with the surface resin affecting the adsorption and desorption mechanisms. In order to avoid this phenomenon, an ultrafiltration process was proposed as a previous stage to adsorption procedure.

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In the following trials microfiltration and ultrafiltration were evaluated to determine their effect on q_e and q_d . The MFE and ultrafiltrated extracts (from 1 kDa and 5 kDa) were standardized to TAC of 15.7 mg L^{-1} .

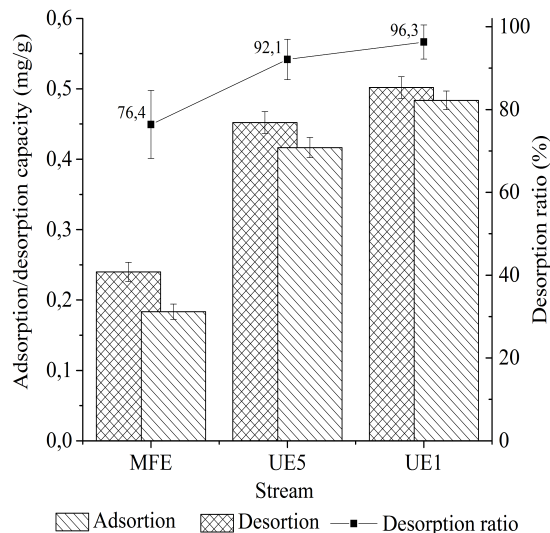


Fig. 2. Adsorption/desorption capacities and desorption ratio of anthocyanins.

Concerning to $\text{pH} \approx 2,0$ was not adjusted because at equilibrium there is no significant effect (Scordino *et al.*, 2004). The adsorption capacity values (q_e) were 0.23, 0.45 and 0.50 $\text{mg anthocyanins/gram resin}$ for ME, UFE5 and UFE1, respectively (see Figure 2). The highest value in q_e and ratio of desorption (D) were found in UFE1, the increasing on D can be related to removal of polymeric substances, resulting a final desorption rate increase to 96.3%.

3.2 Adsorption isotherms

To obtain the adsorption isotherms the initial anthocyanin concentration was standardized at 25 mg L^{-1} for MFE and UFE5, and 15.7 mg L^{-1} for UFE1. At equilibrium, the partition of anthocyanins between extract and adsorbent were correlated with Langmuir and Freundlich linearized equations as reported by Chandrasekhar *et al.* (2012). The results are shown in Table 1, the correlation coefficients were more suitable for Langmuir than Freundlich models.

3.3 Dynamic adsorption and desorption

The dynamic break-through curve on SP-207 resin was obtained to determine the processing volume at which the concentration of anthocyanins in the leaking solution from the adsorption column reaches 10% (breakthrough point), in a scale process at this point the column or series column must be replaced in order to avoid the losses, when the concentration of the leaking solution reaches 90%,

Table 1. Isotherms correlations for anthocyanins adsorption

Stream	Langmuir Equations	R^2	Freundlich Equations	R^2
MFE	$q_e = \frac{2.0131C_e}{0.00149+C_e}$	0.995	$q_e = 0.00222C_e^{1.4678}$	0.845
UFE5	$q_e = \frac{3.9072C_e}{0.00114+C_e}$	0.988	$q_e = 0.00014C_e^{3.0317}$	0.964
UFE1	$q_e = \frac{4.08605C_e}{0.00018+C_e}$	0.969	$q_e = 0.000059C_e^{0.56246}$	0.898

Table 2. Concentration values in the main process streams

Stream	Volume (mL)	TSS (mg mL ⁻¹)	TOC (mg mL ⁻¹)	TAC (mg L ⁻¹)
CE	10000 ± 15	36.646 ± 0.373		
PCE	8929.0 ± 15	29.590 ± 0.370	11.98 ± 0.353	37.85 ± 0.634
MFE	8518.0 ± 15	27.363 ± 0.440	11.18 ± 0.338	34.79 ± 0.700
UFE5	8077.0 ± 14	23.532 ± 0.440	9.53 ± 0.318	27.15 ± 0.480
UFE1	7702.0 ± 13	20.708 ± 0.452	8.71 ± 0.333	15.95 ± 0.349
EPA	482.00 ± 3	25.670 ± 0.573	14.22 ± 0.447	238.96 ± 11.11

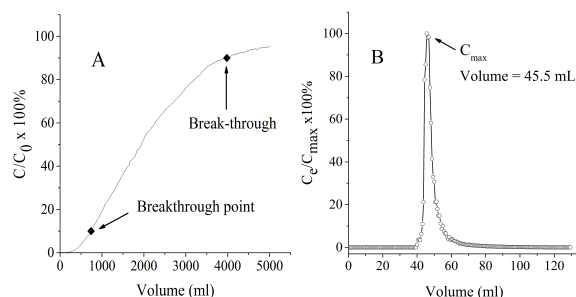


Fig. 3. Dynamic leakage (A) and desorption (B) curves for anthocyanins.

the column is considered saturated (adsorption break-through) and then the purified compounds could be washed and eluted. The optimum adsorption process conditions for the purification of ultrafiltered blue corn anthocyanins extract (at C_0 of 15.95 mg L⁻¹) were found at flow rate of 7 mL min⁻¹ and 4067 ± 133 mL total processed volume of sample (about 239.2 ± 7.8 effective column volume) as shown in Figure 3-A. The flow rate for desorption was at 2.5 mL min⁻¹ allowed the elution with 6.2 ± 0.2 volume column). Finally, Figure 3-B show the narrowest and highest pick in the desorption curve.

The scale up of the adsorption process implemented showed less losses under following conditions: 150 mm column length and 40 mm diameter, flow rate of 75 mL min⁻¹ and 7700 mL of processing volume at room temperature (20 ± 2°C).

3.4 Purification advancement to the adsorption process

Table 2 shows TSS, TOC and TAC values from pre-clarification to adsorption processes. Pre-clarification removed about 27.9 % of the TSS content from the initial extract which contained starch and pericarp particles while adsorption process removed around 40.1%. Basically, the retained compounds included low molecular weight solutes as flavonoids. The above-mentioned operations removed most of TSS in the global process.

According to Cassano *et al.* (2011) membrane filtration processes in tangential configuration does not only reduce the TSS content but also the TAC. After microfiltration, the turbidity of the extract decreased due to removal of suspended colloidal particles and other high molecular weight soluble solids, which cause turbidity. The decreasing in TSS content in feed extract to permeate samples was about 7.52 % while TOC and TAC were 6.67% and 8.08%, respectively. In addition, total rejections in total mass balance on TSS, TOC and TAC were 11.78%, 10.97% and 12%, respectively.

Although, the rejection on anthocyanins in MF is similar compared to Cissé *et al.* (2011) using ultrafiltration process, the phenomenon can be attributed as result of interaction of anthocyanins with bigger retained particles and also affected by TMP, such results reported a 97% of anthocyanins retention when using a 5kDa polyethersulfone ultrafiltration membrane at 3000 KPa. In this work, a TMP of 206.8 KPa was used; nevertheless, the total

anthocyanin retentions were 26.0% and 43.9 % for UFE5 and UFE1, respectively. High anthocyanin rejection could be attributed to the low TMP used and the characteristic of regenerated cellulose membrane material for recovering hydrophilic compounds. Moreover, the interactions between anthocyanins and macromolecules are evident under weak acid conditions (pH 4-6) where anthocyanins are in their colourless form (Castañeda-Ovando *et al.*, 2009; Pina *et al.*, 1999). The clarification was carried out at pH 2 where interactions were lower, resulting lower retention values.

The relation TOC/TAC indicates the milligrams of organic carbon per milligram of anthocyanins. After adsorption TOC/TAC ratio show there is 59.5 ± 0.53 mg of organic carbon per milligram of anthocyanins. This value indicates low purity in adsorption-purified anthocyanins respect to other organic substances.

3.5 Electrophoresis results

The flavylium cation structure show movement towards the negative pole though the agarose gel in the electrophoresis chamber, separating them from other species negatively charged or with no charge, contrary to what could be expected, according to the latest studies regarding pH relationship with colour and charge (Abd-Aal *et al.*, 2006; Moldovan *et al.*, 2012) buffer at pH=3 showed the best results because it allowed the jellification of agarose and anthocyanin movement at 220 volts, several trials were carried out, all samples started at an amperage around 229.9 ± 6.59 mA, the current intensity increased on function on time, and after 20 min was 345.6 ± 11.19 mA while temperature at the beginning was 8°C and at the end increased to $21.6 \pm 0.968^\circ\text{C}$.

The path made by the anthocyanin is shown in Figure 4, which was large due to high concentration, different anthocyanin structures (different mobility), diffusion and sample volume ($250\ \mu\text{L}$) run in each well. For anthocyanin from blue corn, the band length and path length of the coloured strip was 1.292 ± 0.114 cm and 2.958 ± 0.185 cm, respectively. Best purification was obtained by electrophoresis process, the TOC/TAC ratio decreased up to 3.966 ± 0.257 , while the TSS/TAC ratio was 6.217 ± 0.461 mg of total solids per milligram of anthocyanins. The results are promising respect to data reported by Leandro *et al.* (2013), they employed integrated process extraction-adsorption obtaining 51.5 in TSS/TAC ratio, whilst Chandrasekhar and Raghavarao (2015) evaluated ATFE-osmotic membrane distillation, ATFE-forward

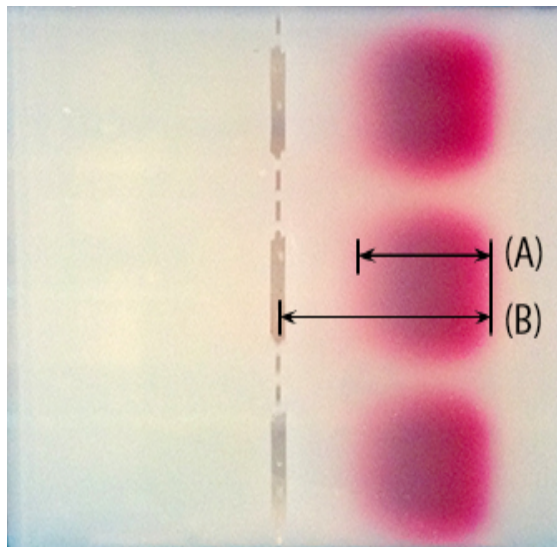


Fig. 4. Band length (A) and Path length (B) for electrophoresis.

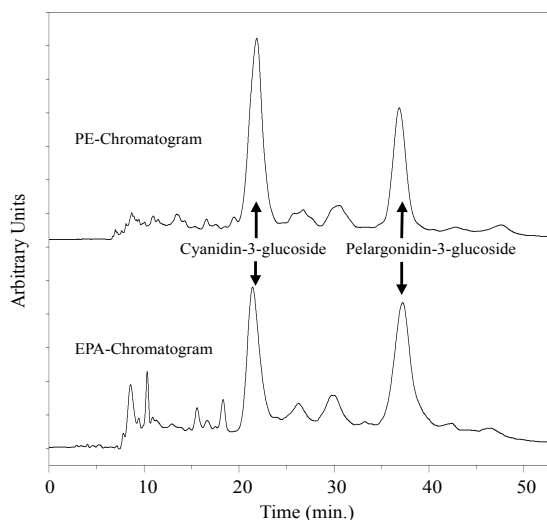


Fig. 5. HPLC Chromatogram of PE and EPA samples.

osmosis, ultrafiltration-forward osmosis and Thermal evaporation getting 275 ± 10 , 137 ± 4 , 169 ± 5 and 148 ± 3 TSS/TAC ratio respectively.

The same sequence was used for blue corn, red cabbage and blueberries. After electrophoresis, TOC/TAC ratios were decreased from 83.6 ± 0.87 to 3.40 ± 0.31 and 136.1 ± 2.1 to 8.86 ± 1.03 , respectively. These results show strong evidence that the methodology proposed is useful to purify anthocyanins in others sources.

3.6 Analysis of downstream processes by HPLC

Figure 5 shows chromatogram of EPA sample from the extract purified by adsorption; the retention times of cyanidin-3-glucoside and pelargonidin-3-glucoside were about 23 min and 38 min, respectively. Other anthocyanins were also detected with lower peaks, similar results were reported by Pascual-Teresa *et al.* (2002). All HPLC chromatograms from previous separations no present difference, i.e. the behaviour seems to be similar for EPA and CE. The HPLC analysis in PE (purified extract by electrophoresis) shows the prevalence of cyaniding-3-glucoside and pelargonidin-3-glucoside while initial peaks were decreased, this could be the result of removal, the frontal eluting sample in C-18 reverse phase chromatography to avoid gel carbohydrates contamination.

Conclusions

An integrated process was established in order to obtain an anthocyanin preliminary purified extract. The purification was highly dependent on previous clarification in order to remove interfering components such as starch, proteins and other chemical compounds from anthocyanins blue corn extract. The adsorption purification process was optimized by clarification at 1 KDa, resulting in better adsorption capacity (0.502 mg g^{-1}), desorption ratio (96.3%) and clean of the resin. Important purification progress was achieved by electrophoresis resulting in organic carbon decreasing per milligram of anthocyanins. The optimal conditions to obtain good electrophoresis mobility were 220 volts using 5% agarose gel in buffer solution at pH 3 and 8°C initial temperature. The results suggest that electrophoresis can be successfully used in anthocyanins preliminary purification from different source.

Nomenclature

TAC	total anthocyanin content, mg mL^{-1}
TOC	total organic carbon, mg mL^{-1}
TSS	total suspended solids, mg mL^{-1}
TMP	transmembrane pressure, KPa
Q_f	axial flow, L min^{-1}
P_{in}	inlet pressure, KPa

P_{out}	outlet pressure, KPa
R	rejection, %
TL	total percent losses, %
V_f	feed volume, mL
V_R	retentate volume, mL
C_p	concentration of a specific component in permeate, mg mL^{-1}
C_f	concentration of a specific component in feed, mg mL^{-1}
q_e	adsorption capacity, mg g^{-1}
q_d	desorption capacity, mg g^{-1}
D	desorption ratio, mg mg^{-1}
V_i	volume of initial sample, mL
V_d	volume of eluent, mL
C_0	initial concentration, mg L^{-1}
C_e	equilibrium concentration, mg L^{-1}
M	mass of adsorbent, g
q_m	Langmuir empirical constant
K_L	Langmuir adsorption equilibrium constant
K_F	Freundlich constant
$1/n$	empirical Freundlich constant

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