JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Adsorption/Desorption Characteristics and Separation of Anthocyanins from Muscadine (*Vitis rotundifolia*) Juice Pomace by Use of Macroporous Adsorbent Resins

Amandeep K. Sandhu and Liwei Gu*

Food Science and Human Nutrition Department, Institute of Food and Agriculture Sciences, University of Florida, Gainesville, Florida 32611-0370, United States

ABSTRACT: In this study, the adsorption/desorption characteristics of anthocyanins on five Amberlite resins (FPX-66, XAD-7HP, XAD-16N, XAD-1180, and XAD-761) were evaluated. FPX-66 and XAD-16N showed the highest adsorption and desorption capacities and ratios for anthocyanins from muscadine pomace extract, while XAD-7HP had the lowest adsorption and desorption capacities and ratios. On the basis of static adsorption and desorption tests, three resins (FPX-66, XAD-16N, and XAD-1180) were selected for adsorption kinetics and isotherms. The adsorption mechanism was better explained by the pseudo-first-order kinetics for FPX-66 and XAD-16N; however, for XAD-1180, pseudo-second-order kinetics was the most suitable model. The experimental data fitted best to Langmuir isotherm model for all three resins. Dynamic testing was done on a column packed with FPX-66 resin and breakthrough volume was reached at 17 bed volumes of muscadine pomace water extract during adsorption. Three bed volumes of aqueous ethanol (70%) resulted in complete desorption. Resin adsorption resulted in a concentrated pomace extract that contained 13% (w/w) anthocyanins with no detectable sugars.

KEYWORDS: *muscadine, pomace, anthocyanins, resin, adsorption, desorption*

INTRODUCTION

Muscadine grapes (*Vitis rotundifolia*) are native to the southeastern United States. They contain a wide variety of antioxidants and possess a unique phytochemical profile. They are reported to contain anthocyanins and other polyphenols including ellagic acid, quercetin, myricetin, and kaempferol.[1−3] Cell culture studies have suggested that polyphenols from muscadine grapes can inhibit proliferation of colon cancer cells and induce apoptosis.[4,5] The anthocyanins from muscadine grapes are also reported to be potent inhibitors of α-glucosidase and pancreatic lipase, the two major enzymes involved in diabetes.[6]

During juice processing or winemaking, 40% of the fruit is lost as pomace, which consists of seeds, skin, pulp, and residual solids. Phenolic compounds in muscadine grapes are concentrated in seeds and skin;[7] thus, even after their extraction in juice or wine, considerable quantities are still left in pomace. Therefore, muscadine pomace is an attractive source of bioactive phytochemicals including anthocyanins, ellagic acid, and flavonols.[8] These bioactive compounds, also known as nutraceuticals, possess high antioxidant capacity and may provide protective effects against chronic diseases such as cancer, diabetes, and cardiovascular diseases.[9,10] There is an increasing demand by the grape industry for the extraction of these phenolic compounds from the pomace and utilization for the production of functional foods. Muscadine pomace is currently utilized as compost or animal feed. Utilization of pomace to extract the phenolic compounds will increase the economic value of muscadine grapes and wines.

Several extraction and separation methods are used for the enrichment of phenolic compounds from plant-based materials, such as liquid−liquid extraction, membrane filtration, ion exchange, and chromatography. However, these methods have several disadvantages; for example, they are time-consuming, laborious, expensive with poor recovery, and not suitable for large-scale industrial production. Alternatively, great progress has been made in recent years to separate these compounds from the plant materials with macroporous resins. The resins are polar or nonpolar polymers having characteristics of good selectivity, different surface properties, high mechanical strength, and fast adsorption speed.[11] The advantages of using macroporous resins outweigh other methods of separation and enrichment as they are relatively low cost, easy to use and regenerate, have high efficiency, and are suitable for industrial scale-up. Moreover, some of these resins meet food-grade standards of the U.S. Food and Drug Administration; therefore, the extracted compounds can be used as food ingredients or dietary supplements. Adsorption by macroporous resins has been successfully applied for the recovery of various phenolic compounds such as hesperidin from citrus peels during citrus processing,[12] flavonoids from mulberry leaves,[13] lycopene from tomato skins,[14] vitexin and isovitexin from pigeon pea,[15] and polyphenols from apple juice.[16−18] No studies have been done to investigate the use of resins for the separation and enrichment of phenolic compounds from muscadine pomace.

The objectives of this study were to investigate the adsorption/desorption behaviors of anthocyanins from muscadine juice pomace extracts on different Amberlite resins and to optimize the conditions for selection of one resin to conduct dynamic adsorption and desorption studies.

MATERIALS AND METHODS

Chemicals and Reagents. Gallic acid, HPLC-grade ethanol, acetonitrile, methanol, formic acid, hydrochloric acid, Folin−Ciocalteau

Received: August 20, 2012
Revised: January 29, 2013
Accepted: January 31, 2013
Published: January 31, 2013

reagent, sodium hydroxide, sodium carbonate, sucrose, glucose, and fructose were purchased from Fischer Scientific Co. (Pittsburgh, PA). Standards of the 3-*O*-*β*-glucosides of pelargonidin, cyanidin, peonidin, delphinidin, petunidin, and malvidin (six mixed anthocyanin standard, HPLC-grade), were purchased from Polyphenols Laboratories (Sandnes, Norway). Ellagic acid, myricetin, quercetin, kaempferol, cyanidin 3-rutinoside, (+)-catechin, and (−)-epicatechin were obtained from Sigma−Aldrich (St. Louis, MO). Resins FPX-66, XAD-16N, XAD-1180, XAD-7HP, and XAD-761 were products of Rohm and Haas Co. (Philadelphia, PA).

Muscadine Juice Pomace Preparation. Muscadines of Noble cultivar were purchased from a local vineyard in central Florida. Muscadine juice was extracted by a hot-pressing technique in which crushed Noble grapes were heated for 30 min at 60 °C prior to pressing. Pomace was ground to a fine paste in a mill (Robot Coupe USA, Inc. Jackson, MS), packaged into plastic bags, and kept in the freezer (−20 °C) until used for extraction.

Preparation of Juice Pomace Water Extracts. Juice pomace was extracted twice with acidified hot water (1% formic acid) at 90 °C. For the first extraction, pomace (200 g) was mixed with 500 mL of acidified hot water (1% formic acid) at 90 °C, sonicated for 15 min, and kept in the dark at room temperature for 1 h. The samples were sonicated again for 15 min before filtration through muslin cloth. The residue from the muslin cloth was extracted with 400 mL of acidified hot water (1% formic acid) by the same procedure. The filtered extracts from the two extractions were pooled and transferred into a 1-L volumetric flask. Acidified water (1% formic acid) was added to make up the final volume to 1 L. The final extract was filtered through Whatmann no. 4 filter paper.

Characterization and Phytochemical Analysis of Extracts. The extracts were diluted to appropriate concentration for analysis. Total anthocyanin content in pomace extracts and concentrated extracts obtained after resin adsorption/desorption processes was measured by the pH differential spectrophotometric method described by Giusti and Wrolstad.[19] The extracts were dissolved in 0.025 mol/L potassium chloride buffer, pH 1.0, and 0.4 mol/L sodium acetate buffer, pH 4.5, with predetermined dilution factor. Absorbance at 520 and 700 nm was measured on a DU 730 Life Science UV/vis spectrophotometer (Beckman Coulter, Fullerton, CA) after 30 min of incubation at room temperature. The absorbance (A) of the diluted sample was then calculated as $(A_{520} - A_{700})_{pH1.0} - (A_{520} - A_{700})_{pH4.5}$. The monomeric anthocyanin concentration in the original sample was calculated in cyanidin 3,5-diglucoside equivalents according to this formula: $A(MW)(DF)1000/(\varepsilon \times 1)$, where the molecular weight (MW, 611) of cyanidin 3,5-diglucoside wass used, the molar absorptivity ε was 30 175, DF is the dilution factor, 1000 is the factor to convert grams to milligrams, and A is absorbance. Results for total anthocyanin content were expressed as milligrams of cyanidin 3,5-diglucoside equivalent per gram of fresh pomace (mg of cyanidin 3,5-diglucoside/g). The total phenolic content was determined as reported previously.[7] The results were expressed as milligrams of gallic acid equivalents per gram of fresh pomace (mg of GAE/g).

HPLC analysis of anthocyanins, other phenolic phytochemicals, and sugars was performed on an Agilent 1200 HPLC system consisting of an autosampler, a binary pump, a column compartment, a diode array detector, a fluorescent detector, and a refractive index detector (Agilent Technologies, Palo Alto, CA). Reversed-phase chromatography was used for the quantification of anthocyanins, ellagic acid, flavonols, catechin, and epicatechin. An Agilent Zorbax Stablebond SB-C18 column (250 mm × 4.6 mm, 5 μm particle size; Agilent Technologies, Palo Alto, CA) was used for the separation of phenolic compounds. For anthocyanin analysis, the extracts (1 mL) were filtered through 0.45 μM filter units and 5 μL was injected directly without any purification. Elution was performed with mobile phase A (5% formic acid aqueous solution) and mobile phase B (methanol). The flow rate was 1 mL/min with a 50 min gradient as follows: 0−2 min, 5% B; 2−10 min, 5−15% B; 10−25 min, 15−25% B; 25−30 min, 25−30% B; 30−45 min, 30% B; 45−47 min, 30−70% B; and 47−50 min, 70−5% B; followed by 5 min of re-equilibration of the column before the next run. UV−vis spectra were scanned from 220 to 600 nm on a diode array detector, and the detection wavelength for the anthocyanins was 520 nm. The individual anthocyanins were quantified by use of standards. The analysis of ellagic acid, flavonols, catechin, and epicatechin was done by HPLC after acid hydrolysis of the samples. The solids were dissolved in 5 mL of methanol (50%) containing 1.2 N HCl and sonicated for 5 min. Hydrolysis was conducted in a precision water bath (Thermo Scientific, Waltham, MA) for 80 min at 90 °C and aglycones were separated and quantified by HPLC. The binary mobile phase consisted of (A) 0.5% aqueous formic acid solution and (B) acetonitrile. The flow rate was 1 mL/min with a 25 min gradient as follows: 0−5 min, 10−30% B; 5−10 min, 30−40% B; 10−20 min, 40−50% B; 20−23 min, 50−70% B; and 23−25 min, 70−10% B; followed by 5 min of re-equilibration of the column before the next run. The column temperature was maintained at 30 °C. The detection wavelengths for ellagic acid and flavonols were 260 and 360 nm, respectively. Catechin and epicatechin were quantified by fluorescent detection. Excitation and emission of the fluorescent detector were set at 230 and 321 nm, respectively. Sugar analysis was conducted on a Restek ultra amino column (5 μm, 250 × 4.6 mm). Acetonitrile/water (65:35 v/v) was used as the mobile phase at a constant flow rate of 1.0 mL/min. The column temperature was maintained at 30 °C, and a 5 μL of sample was injected. The optical unit temperature was set at 35 °C, and the refractive index detector signal was monitored in positive polarity. The run time for each sample was 15 min, followed by 5 min post time before the next run. Calibration curves were constructed by use of pure standards of glucose and fructose.

Pretreatment of Macroporous Resins. The physical characteristics of five different resins used in this study are summarized in Table 1.

Table 1. Physical Characteristics of Adsorbent Resins

resin	chemical nature[a]	polarity	surface area (m^2/g)	avg pore diameter (Å)
XAD-7HP	aliphatic ester	polar	500	450
XAD-761	phenol−formaldehyde	polar	200	600
XAD-16N	polystyrene−DVB	nonpolar	800	150
XAD-1180	polystyrene−DVB	nonpolar	700	400
FPX-66	polystyrene−DVB	nonpolar	700	200−250

[a]DVB = divinylbenzene.

Resins were soaked in ethanol overnight and then treated with 2 bed volumes (BV) of 4% HCl and 5% NaOH solutions to remove salts and other impurities trapped inside the pores due to synthesis process. After acid and base wash, resins were neutralized with distilled water. To determine the moisture content of resins, three samples of each kind of pretreated resin were weighed and dried at 60 °C for 24 h.

Static Adsorption and Desorption Tests for Screening of Resins. The static adsorption and desorption experiments were performed as follows: 1 g of resin (wet weight) was introduced into a 125 mL Erlenmeyer flask. Then 50 mL of juice pomace water extract was added to each flask. A control sample was employed to monitor any change in the initial concentration values, to exclude effects on measured absorbance. The flasks were then shaken (120 rpm) for 24 h at room temperature (25 °C) in a shaking water bath. After adsorption, resins were filtered and washed with 50 mL of distilled water. For desorption, 70% and 95% acidified (with 1% formic acid) ethanol solutions were tested. Fifty milliliters of ethanol−water solution was added to the flasks containing the adsorbate-laden resins. The flasks were shaken (120 rpm) for 24 h at room temperature (25 °C) in a shaking water bath. The content of anthocyanins in the liquid phase after adsorption and desorption was analyzed for total anthocyanins by the pH differential method. Candidate resins were selected in terms of their adsorption capacities, adsorption and desorption ratios, and recovery (percent).The following equations were used to quantify the capacities of adsorption and desorption as well as their ratios.

Adsorption evaluation

$$Q_e = \frac{(C_0 - C_e)V_0}{m} \quad (1)$$

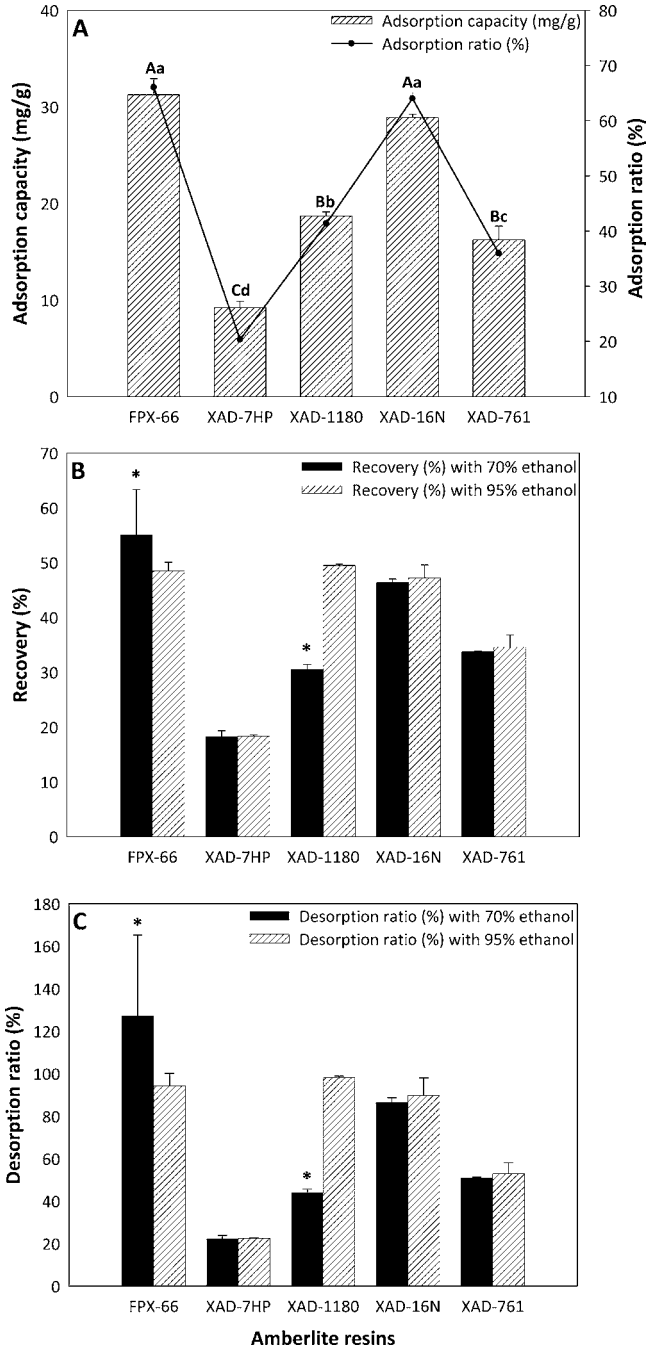

Figure 1. Static adsorption results based on total anthocyanin content on different resins: (A) Adsorption capacity and ratio. Different uppercase letters indicate significant differences between bars ($p \leq 0.05$). Different lowercase letters indicate significant differences between lines ($p \leq 0.05$). (B) Recovery rate (percent) with 70% and 95% ethanol. Asterisks indicate significant differences ($p \leq 0.05$) in recovery. (C) Desorption ratio (percent) with 70% and 95% ethanol. Asterisks indicate significant differences ($p \leq 0.05$) in the desorption ratio. Results are the mean of three determinations.

$$E = \frac{(C_0 - C_e)}{C_0} \times 100 \quad (2)$$

where Q_e is the adsorption capacity at adsorption equilibrium (milligrams per gram of resin); E is the adsorption ratio (percent); C_0 and C_e are initial and equilibrium concentrations (milligrams per liter) of solute in the solution, respectively; V_0 is the volume of initial sample solution (milliliters); and m is the weight of dry resin (grams).

Desorption evaluation

$$D = \frac{(C_d V_d)}{(C_0 - C_e)V_0} \times 100 \quad (3)$$

$$R = \frac{C_d V_d}{C_0 V_0} \times 100 \quad (4)$$

where D is the desorption ratio (percent), C_d is the concentration of solute in desorption solution (milligrams per liter), V_d is the volume of desorption solution (milliliters), and R is the recovery (percent). C_0, C_e, and V_0 are the same as described above.

Adsorption Kinetics. The adsorption kinetics was studied on preliminary selected resins. The test for adsorption kinetics on the selected resins was conducted by adding 50 mL of water extracts of juice pomace with 1 g (wet weight) of resin and then shaking (120 rpm) for 8 h at room temperature (25 °C). Aliquots (1 mL) of sample solution were taken at equal time intervals until equilibrium was reached, and they were analyzed for total anthocyanins. Two mathematical models were applied to simulate the uptake of phenolic compounds on the selected resins with time, that is, pseudo-first-order (eq 5)[20] and pseudo-second-order kinetic model (eq 6).[21]

$$\ln(Q_e - Q_t) = \ln Q_e - k_a t \quad (5)$$

$$\frac{t}{Q_t} = \frac{1}{k_b Q_e^2} + \frac{1}{Q_e} t \quad (6)$$

where Q_e and Q_t (milligrams per gram) are the amount of phenolic compounds adsorbed per gram of resin at equilibrium and at time t (minutes), respectively; k_a (per minute) is the equilibrium rate constant; and k_b (grams per milligram per minute) is the second-order model rate constant.

Adsorption Isotherms. The tests for adsorption isotherms on the selected resins were conducted at room temperature (25 °C) and at 30 and 35 °C. Briefly, 50 mL of water extracts of juice pomace at different initial concentrations were mixed with 1 g (wet weight) of resin, and then the samples were shaken (120 rpm) for 8 h at selected temperatures. At equilibrium, samples were analyzed for total anthocyanins. Two standard theoretical models, Langmuir and Freundlich models, are used to describe the adsorption behavior between adsorbate and adsorbent.[22]

Langmuir model

$$\frac{C_e}{Q_e} = \frac{1}{K_L Q_m} + \frac{C_e}{Q_m} t \quad (7)$$

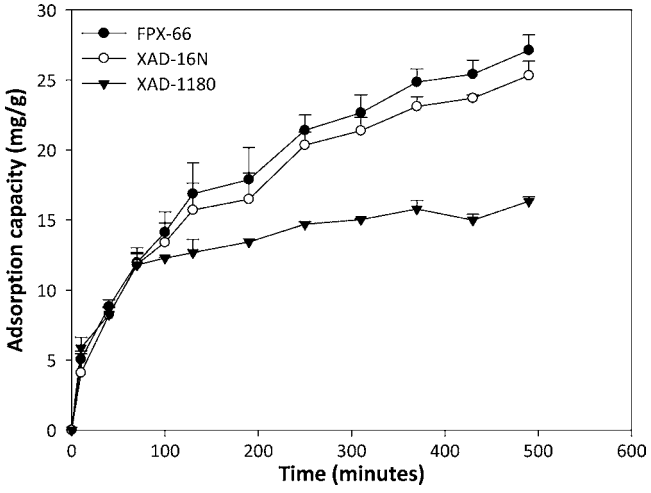

Figure 2. Adsorption kinetic curves for total anthocyanin content on FPX-66, XAD-16N, and XAD-1180. Results are the mean of three determinations.

dx.doi.org/10.1021/jf3036148 | J. Agric. Food Chem. 2013, 61, 1441−1448

Table 2. Kinetic Parameters of Muscadine Juice Pomace Adsorption on Resins[a]

		pseudo-first-order			pseudo-second-order		
resins	Q_e exp (mg/g)	Q_e calc (mg/g)	K_a (min^{-1})	R^2	Q_e calc (mg/g)	K_b (g/mg min)	R^2
FPX-66	27.12	25.27	0.0063	0.9883	31.55	2.96×10^{-4}	0.9784
XAD-16N	25.32	23.23	0.0062	0.9907	29.50	4.06×10^{-4}	0.9819
XAD-1180[b]	16.32	7.57	0.0051	0.8387	16.86	1.64×10^{-3}	0.9920

[a]Adsorption is at room temperature (25 °C) and is based on total anthocyanins. exp, experimental; calc, calculated. Data are based on three replicates except as noted. [b]Based on two determinations.

Freundlich model

$$\ln Q_e = \ln K_F + \frac{1}{n}\ln C_e \quad (8)$$

where Q_m (milligrams per gram) is the maximum amount of adsorption and K_L is the affinity constant in the Langmuir model, which can be calculated from the slope and intercept of the linear plot of C_e/Q_e versus C_e, respectively. K_F and $1/n$ are the constants as measures of adsorption capacity and adsorption intensity in the Freundlich model and can be determined from the intercept and slope of linear plot of ln Q_e versus ln C_e, respectively. The essential characteristics of the Langmuir isotherm can be expressed by means of R_L, a dimensionless constant called the separation factor or equilibrium parameter. R_L can be calculated from the following equation:

$$\frac{1}{1 + K_L C_o} \quad (9)$$

R_L values indicate the type of isotherm: irreversible ($R_L = 0$), favorable ($0 < R_L < 1$), linear ($R_L = 1$), or unfavorable ($R_L > 1$).[23]

Dynamic Adsorption and Desorption Tests. Dynamic adsorption and desorption tests were carried out on a glass column (22 mm × 350 mm) packed with 28 g (wet weight) of the selected FPX-66 resin. The bed volume (BV) of resin was 30 mL and the packed length of resin bed was 19 cm. The adsorption process was performed by loading juice pomace water extract onto the pretreated resin filled in a glass column. Subsequently, the adsorbate-laden column was washed first with 4 BV of distilled water and then desorbed with 70% acidified (with 1% formic acid) ethanol solution. The effect of sample flow rate and ethanol solution flow rate on adsorption and desorption phenomena were studied.

Statistical Analyses. All data are expressed as mean ± standard deviation. Two-way analyses of variance (2-way ANOVA) were performed for static desorption tests by use of Sigma Stat (version 11.0, Systat Software Inc., Chicago, IL). One-way analyses of variance (ANOVA) with Tukey−HSD pairwise comparison of the means were performed by use of JMP software (version 8.0, SAS Institute Inc., Cary, NC) for other results. The mean values were considered significantly different at $p \leq 0.05$.

RESULTS AND DISCUSSION

Screening of Resins. Resins differ in chemical structure, polarity, particle size, porosity, and surface area. The adsorption of a solute on an adsorbent is a complex process that involves the interactions among three components, the adsorbate (the solute), the adsorbent, and the solvent, involving a physical action through van der Waals force or hydrogen bonding. The adsorption and desorption properties of anthocyanins on different resins were tested in juice pomace water extracts.

Adsorption capacity describes the amount of anthocyanins adsorbed on 1 g of resin. Adsorption ratio is the percentage of anthocyanins adsorbed by resins from the aqueous extract. Adsorption capacity was highest for FPX-66 and XAD-16N, followed by XAD-1180 and XAD-761. Similarly, the highest adsorption ratio was observed for FPX-66 and XAD-16N, followed by XAD-1180. However, XAD-7HP showed the lowest adsorption capacity and ratio (Figure 1A). Recovery rate is the percentage of anthocyanins recovered from the resins by the desorbing solvent based on the initial concentration of the extract. Desorption ratio is the percentage of anthocyanins desorbed from the resins by the desorbing solvent. The recovery was highest in FPX-66 and XAD-16N, followed by XAD-1180 and XAD-761, based on total anthocyanin content (Figure 1B). However, recovery of anthocyanins with 95% ethanol was similar for FPX-66, XAD-1180, and XAD-16N. The lowest recovery was seen in XAD-7HP with both 70% and 95% ethanol. Significant differences were observed among resins in desorption ratios with 70% ethanol. The highest desorption ratio was observed in FPX-66, followed by XAD-16N, with 70% ethanol as desorption solvent (Figure 1C). In contrast, the desorption ratio was similar for FPX-66, XAD-1180, and XAD-16N with 95% ethanol. Similar to anthocyanin recovery, XAD-7HP showed the lowest desorption ratios with 70% and 95% ethanol. There was a statistically significant interaction between resins and perrcentage of ethanol ($p < 0.001$).

The adsorption and desorption of anthocyanins was highest on nonpolar resins (FPX-66, XAD-16N, and XAD-1180). The pore size of the porous adsorbents and the size of adsorbate molecules play an important role in the process of adsorption. If the pore diameter is too small, it can restrict the diffusion of adsorbate molecules. On the other hand, if the pore diameter is too large, the molecules adsorbed will be prone to desorption at the same time.[24] In addition, the affinity between different polyphenols for the adsorption sites may vary, with some phenolics being preferentially selected over others. Anthocyanins are small molecules compared to other phenolic compounds present in pomace. They contain both polar hydroxy groups and nonpolar phenylallyl groups, which could explain the differences in adsorption behaviors of resins with different polarities. Except for XAD-7HP, the amount of anthocyanins adsorbed seems to be proportional to the adsorbent surface area.[12,25] Resins showing high adsorption capacity had lower pore diameters, which could be another factor in their better adsorption of anthocyanins. Desorption of anthocyanins was almost complete for FPX-66, XAD-16N, and XAD-1180 resins. However, the desorption ratio was very low for polar resins, such as XAD-761 and XAD-7HP, which might be due to strong interaction between polar hydroxy groups of anthocyanins in the solute with the adsorbent material. Large surface area and ideal pore diameter for anthocyanins could be a possible explanation for better adsorption and desorption characteristics of FPX-66, XAD-16N, and XAD-1180 resins.

On the basis of the initial screening, three resins (FPX-66, XAD-16N, and XAD-1180) were selected for further testing of adsorption kinetics and thermodynamics.

Adsorption Kinetics. The results of the adsorption kinetics of anthocyanins on the three selected resins are shown in Figure 2. Adsorption of phenolic compounds from the aqueous solution on all three resins increased quickly in the first 90 min and then increased slowly until adsorption equilibrium was reached at 490 min. Different kinetic models are used to

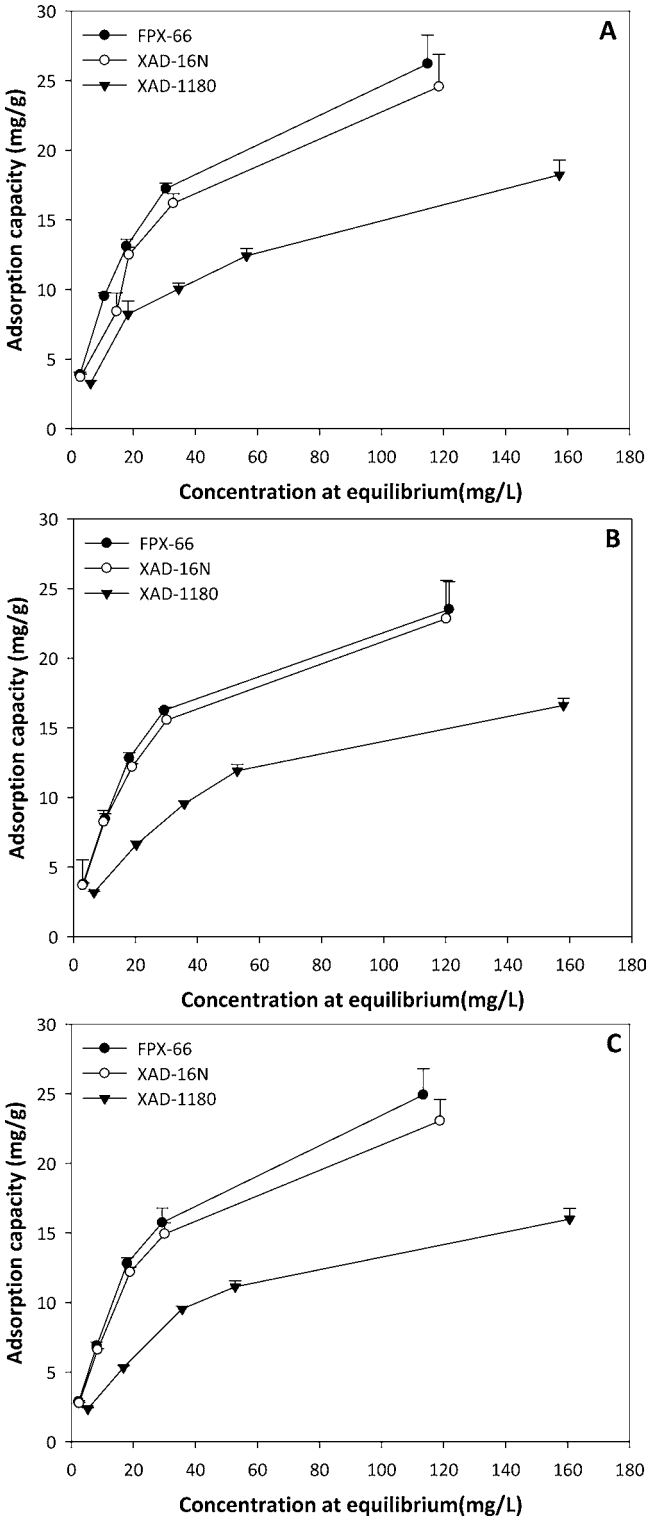

Figure 3. Adsorption isotherms for total anthocyanin content on FPX-66, XAD-16N, and XAD-1180 at (A) room temperature (25 °C), (B) 30 °C, and (C) 35 °C. Results are the mean of three determinations.

determine the rate of adsorption processes and mechanism of adsorption. The commonly used kinetic models are pseudo-first-order and pseudo-second-order models.[26] The pseudo-first-order model is generally applicable over the initial stage of an adsorption process, while the pseudo-second-order model assumes that the rate-limiting step is chemisorption and predicts the behavior over the whole range of adsorption.[27]

Table 3. Langmuir and Freundlich Parameters for Adsorption of Muscadine Juice Pomace on Resins[a]

		Langmuir equation			Freundlich equation		
temp (°C)	Q_m (mg/g)	K_L (L/mg)	R_L	R^2	n	K_F (L/mg)	R^2
			FPX-66				
25	31.35	0.043	0.09	0.9974	1.94	2.65	0.9654
30	27.70	0.047	0.08	0.9994	1.97	2.49	0.9358
35	30.49	0.039	0.10	0.9979	1.76	2.04	0.9601
			XAD-16N				
25	30.22	0.035	0.10	0.9831	1.90	2.27	0.9627
30	26.81	0.047	0.08	0.9989	2.02	2.51	0.9543
35	27.86	0.040	0.09	0.9987	1.79	1.95	0.9555
			XAD-1180				
25	22.33	0.026	0.13	0.9915	1.94	1.52	0.9478
30	20.84	0.025	0.14	0.9981	1.88	1.29	0.9669
35	20.24	0.023	0.15	0.9987	1.74	1.03	0.9609

[a]Adsorption is based on total anthocyanins. Data are based on three replicates.

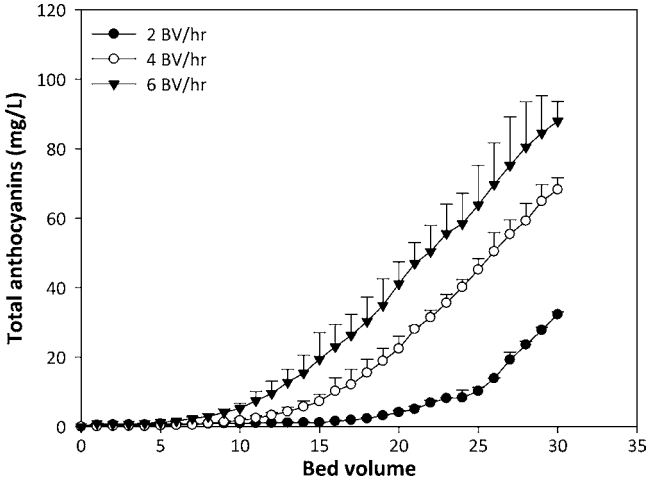

Figure 4. Dynamic breakthrough curves of total anthocyanins from muscadine juice pomace on column packed with FPX-66 resin at different flow rates. Results are the mean of two determinations.

The experimental data were fitted to these two models in order to determine which model best described the adsorption rate of phenolic compounds. A summary of the results is reported in Table 2. In the case of FPX-66 and XAD-16N resins, the higher correlation coefficients and agreement between experimental and calculated Q_e values indicate that the pseudo-first-order model gives a more favorable fit for the adsorption of total anthocyanins from juice pomace extracts. However, for XAD-1180, the pseudo-second-order model seems to be a better fit. Similar findings were reported by other studies.[25,28]

Adsorption Isotherms. The equilibrium adsorption isotherms of anthocyanins on three selected resins were investigated at three temperatures [room temperature (25 °C) and 30 and 35 °C] as shown in Figure 3 . The Langmuir and Freundlich isotherms are two of the most commonly used models for describing adsorption isotherms. The Langmuir model describes a monolayer adsorption with energetically identical sorption sites and without mutual interactions between the adsorbed molecules. The Freundlich model assumes adsorption to heterogeneous surfaces, which is characterized by sorption sites at different energies. This model can be used to describe the adsorption behavior of a monomolecular

dx.doi.org/10.1021/jf3036148 | J. Agric. Food Chem. 2013, 61, 1441−1448

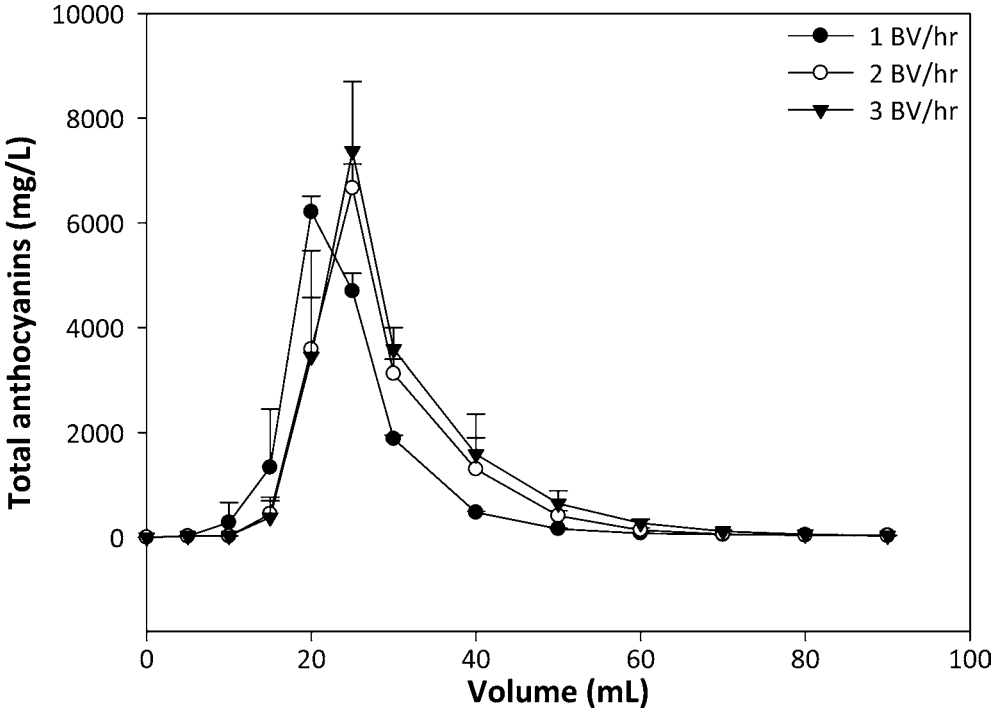

Figure 5. Dynamic desorption curves of total anthocyanins from muscadine juice pomace on column packed with FPX-66 resin at different flow rates. Results are the mean of two determinations.

layer as well as that of a multimolecular layer.[27] Correlation coefficients for each model on three selected resins at different temperatures are listed in Table 3. For all three resins, the Langmuir model was considered as a better model for describing adsorption equilibrium due to higher correlation coefficients (R^2 = 0.9831−0.9994). The values of Q_m (maximum adsorption capacity) increased in the order FPX-66 > XAD-16N > XAD-1180. Our results show that adsorption of anthocyanins on the selected resins is favorable and has R_L values between 0 and 1 (Table 3). The Freundlich isotherm constant n represents the strength of the adsorption process, and its value should be greater than 1 and less than 10 for favorable adsorption conditions.[27] The n values obtained from Freundlich plots were greater than 1 for all resins, indicating favorable adsorption conditions.

Dynamic Breakthrough Curve on FPX-66 Resin. In order to optimize the process of dynamic adsorption and desorption, important factors such as feed volume, flow rate of feed and eluent, and volume of eluent were taken into consideration and tested. The process of adsorption involves diffusion of adsorbate and interaction between the adsorbate molecules and the resin, including hydrogen bonding, simple stacking, or hydrophobic interactions. Resins can easily adsorb the molecules due to their large surface area and highly porous structure. When adsorption reaches the breakthrough point (the point of maximum saturation, when the resin cannot hold the adsorbate molecules), the adsorption effect decreases and solute starts to leak from the resin. Thus, it is important to define the breakthrough point in order to calculate the resin quantity, processing volume of sample, and proper sample flow rate. The best adsorption performance was observed at the slowest flow rate (2 BV/h) (Figure 4). The low flow rate allows more time for the adsorbate molecules to interact with the active sites of the resin at the expense of longer processing time. In contrast, a faster flow rate requires less time but has a negative impact on the adsorption capacity since the breakthrough point is reached more quickly.[25] The 5% ratio of exit to the inlet solute was defined as the breakthrough point in this study. On the basis of the results and taking into consideration the processing time, a flow rate of 4BV/h was selected as the optimum for adsorption, with approximately 17 BV processing volume of the sample solution.

Dynamic Desorption Curve on FPX-66 Resin. These curves were based on the volume of desorption solvent used and the concentration of anthocyanins in the desorption solvent. A 70% acidified (with 1% formic acid) ethanol solution was used as desorption solvent. It is important to select the proper flow rate for better desorption performance. As can be seen from Figure 5, the best desorption performance was observed at the slowest flow rate of 1 BV/h. There was no difference in the dynamic desorption at flow rates of 2 and 3 BV/h. Therefore, 2 BV/h was chosen as the proper flow rate for dynamic desorption on account of short working time. Approximately 3 BV of desorption solution was used to completely desorb the anthocyanins from the resin.

Under the above optimized conditions for adsorption and desorption, the 70% ethanol eluent was collected and concentrated to dryness in a Speed Vac concentrator (Thermo Scientific ISS110, Waltham, MA) under reduced pressure at 25 °C to remove the solvent. The yield of concentrated powder was 5.7 mg/g of fresh juice pomace. The percent recovery of total anthocyanins was 73%, which was comparable with those in some previous studies.[14,29] Choice of organic solvent and poor stability of anthocyanins may also adversely affect the recovery.[30]

Characterization of Concentrated Extracts from Muscadine Juice Pomace. The content of individual phytochemicals in the concentrated extracts obtained after the resin adsorption and desorption process is shown in Table 4. The values were compared with those in the initial water extract of muscadine juice pomace. The extracts were highly concentrated solutions of anthocyanins, ellagic acid, flavonols, catechin, and (epi)catechin without sugars.[31] Resin adsorption increased the content of peonidin 3,5-diglucoside by 38 times. Similarly, total anthocyanins

Table 4. Comparison of Phytochemical and Sugar Content of Muscadine Juice Pomace Water Extract with Concentrated Extract

compound	juice pomace water extract (mg/g dry weight)	concentrated extract (mg/g dry extract)	enrichment factor
	Anthocyanins		
delphinidin 3,5-diglucoside	1.69 ± 0.01	48.86 ± 0.98	28.9
cyanidin 3,5-diglucoside	0.44 ± 0.00	12.77 ± 0.23	29.1
petunidin/pelargonidin 3,5-diglucoside[a]	0.87 ± 0.01	29.54 ± 0.66	34.1
peonidin 3,5-diglucoside	0.87 ± 0.00	33.22 ± 0.60	38.2
malvidin 3,5-diglucoside	0.53 ± 0.00	18.16 ± 0.45	34.1
total anthocyanins[b]	3.41 ± 0.20	130.04 ± 2.73	38.1
	Other Phenolic Compounds		
ellagic acid	7.23 ± 0.49	42.86 ± 2.19	5.9
myricetin	1.24 ± 0.35	7.53 ± 0.24	6.1
quercetin	0.60 ± 0.10	2.79 ± 0.05	4.7
kaempferol	0.30 ± 0.04	1.33 ± 0.00	4.4
catechin	ND	17.32 ± 0.24	NA
epicatechin	ND	10.56 ± 0.29	NA
total phenolic content (mg of GAE/g)	16.61 ± 0.43	691.75 ± 65.6	41.6
	Sugars		
fructose	165.66 ± 4.06	ND	NA
glucose	137.44 ± 4.74	ND	NA

[a]Peaks coeluted on HPLC. Data are mean ± standard deviation for triplicate tests. [b]Determined by pH differential method. ND, not detected. NA, not applicable.

and total phenolics were 38 and 42 times higher in the concentrated extract compared to the water extract. A study on muscadine pomace showed an increase in total phenolics and anthocyanins by 25 times after the resin adsorption/desorption process.[32] Similarly, anthocyanins were increased 7−20 times from a byproduct of blood orange juice processing.[31] The adsorption of phenolic phytochemicals on resin was not completely selective for anthocyanins. In addition to anthocyanins, the content of ellagic acid and total flavonols (myricetin, quercetin, and kaempferol) in the concentrated extract also increased by 6 and 5 times, respectively. Catechin and epicatechin were detected in the concentrated extract only but not in the initial water extract. Muscadine pomace water extract contained significant amount of glucose and fructose (Table 4). These sugars were completely removed by water wash during the resin adsorption process.

In summary, our results indicate that FPX-66 is the most suitable resin among the selected commercial adsorbents for recovery of anthocyanins from muscadine juice pomace. This is due to its high adsorption and desorption capacity and greater affinity toward these phenolic compounds with respect to the other tested resins under the same conditions. The optimization of resin adsorption process in the present study sets parameters for the development of pilot-scale separation and concentration of anthocyanins from muscadine pomace. This extract could potentially find application as a natural colorant, dietary supplement, antioxidant ingredient for functional foods, and/or raw material in cosmetic and pharmaceutical industry preparations.

AUTHOR INFORMATION

Corresponding Author

*Phone (352) 392-1991, ext 210; fax (352)392-9467; e-mail LGu@ufl.edu.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This research is supported by a grant from the Florida Department of Agriculture and Consumer Service, Viticulture Research Program.

REFERENCES

(1) Ector, B. J.; Magee, J. B.; Hegwood, C. B.; Coign, M. J. Resveratrol concentration in muscadine berries, juice, pomace, purees, seeds, and wines. *Am. J. Enol. Vitic.* **1996**, *47*, 57−62.

(2) Huang, Z.; Wang, B.; Williams, P.; Pace, R. D. Identification of anthocyanins in muscadine grapes with HPLC-ESI-MS. *LWT—Food Sci. Technol.* **2009**, *42*, 819−824.

(3) Lee, J. H.; Johnson, J. V.; Talcott, S. T. Identification of ellagic acid conjugates and other polyphenolics in muscadine grapes by HPLC-ESI-MS. *J. Agric. Food Chem.* **2005**, *53*, 6003−6010.

(4) Mertens-Talcott, S. U.; Lee, J. H.; Percival, S. S.; Talcott, S. T. Induction of cell death in Caco-2 human colon carcinoma cells by ellagic acid rich fractions from muscadine grapes (*Vitis rotundifolia*). *J. Agric. Food Chem.* **2006**, *54*, 5336−5343.

(5) Yi, W.; Fischer, J.; Akoh, C. C. Study of anticancer activities of muscadine grape phenolics in vitro. *J. Agric. Food Chem.* **2005**, *53*, 8804−8812.

(6) You, Q.; Chen, F.; Wang, X.; Luo, P. G.; Jiang, Y. Inhibitory effects of muscadine anthocyanins on α-glucosidase and pancreatic lipase activities. *J. Agric. Food Chem.* **2011**, *59*, 9506−9511.

(7) Sandhu, A. K.; Gu, L. Antioxidant capacity, phenolic content, and profiling of phenolic compounds in the seeds, skin, and pulp of *Vitis rotundifolia* (muscadine grapes) as determined by HPLC-DAD-ESI-MS^n. *J. Agric. Food Chem.* **2010**, *58*, 4681−4692.

(8) Cardona, J. A.; Lee, J. H.; Talcott, S. T. Color and polyphenolic stability in extracts produced from muscadine grape (*Vitis rotundifolia*) pomace. *J. Agric. Food Chem.* **2009**, *57*, 8421−8425.

(9) Arts, I. C. W.; Hollman, P. C. H. Polyphenols and disease risk in epidemiologic studies 1−4. *Am. J. Clin. Nutr.* **2005**, *81*, 317S−325S.

(10) Kaur, C.; Kapoor, H. C. Antioxidants in fruits and vegetables-The millenium health. *Int. J. Food Sci. Technol.* **2001**, *36*, 703−725.

(11) Wei-min, Z. Chapter 2: Introduction to natural products chemistry. In *Extraction and isolation of natural products*; Xu, R., Yang, Y., Wei-min, Z., Eds.; Science Press: Beijing, 2012; Vol. *1*, pp 18−19.

(12) Scordino, M.; Di Mauro, A.; Passerini, A.; Maccarone, E. Adsorption of flavonoids on resins: hesperidin. *J. Agric. Food Chem.* **2003**, *51*, 6998−7004.

(13) Wang, J.; Wu, F. A.; Zhao, H.; Liu, L.; Wu, Q. S. Isolation of flavonoids from mulberry (*Morus alba* L.) leaves with macroporous resins. *Afr. J. Biotechnol.* **2008**, *7*, 2147−2155.

(14) Liu, Y.; Liu, J.; Chen, X.; Liu, Y.; Di, D. Preparative separation and purification of lycopene from tomato skins extracts by macroporous adsorption resins. *Food Chem.* **2010**, *123*, 1027−1034.

(15) Fu, Y.; Zu, Y.; Liu, W.; Hou, C.; Chen, L.; Li, S.; Shi, X.; Tong, M. Preparative separation of vitexin and isovitexin from pigeonpea extracts with macroporous resins. *J. Chromatogr. A* **2007**, *1139*, 206−213.

(16) Kammerer, D. R.; Saleh, Z.; Carle, R.; Stanley, R. Adsorptive recovery of phenolic compounds from apple juice. *Eur. Food Res. Technol.* **2007**, *224*, 605−613.

(17) Kammerer, D. R.; Carle, R.; Stanley, R. A.; Saleh, Z. S. Pilot-scale resin adsorption as a means to recover and fractionate apple polyphenols. *J. Agric. Food Chem.* **2010**, *58*, 6787−6796.

(18) Saleh, Z. S.; Wibisono, R.; Lober, K. Recovery of polyphenolics from apple juice utilizing adsorbent polymer technology. *Int. J. Food Eng.* **2008**, *4* (1), 1−20.

(19) Giusti, M. M.; Wrolstad, R. E. Unit F1.2: Anthocyanins. Characterization and measurement of anthocyanins by UV-visible spectroscopy. In *Current protocols in food analytical chemistry*; Wrolstad, R. E., Ed.; John Wiley & Sons: New York, 2001; pp 1−13.

(20) Lagergren, S. Zur theorie der sogenannten adsorption geloster stoffe. *K. Sven. Vetenskapsakad. Handl.* **1898**, *24* (4), 1−39.
(21) Ho, Y. S.; McKay, G. Pseudo-second order model for sorption processes. *Process Biochem.* **1999**, *34*, 451−465.
(22) Pompeu, D. R.; Moura, F. G.; Silva, E. M.; Rogez, H. Equilibria, kinetics, and mechanisms for the adsorption of four classes of phenolic compounds onto synthetic resins. *Sep. Sci. Technol.* **2010**, *45*, 700−709.
(23) Webi, T. W.; Chakravort, R. K. Pore and solid diffusion models for fixed-bed adsorbers. *J. Am. Inst. Chem. Eng.* **1974**, *20*, 228−238.
(24) Xu, Z.; Zhang, Q.; Chen, J.; Wang, L.; Anderson, G. K. Adsorption of napthalene derivatives on hypercrosslinked polymeric adsorbents. *Chemosph.* **1999**, *38*, 2003−2011.
(25) Chang, X. L.; Wang, D.; Chen, B. Y.; Feng, Y. M.; Wen, S. H.; Zhan, P. Y. Adsorption and desorption properties of macroporous resins for anthocyanins from the calyx extract of roselle (*Hibiscus sabdariffa* L.). *J. Agric. Food Chem.* **2012**, *60*, 2368−2376.
(26) Rudzinski, W.; Plazinski, W. Kinetics of dyes adsorption at the solid-solution interfaces: a theoretical description based on the two-step kinetic model. *Environ. Sci. Technol.* **2008**, *42*, 2470−2475.
(27) Duran, C.; Ozdes, D.; Gundogdu, A.; Senturk, H. B. Kinetics and isotherm analysis of basic dyes adsorption onto almond shell (*Prunus dulcis*) as a low cost adsorbent. *J. Chem. Eng. Data* **2011**, *56*, 2136−2147.
(28) Kumar, N. S.; Min, K. Removal of phenolic compounds from aqueous solutions by biosorption onto *Acacia leucocephala* bark powder: Equilibrium and kinetic studies. *J. Chil. Chem. Soc.* **2011**, *56*, 539−545.
(29) Jia, G.; Lu, X. Enrichment and purification of madecassoside and asiaticoside from *Centella asiatica* extracts with macroporous resins. *J. Chromatogr. A* **2008**, *1193*, 136−141.
(30) Kammerer, D.; Kljusuric, J. G.; Carle, R.; Schieber, A. Recovery of anthocyanins from grape pomace extracts (*Vitis vinifera* L. cv. Cabernet Mitos) using a polymeric adsorber resin. *Eur. Food Res. Technol.* **2005**, *220*, 431−437.
(31) Scordino, M.; Di Mauro, A.; Passerini, A.; Maccarone, E. Selective recovery of anthocyanins and hydroxycinnamates from a byproduct of citrus processing. *J. Agric. Food Chem.* **2005**, *53*, 651−658.
(32) Biswas, R. Development of technologies for the production of polyphenolic nutraceuticals from muscadine grapes and rabbiteye blueberries. Ph.D. Dissertation, 2007.