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Author: Liu, Xuan; Xu, Zhenghong; Gao, Yangxiang; Yang, Bin; Zhao, Jian; Wang, Lijun

Email address:- liuxuancau@126.com

xzhhtwl@163.com

gyxcau@126.com

Year:- 2007

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Journal International Journal of Food Engineering

Volume: 5

Issue: 3

Pages: pp1-16

ISSN: 1556-3758

URL: <http://www.bepress.com/ijfe/>

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Adsorption Characteristics of Anthocyanins from Purple-fleshed Potato (*Solanum tuberosum* Jasim) Extract on Macroporous Resins

Xuan Liu*, Zhenghong Xu*, Yangxiang Gao*[‡] Bin Yang, Jian Zhao[†] Lijun Wang*

*College of Food Science and Nutritional Engineering, China Agricultural University, Beijing 100083, China

[†]School of Wine & Food Sciences, EH Graham Center for Agricultural Innovation, Charles Sturt University, Wagga Wagga, New South Wales, 2678, Australia,

[‡]Corresponding author.

Tel.: + 86-10-62737034

Fax: + 86-10-62737986

E-mail address: gyxcau@126.com

Abstract

The adsorption characteristics of anthocyanins from purple-fleshed potatoes on 11 macroporous SDVB, acrylic and methacrylic resins were investigated. Anthocyanins were first extracted from purple-fleshed potatoes using dilute acid solutions, and then partially purified by membrane filtration, which resulted in the removal of more than 90% of the total sugar and proteins from the crude extract. The partially purified extract were mixed with the resins at varying extract/resin ratios until equilibrium adsorption was reached and the adsorption data were fitted to the Langmuir and Freundlich isotherms. Both models were found suitable to describe the adsorption isotherms of anthocyanins on the resins tested. Adsorption capacities for anthocyanins increased with the rise of temperature for SDVB resins but decreased for methacrylic resins. The resin with the highest adsorption capacity for anthocyanins was XAD-1600, on which anthocyanin adsorption was an endothermic process and favored at higher temperature.

KEYWORDS: purple-fleshed potato, anthocyanins, macroporous resin, adsorption, Langmuir isotherm, Freundlich isotherm

1. Introduction

Anthocyanins are intensely colored water-soluble pigments that are widely distributed in various plant organs including flowers, fruits, leaves, roots, tubers and seeds. With the worldwide tendency towards using natural pigments to replace synthetic colorants, anthocyanins have received growing attention and increasingly been used in food and pharmaceutical products. Traditionally, grape skin and red cabbage have been the main commercial sources of anthocyanins (Gao & Mazza, 1996). Anthocyanins from red-fleshed sweet potatoes and red radishes have also been considered as potential sources for food colorants (Rodriguez-Saona, et al., 1999). As the applications of natural pigments continue to grow, however, more anthocyanin sources are required to meet the market demands.

Purple-fleshed potato is a sweet potato cultivar which is noteworthy for its purple flesh color due to its high content of anthocyanins (Shi et al., 1992; Lewis et al., 1998). The extract from purple-fleshed potato has similar color characteristics to those of FD&C Red #40 (Rodriguez-Saona, 1998). The major pigment in the purple-fleshed potato extract has been identified as pelargonidin-3-rutinoside-5-glucoside acylated with *p*-coumaric acid. The acylation shifts the orange-red hue of pelargonidin to an intense red (Lewis et al., 1998), and also improves the stability of anthocyanins through intramolecular copigment (Giusti & Wrolstad, 1996a; Giusti & Wrolstad, 1996b).

Adsorbent resins have frequently been used to recover anthocyanins and other flavonoids from plant extracts (Di Mauro et al., 2002; Scordino et al., 2003; Scordino et al., 2004; Kammerer et al., 2005). These resins are usually styrene-divinylbenzene

(SDVB) or acrylic polymers which have high adsorption capacities and long durability and are easy to regenerate (Scordino et al., 2004). Furthermore, the resins are rather inexpensive and their use for food contact has been approved by the Food and Drug Administration (Scordino et al., 2004). Despite the wide spread use of these resins for the recovery of many products, there is a lack of understanding of the adsorption process and many of the applications are based on empirical data rather than predictable models. The adsorption of a solute on an adsorbent is a complex process which involves the interactions among three components: the adsorbate (the solute), the adsorbent and the solvent (Scordino et al., 2003). For a given solute and solvent, both the intrinsic factors, such as the structure, polarity, particle size, porosity and surface area of the adsorbent and external factors such as temperature, pH and competing compounds may affect the adsorption process (Di Mauro et al., 2002; Scordino et al., 2003). Therefore, the development of reliable mathematical models that can accurately describe and predicate experimental data of adsorption would be extremely helpful in understanding the adsorption process and the driving forces involved. Several such models have been developed for the adsorption of pure flavonoid compounds on SDVB and acrylic resins (Scordino et al., 2003; Scordino et al., 2004), however, few studies have attempted to develop models for the adsorption of anthocyanins in plant extracts on these resins.

The objectives of this work were: (1) to investigate the adsorption characteristics of anthocyanins in purple-fleshed potato extract on 11 commercial resins with different physiochemical properties; (2) to test the suitability of the Langmuir and Freundlich adsorption models for describing the adsorption isotherms of anthocyanins on the resins.

2. Materials and Methods

2.1. Preparation of anthocyanin extract

Fresh purple-fleshed potatoes were obtained from a farm in Shandong Province, China. The potatoes were washed, trimmed and sliced to 3 mm thick slices, which were air dried to a moisture content of 5% (w/w) and ground to pass 80 mesh. Samples (4 kg) of the ground potato were mixed with 60 L of 0.3% (v/v) aqueous hydrochloric acid solution preheated to 60 °C. The mixture was allowed to stand at 60 °C for 4 h, then moved to 4 °C where it was kept for 24 h, and then centrifuged (4,000 g, 20 min) to obtain the crude anthocyanin extract.

2.2. Membrane filtration and concentration of the crude anthocyanin extract

The crude anthocyanin extract was filtrated with a nanofiltration system (Henghui Environmental Industry Co. Ltd., Xiangtan, China) using a ceramic membrane of 50 nm in pore size with a membrane area of 0.05 m², at an operating pressure of 0.5 MPa. The nanofiltrates obtained were concentrated approximately 2.5 times with a polyamide membrane (200~300 Da molecular weight cutoff, 0.2 m² membrane area, supplied by Getrain Purification Technology Co. Ltd. Shanghai, China) at an operating pressure of 1 MPa. The crude and nanofiltrated extract were analyzed for pH, anthocyanins, sugars and proteins.

2.3. Analysis of the purple-fleshed potato extract

Anthocyanins in the crude and nanofiltered purple-fleshed potato extracts were determined according to the spectrometry method of Buckmire and Francis (Buckmire & Francis, 1976) using a UV-Vis spectrophotometer (UV 757 CRT, Shanghai

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Precision & Scientific Instrument Co., Shanghai, China) at 533 nm. Reducing sugar and total sugar were determined using the 3, 5-dinitrosalicylic acid (DNS) reagent (Guan, 1999) and phenol-sulphate acid methods (Xu et al., 2005), respectively. Protein was determined according to the Coomassie blue method of Bradford (1976) with bovine serum albumin as the standard, and pH was measured with a Hanna 211 pH meter (Hanna Instruments Co., Padova, Italy). All analyses were performed at least in duplicate.

2.4. Adsorption of anthocyanins in the nanofiltrated extract with macroporous resins.

2.4.1. Resin activation

All resins used in this study were commercial products of food grade. Amberlite XAD-7HP, XAD-1180, XAD-4 and XAD-1600 resins were purchased from Rohn and Haas, Co. (Philadelphia, USA); EXA-31, EXA-117, EXA-32, EXA-90, EXA-45, EXA-50 and EXA-118 resins were kindly provided by Resindion, Mitsubishi Chem. Co. (Milan, Italy). EXA-31 and XAD-7HP were methacrylic and acrylic polymers, respectively; other resins are macroporous styrene-divinylbenzene (SDVB) copolymers with no functional groups. The resins were characterized by a wide range of surface areas (500 ~ 1200 m²/g) and pore radii (38 ~ 450 Å). Chemical and physical properties of the resins are shown in Table 1. In accordance with the manufacturers' recommendations, the resins were washed first with ethanol (95%, V/V) then distilled water, followed by drying at 70 °C for 24 h. Appropriate amounts (see below) of each dried resin were soaked in 100 ml ethanol (95%, V/V) overnight. After that, the resins were rinsed with distilled water until all excess ethanol was

eliminated, and then transferred into a 250 ml conical flask, to which appropriate volumes of anthocyanin extracts (crude or nanofiltrated) were added.

2.4.2. Adsorption kinetics of anthocyanins on different resins

Adsorption kinetics of anthocyanins was evaluated on 11 different types of resins: XAD-7HP, XAD-1180, XAD-4, XAD-1600, EXA-31, EXA-117, EXA-32, EXA-90, EXA-45, EXA-50 and EXA-118. For each type, 0.3 g (dry weight) of the activated resin was mixed with 70 ml of the nanofiltrated anthocyanin extract in a 250 ml conical flask mounted on a rotating platform (100 rpm) in a 30 ± 0.1 °C water bath for 24 h. Small aliquots were withdrawn at regular intervals to measure the concentration of anthocyanins. The concentration of anthocyanins in the resin phase was estimated by the following mass balance equation (Scordino et al., 2003):

$$Q_t = \frac{(C_o - C_t)V}{m} \quad (1)$$

Where Q_t (mg/g) is the quantity (mg) of anthocyanins on a unit amount (g, dry weight) of absorbent at time t ; Q_s (mg/g) is the Q_t value at absorption equilibrium. C_o and C_t are the concentrations of anthocyanins (mg/L) in liquid phase at the initial stage and at t time. V is the volume of the solution (L), and m is the weight (g) of dry resin.

To compare the adsorption of anthocyanins in the crude and nanofiltered extracts, 0.3 g (dry weight) of the best resins (obtained from the above experiments) was mixed separately with 70 ml each of the crude and nanofiltrated extracts, standardized to the same anthocyanin concentration, in a 250 ml conical flask mounted on a rotating platform (100 rpm) in a 30 ± 0.1 °C water bath until equilibrium was reached. The

anthocyanin concentrations in the liquid phase were determined using the method described already. The adsorption ratio (%) for anthocyanins on the resin, which is the percent of the mass of total adsorbate being adsorbed after reaching equilibrium, was calculated according to the following equation (Fu et al., 2005):

$$AR(\%) = \frac{(C_o - C_s)}{C_o} \times 100 \quad (2)$$

Where C_o and C_s are the concentrations of anthocyanins (mg/L) in liquid phase at the initial stage and at equilibrium.

2.4.3. Equilibrium adsorption isotherms and models

A fixed amount of the 11 activated macroporous resins (0.025 ~ 0.5 g of dry weight) was mixed with 70 ml of filtered anthocyanin extracts in a 250 ml conical flask mounted on a rotating platform (100 rpm) in a 30 ± 0.1 °C water bath until adsorption equilibrium was reached. The concentration of anthocyanins in the liquid phase was determined using the method described already and the concentration of anthocyanins in the resin phase was calculated according to equation (1). The adsorption data were used to obtain adsorption isotherms.

Langmuir isotherm is the simplest theoretical model for describing monolayer adsorption (Di Mauro et al., 2002; Scordino et al., 2003), which assumes a uniform adsorbent surface with energetically identical sorption sites. The Langmuir isotherm is:

$$Q_s = \frac{Q_m a_L C_s}{1 + a_L C_s} = \frac{K_L C_s}{1 + a_L C_s} \quad (3)$$

where Q_s (mg/g) is the adsorbate concentration per unit weight of adsorbent (solid phase) and can be calculated from equation (1). C_s (mg/L) is the concentration of adsorbate in solution (liquid phase) at equilibrium. K_L (L/g) and a_L (L/mg) are the

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adsorption equilibrium constants. K_L/a_L is defined as the monolayer adsorbent capacity Q_m (mg/g); in other words, it means the maximum concentration retained by the adsorbent surface corresponding to the complete monolayer coverage. The above equation can be rearranged to the following linear form:

$$\frac{1}{q_s} = \frac{1}{K_L C_s} + \frac{1}{Q_m} \quad (4)$$

in which K_L and Q_m can be obtained from the slope and the intercept by plotting $1/q_s$ against $1/C_s$.

Another common isotherm model is the Freundlich isotherm, it describes the equilibrium conditions on heterogeneous surfaces and therefore does not assume the monolayer capacity. It is expressed as:

$$Q_s = K_F C_s^{b_F} \quad (5)$$

where Q_s (mg/g) and C_s (mg/L) are defined as above; K_F (L/g) provides an indication of the adsorption capacity of the adsorbent; and b_F (dimensionless) represents the adsorption intensity. K_F and b_F are the empirical constants dependent on several environmental factors. This equation is usually applied in the linear form:

$$\log Q_s = \log K_F + b_F \log C_s \quad (6)$$

By plotting the equation on a log-log scale, K_F and b_F can be calculated from the intercept and the slope, respectively.

2.4.4. Effects of temperature

The effect of temperature on the anthocyanin adsorption capacity of the different resins (XAD-7HP, XAD-4, XAD-1600, EXA-31, EXA-32, EXA-45) with 3 different structures were investigated at the temperature range of 30-60 °C. Based on the results

of these experiments, the resin with the largest adsorption capacity (XAD-1600) was further tested at four different temperatures (30, 40, 50, 60 °C) to investigate the effect of temperature on equilibrium adsorption isotherms. The thermal parameters for the adsorption process were then estimated from the adsorption data.

The adsorptive free energy is calculated with Gibbs equation (Wei et al., 2004):

$$\Delta G = -RT \ln a_L \quad (7)$$

Where a_L is the constant in the Langmuir isotherm, and a_L changes with temperature and adsorptive enthalpy as expressed by the following equation (Wei et al., 2004):

$$\ln a_L = \ln K_0 + \left(\frac{-\Delta H}{RT} \right) \quad (8)$$

Where ΔH is the isosteric enthalpy of adsorption, R is the gas constant (8.3144×10^{-3} kJ/ (mol· K), T is the absolute temperature (K) and K_0 is a constant.

Gibbs-Helmholtz equation shows (Wei et al., 2004):

$$\Delta S = \left(\frac{\Delta H - \Delta G}{T} \right) \quad (9)$$

thus, ΔH and ΔS (adsorptive entropy) can be calculated respectively from the slope and the intercept of the following equation (Wei et al., 2004):

$$\ln a_L = \frac{\Delta S}{R} - \frac{\Delta H}{RT} \quad (10)$$

3. Statistical analysis

The whole experiment was repeated twice. Statistical analysis of the data collected was carried out using the software package SPSS 12.0 for Windows (SPSS Inc., Chicago, USA).

4. Results and Discussion

4.1. Partial purification of crude anthocyanin extract from purple-fleshed potatoes by nanofiltration

The crude extract contained about 1181.4 µg/ml protein and 44.4 mg/ml total sugar, which were reduced by nearly 10- fold (126.32 µg/ml) and 7-fold (7.84 mg/ml), respectively, by nanofiltration. However, only a relatively small proportion of the reducing sugar (about 11.4%) was removed and there was no significant difference between the pH values of the crude and filtered extracts ($p < 0.05$).

To study the effect of nanofiltration on the adsorption of anthocyanins on resins, the XAD-1600 resin was mixed separately with excessive amounts of crude and nanofiltered extracts standardized to the same anthocyanin concentration, until adsorption equilibrium was reached. The adsorption ratio for anthocyanins in the crude extract was $68.05 \pm 0.15\%$, whereas the value for the nano-filtered extract was much higher at $98.08 \pm 0.06\%$. This result was expected as impurities in the crude extract, such as carbohydrates and proteins, may block the pores of the resin and compete with anthocyanins for adsorption sites on the adsorbent.

4.2. Adsorption kinetics of anthocyanins on resins

Fig. 1 shows the uptake of anthocyanins by the tested resins over time. The amount of adsorbed anthocyanins increased with the extension of contact time for each adsorbent tested. The anthocyanin concentrations in the resin phase increased very quickly during the first hour, but thereafter the increase became more gradual.

Q_t values varied significantly with the type of resins ($p < 0.05$). XAD-1600 and XAD-1180 were the two best resins with equilibrium concentrations over 10.2 mg/g. XAD-4 was the worst with an equilibrium concentration of 6.56 mg/g. EXA-32, EXA-118 and XAD-7HP with similar equilibrium concentrations had intermediate adsorption capacities ($p > 0.05$).

4.3. Equilibrium adsorption isotherms and models

The adsorption isotherm is the equilibrium relationship between the concentration of adsorbate in the fluid phase and the adsorbent at a given temperature. Fig. 2 shows the adsorption isotherms of anthocyanins on 11 different resins at 30°C.

The Langmuir and Freundlich isotherms are two of the most commonly used models for describing adsorption isotherms. The Langmuir isotherm is a theoretical model suitable for the ideal conditions of monolayer adsorption, while the Freundlich isotherm is an empirical model for non-ideal adsorption on heterogeneous surfaces (Scordino et al., 2003). By fitting the data in Fig. 2 to the Langmuir and Freundlich equations (Eq. 4 and 6, respectively) and applying regression analysis, the parameters for the two models can be obtained as shown in Tables 2 and 3. The high values of all the regression coefficients ($R^2 > 0.97$) showed that the experimental data for all 11 resins could be well fitted to both models, suggesting that both isotherms were suitable for describing the adsorption process.

The Q_m and KF values for XAD-1600 were the highest and those for XAD-4 were the lowest, which was consistent with the results of the adsorption isotherms. When compared with adsorption capacities, usually the resin with the highest amount of

solute adsorbed (Q_s) at a specified residual concentration (C_s) would be preferred. The adsorption isotherms (Fig. 2) showed that XAD-1600 had greater Q_s value than the other resins at all residual concentrations of anthocyanins and, therefore, was the best material for removing anthocyanins from aqueous solutions. In contrast, XAD-4 was the least effective resin for anthocyanins removal.

4.4. Effect of temperature on the adsorption of anthocyanin on different resins

Fig. 3 shows the amount of anthocyanins adsorbed on six resins at equilibrium over the temperature range of 30-60 °C. The equilibrium adsorption (Q_s) increased with temperature for SDVB resins, whereas temperature had a negative effect on the equilibrium adsorption on methacrylic resins. These results were consistent with previous reports that elevated temperatures facilitated the removal of phenolic compounds from apple juice and hesperidin from orange juice with SDVB resins (Carabasa et al., 1998; Gökmen & Serpen, 2002; Scordino et al., 2004). However, some studies have shown that temperatures had a negative effect on the adsorption of phenolic compounds on SDVB resins, which was attributed to the greater mobility of the molecules at higher temperatures (Juang & Shiau, 1999; Ku & Lee, 2000).

4.5. Adsorption isotherms and thermodynamic parameters for the adsorption of anthocyanins on XAD-1600 at different temperatures

Fig. 4 shows the equilibrium adsorption isotherms of anthocyanins on the XAD-1600 resin at 30, 40, 50 and 60 °C. Data showed that the adsorption capacity of anthocyanins on XAD-1600 increased with the rise of temperature. This was consistent with the positive effect of temperature on the adsorption capacity of SDVB resins described above.

Enthalpy change (ΔH) and entropy change (ΔS) for the adsorption of anthocyanins on XAD-1600 were calculated from the slope and intercept of the line obtained by plotting $\ln aL$ vs. $1/T$ (Fig. 5). The thermodynamic parameters of Gibbs free energy (ΔG) was calculated according to the equation (7), and the values were given in Table 4. The change of Gibbs free energy (ΔG) for the adsorption decreasing with increasing temperature showed that, for XAD-1600, the reaction was easier at higher temperatures. The positive ΔG value indicated that the adsorption process was non-spontaneous and the adsorbate was not inclined to be adsorbed from the solution to the surface of the adsorbent (Wei et al., 2004). The positive value of ΔH indicated that the adsorption process was endothermic in nature, which was consistent with the decreasing values of ΔG with the rise of temperature. Gökmen and Serpen (2002) have shown that if the ΔH value is positive and less than 43 kJ/mol, the adsorption is controlled by physical rather than chemical mechanism. The ΔH value of 25.43 kJ/mol would imply that the adsorption of anthocyanins on XAD-1600 was controlled by physical mechanism. The value of ΔS is a measure of the randomness of a reaction (Ajmal et al, 2000) and the positive value showed that the randomness increased at the solid/solution interface during the adsorption of anthocyanins on XAD-1600.

5. Conclusions

Nanofiltration was an effective process for the primary purification of anthocyanins from the purple-fleshed potato extract, and the resin adsorption can be applied directly to recover anthocyanins from the nanofiltered extract. Both Langmuir and Freundlich models were suitable to describe the adsorption isotherms of anthocyanins on the 11 tested SDVB, methacrylic and acrylic resins. Higher temperature led to the increase in

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adsorption capacities for SDVB resins but the decreases for the methacrylic resin. In tested resins XAD-1600 was proved to be the most efficient one for anthocyanin adsorption, and was an endothermic adsorption process which was favored at higher temperatures.

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Table 1. Chemical and physical properties of resins provided by manufacturers

Trade name	Surface area (m ² /g)	Pore radius (Å)	Structure
XAD-7HP	500	450	Acrylic
XAD-1180	700	400	SDVB
XAD-4	750	20	SDVB
XAD-1600	800	150	SDVB
EXA-31	500	170	Methacrylic
EXA-117	570	80	SDVB
EXA-32	600	260	SDVB
EXA-90	600	110	SDVB
EXA-45	1000	38	SDVB
EXA-50	1000	57	SDVB
EXA-118	1200	90	SDVB

Table 2. Langmuir isotherm parameters for the adsorption of anthocyanins on different resins (T = 30 °C, pH=1)

Resins	N	Q _m (mg/g)	a _L (L/mg)	R ²
XAD-4	3	17.6991	0.0263	0.9991
EXA-45	3	18.8324	0.0432	0.9992
EXA-50	3	23.2558	0.0361	0.9929
EXA-90	3	23.8664	0.0443	0.9920
EXA-31	3	27.8552	0.0440	0.9940
EXA-117	3	28.2486	0.0605	0.9987
EXA-32	3	30.8642	0.0796	0.9957
EXA-118	3	32.6797	0.0728	0.9944
XAD-7HP	3	34.8432	0.0736	0.9996
XAD-1180	3	38.6100	0.0894	0.9993
XAD-1600	3	48.0769	0.1049	0.9964

Table 3. Freundlich isotherm parameters the adsorption of anthocyanins on different resins (T = 30 °C, pH=1)

Resins	N	K _F (L/g)	b _F	R ²
XAD-4	3	1.00	0.60	0.9932
EXA-50	3	1.55	0.60	0.9946
EXA-45	3	1.69	0.54	0.9932
EXA-90	3	2.00	0.56	0.9760
EXA-31	3	2.03	0.61	0.9995
EXA-117	3	2.82	0.55	0.9937
EXA-32	3	3.26	0.56	0.9950
EXA-118	3	3.53	0.56	0.9837
XAD-7HP	3	3.76	0.56	0.9860
XAD-1180	3	4.71	0.54	0.9819
XAD-1600	3	6.19	0.54	0.9730

Table 4. Thermodynamic parameters for the adsorption process of anthocyanins on XAD-1600

Resins	T (K)	ΔG (kJ/mol)	ΔH (kJ/mol)	ΔS (kJ/mol)	R^2
XAD-1600	303.15	5.680			
	313.15	5.044			
	323.15	3.989	25.425	0.065	0.9543
	333.15	3.875			

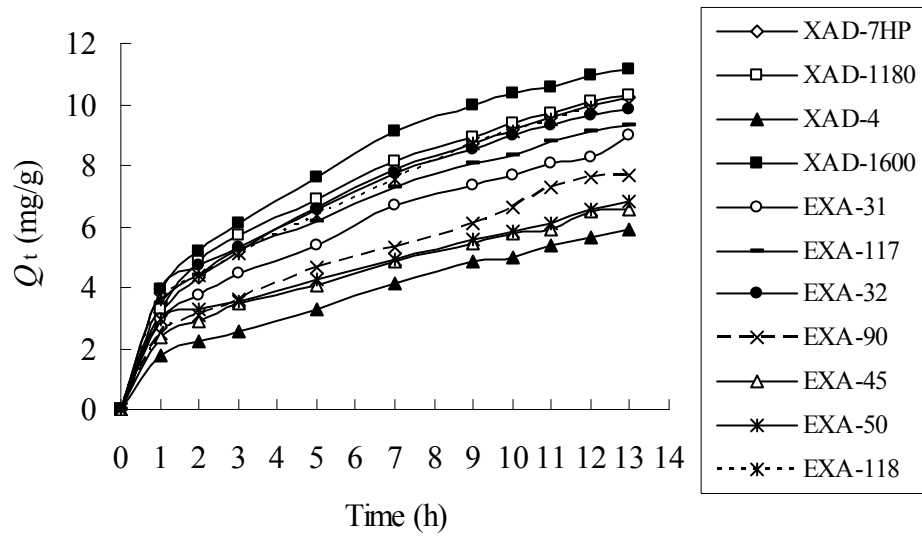


Fig. 1. Absorption kinetics of anthocyanins in nanofiltered extract on different resins at 30 °C

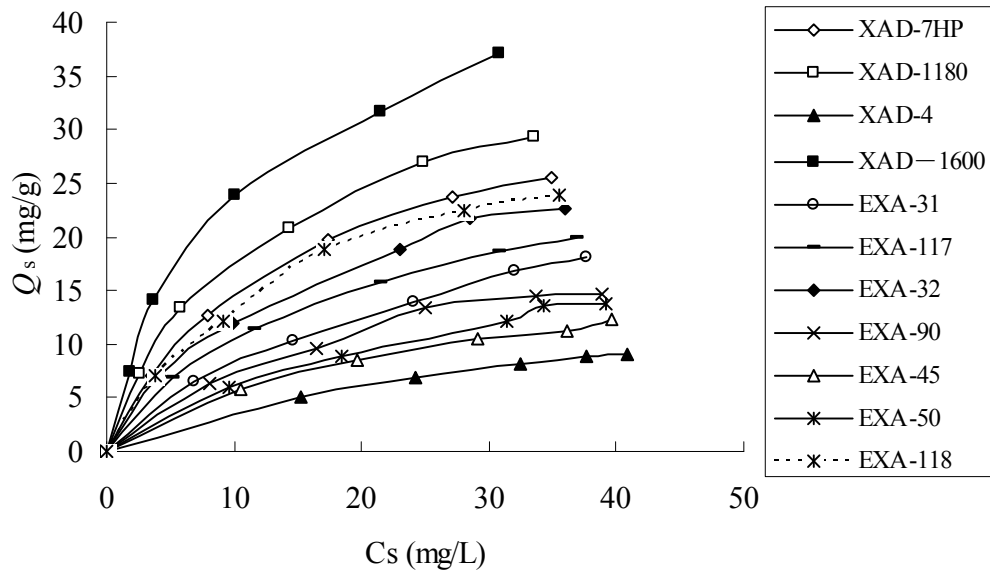


Fig. 2. Equilibrium adsorption isotherms of anthocyanins on different resins at 30 °C

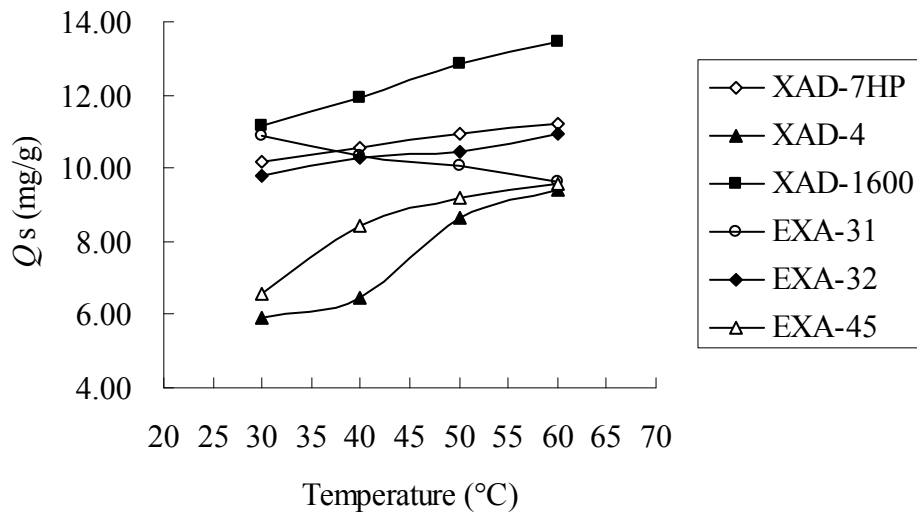


Fig. 3. The effect of temperature on the adsorption of anthocyanin on different resins

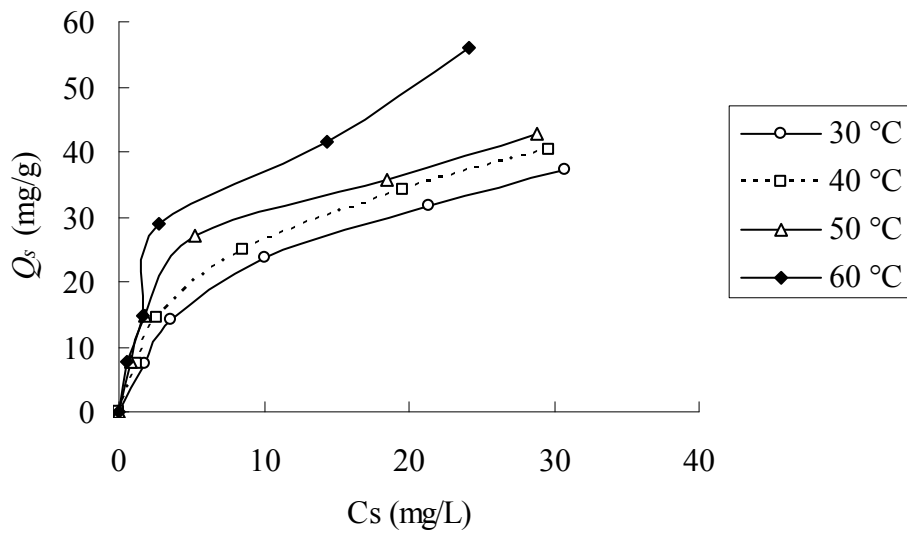


Fig. 4. Equilibrium adsorption isotherms of anthocyanins on XAD-1600 at different temperatures

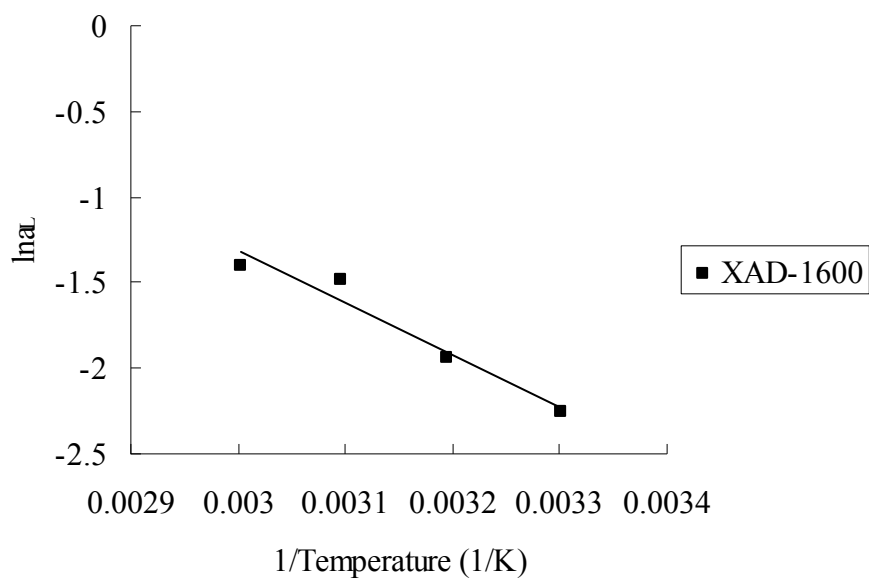


Fig. 5. Determination of isosteric enthalpy changes of the anthocyanin adsorption on XAD-1600