Purification characteristics and parameters optimization of anthocyanin extracted from blueberry

Zheng Xianzhe, Zhang Zhaoguo, Jin Changjiang, Mu Yanqiu, Liu Chenghai, Chen Zhiying, Liu Haijun, Lin Zhen

(*College of Engineering, Northeast Agricultural University, Harbin 150030, China*)

Abstract: To improve the yield and purity of anthocyanin extracted from blueberry, the characteristics and optimization for the separation and purification process of anthocyanin by AB-8 macroporous adsorbent resin was studied. In the absorption stage, the pH value has positive effect in ranges of 1.0-3.0 and the temperature rise has firstly negative then positive effect on adsorption rate of anthocyanin with adsorption time. In the desorption stage, the ethanol concentration increase has firstly positive then steady effect and the temperature rise has positive and then negative effect on desorption rate of anthocyanin. The purification temperature may improve diffusion or induce degradation of anthocyanin. AB-8 macroporous adsorbent resin is suitable to purify the anthocyanin from extract of powdered blueberries and the optimum parameters were obtained by using Design Expert software as adsorption time of 3 h, pH value of 1.0, adsorption temperature of 18°C in absorption stage and desorption time of 30 min, ethanol concentration of 46.5%, desorption temperature of 22°C in desorption stage with the highest absorption rate and desorption rate are 87.65% and 84.80%, respectively.

Keywords: parameters optimization, extraction, purification, adsorption, desorption, anthocyanin, blueberry

DOI: 10.3965/j.ijabe.20150802.1407

Citation: Zheng X Z, Zhang Z G, Jin C J, Mu Y Q, Liu C H, Chen Z Y, et al. Purification characteristics and parameters optimization of anthocyanin extracted from blueberry. Int J Agric & Biol Eng, 2015; 8(2): 135－144.

1 Introduction

Anthocyanins, as the polyphenols of water-soluble, have great potential benefits to human health[1-3] due to its strong oxidation resistance for the somatic cells and organizations[4], such as enhancing immunity capacity, curing cancer[5,6] and cardiovascular disease[7], anti-inflammatory[8] and balancing weight[9]. Blueberry contains abundant phenolic compounds[10,11], as well as anthocyanins[12]. In recent years, extraction, separation, purification and oxidation resistance evaluation[13-15] of anthocyanins from berries are the research hotspot in agricultural products processing and pharmaceutical industry. The operations of separation and purification are the indispensable process after extraction from the raw material, which influence the yield and quality of extracted components, such as lycopene from tomato skins[16], ginseng saponins from American ginseng[17], anthocyanin from blueberry[18], chlorogenic acid from honeysuckle[19], and rosavin from Rhodiola rosea[20].

Analysis of purification characteristics for the extracted materials contributes to the selection of

Received date: 2014-10-22 **Accepted date:** 2015-02-17

Biographies: Zhang Zhaoguo, PhD, Professor, agricultural product processing. Email: zhangzg@neau.edu.cn; **Jin Changjiang**, PhD, Assisted professor, agricultural product processing. Email: Jinchangjiang416@tom.com; **Mu Yanqiu**, PhD, Assisted professor, agricultural product processing. Email: muyanqiu@126.com; **Liu Chenghai**, PhD, Assisted professor, agricultural product processing. Email: liuchenghai@neau.edu.cn; **Chen Zhiying**, PhD, Assisted professor, agricultural product processing. Email: chenzy1997@163.com; **Liu Haijun**, PhD, Assisted professor, agricultural product processing. Email: liuhj1982@163.com; **Lin Zhen**, PhD, Assisted professor, agricultural product processing. Email: linzhen@163.com;

***Corresponding author: Zheng Xianzhe,** PhD, Assisted-professor, agricultural product processing. Mailing address: College of Engineering, Northeast Agricultural University, Harbin, 150030, China. Tel: +86-451-55191021, Email: zhengxz@neau.edu.cn.

absorbent and the elucidation of effects of purification technology parameters. Non-ionic acrylic ester adsorbent, namely Amberlite XAD-7HP, has the highest adsorption capacity and desorption ratio for the extraction of anthocyanins from red cabbage, which may remove the sugars in the anthocyanin solution after purification to improve the stability of anthocyanin[21]. Purification treatment connected to extraction may ensure and improve the function of extracts. Determination of purification mode and medium depends mostly on the extraction method and purification intention, precise analysis or scale-up production. Purification for the precise analysis focuses on the quantitative and qualitative measurement of crude extract ingredients with high precision and costly detection. You et al.[22] purified *Tricholoma mongolicum Imai* polysaccharides (TMIPs) extracted from fruit bodies by chromatography to detect four TMIPs ingredients, which approves powerful antioxidant activity of TMIPs. Hu et al.[23] developed a microwave-low-pressure process to extract total polyphenols with high extraction yield. Oh et al.[24] evaluated the efficiency of different adsorbents for the removal of plant-derived impurities during the pre-purification of paclitaxel from plant cell cultures and found large pore diameter of silica may improve adsorbent capacity in terms of purity and yield. Simultaneous microwave-assisted extraction (MAE) and adsorbent treatment process obviously reduce the procedure and cost of separation/purification[25]. Polymeric adsorbents have feasible adaptability on the purification of total flavonoids from *Ginkgo biloba* L., *Radix puerariae* and *Hypericum perforatum* L. with high purity[26].

Optimization of purification process provides the reasonable combinations parameters for the desired objectives including high yield, premium quality of extracted components, which gives the valuable guide for components extraction in practice. Wu et al.[27] presented the optimal parameters including concentration of alcohol and the quantity of grape juice, extraction time, system pH and temperature in an aqueous two-phase extraction mode for the extraction and preliminary purification of anthocyanins in grape juice to reach recovery of anthocyanin as high as 99%, which proved the effectiveness and great potential of extraction method studied in processing mass grape juice. An optimal extract procedure of chitosan-treatment to purify anthocyanin from radish was developed with many advantages of biodegradable agent, flavor-rich radish anthocyanin extracts for commercial production[28]. High purify and stability of anthocyanin from jamun (*Syzygium cumini* L.) extracts was produced, Amberlite XAD7HP as absorbent, by means of optimization of purification parameters[29].

Adsorption–desorption, macorporous resin as adsorbent, may recovery and purify bioactive components from exact liquid from plant[30]. Jia et al.[31] selected a D141-type macroporous adsorptive resin as adsorbent to recovery the flavonoids and anthocyanins from raw juice with high efficiency. HPD-200 macroporous adsorption resins, a styrene-divinylbenzene copolymer, may provide the optimal adsorption and desorption properties for rosavin from in *Rhodiola rosea*.L[32].

AB-8 macorporous resin, as a crosslinked copolymer with pore and cavity structure, has remarkable enrichment and separation on flavoniods. Zhao et al.[33] reported AB-8 macorporous resin was the most appropriate, among five macroporous resins selected with different physical and chemical properties, to the dynamic adsorption and desorption of rutin and quercetin from *Euonymus alatus* (*Thunb.*) extracts in large-scale preparation from bioresource. Macroporous adsorption resin is applied widely in separation and purification of natural products as a typical organic polymer adsorbent with advantages of high capacity and efficiency of absorption, strong selectivity, and renewable simple[14]. AB-8 macro-porous resin is suitable for the anthocyanins purification from blueberry extraction[34].

However, little information was published about the separation and purification for the anthocyanin extracted from blueberry powder by using microwave extraction method. The objectives of this study were: 1) to analyze adsorption and desorption characteristics of anthocyanins extracted from blueberry powder using AB-8 macroporous resin; 2) to optimize purification process parameters considering high yield of anthocyanins.

2 Materials and methods

2.1 Plant material and reagents

Fresh wild blueberries were supplied by Lesser Khingan area (Heilongjiang, China). Ripe whole blueberries without foreign matter were selected as raw material to be frozen and stored in temperature of -18°C in a freezer (BC/BD-272SC, Haier Group, Qingdao, China). The frozen blueberries were taken out to thaw for 10-12 h in room temperature (25-28°C). Thawed blueberries were smashed by using a household electrical grinder (SJ260C, Lanpu Electrical Equipment Factory, Guangdong, China) into puree. The blueberry puree were frozen in a freezer, followed by the dehydration processing in a freeze vacuum dryer for 24 h (Shanghai Instrument Co. Ltd, Shanghai, China) to get the dehydrated blueberry slices with the moisture content below 0.5% (w.b.). The blueberry slices were pulverized by using a cyclone mill through a 40 mesh sieve to produce fine blueberry powder. These powders were kept in an airtight desiccator for the further experiments. The anthocyanin was extracted from the powdered blueberry in the condition of optimum extraction parameters with extraction time of 7 min, extraction temperature of 47°C, solid to liquid ratio of 1:34 (g/mL) and ethanol concentration of 55.5%. Anthocyanin content in blueberry powder, extract and purification were measured by chemical analysis method provided by Xu[35]. Anthocyanin extract solution was distillated under low pressure conditions, and condensed extraction liquid was kept for the further purification using AB-8 macroporous resin as adsorbent.

AB-8 macroporous adsorbent resin and the standards of anthocyanin were supplied by Tianjin Guangfu Fine Chemical Institute (Tianjin, China) and Tianjing Jianfeng Natural Products Research and Development Company (Tianjin, China), respectively. Analytical grade regents were methanol, ethanol, citric acid, citric acid sodium and vanillic aldehyde, and concentrated hydrochloric acid Tianjin Fuyu Fine Chemical Factory, Tianjin, China). Local distilled water company (Harbin, China) supplied distilled water.

2.2 Equipments

Extraction experiments were performed in an Advanced Microwave Digestion System (Ethos1, Milestone Inc., Italy) and operated via a compact terminal touch screen display with Milestone Easy-Control software. A maximal power of 1 600 W was delivered to a chamber with a rotating sample tray of 10 Teflon vials with each capacity of 100 mL. One of the vials was used as a reference vessel for monitoring temperature and pressure states. A visible spectrophotometer (Type 722, Shanghai Spectral Instrument Inc., Shanghai, China) with a variable wavelength detector was employed to measure the anthocyanin content of blueberry extract. The solvents were removed from the extracts with a evaporator (RE-52AA, Shanghai Yarong Biochemistry Instrument Factory, Shanghai, China). The extract was evaporated in a vacuum freeze-dryer (Shanghai instrument Co. Ltd, Shanghai, China).

2.3 Pretreatment experiment

In pretreatment of macroporous resin, AB-8 macroporous resin powder was soaking in pure alcohol aqueous in 24 h until it was fully swelling, then cleaned by leaching pure alcohol to neatness for the purification experiments.

2.4 Experimental design

2.4.1 Adsorption combination design

Taken value range of each factor was determined based on the results from the preliminary result from the single factor experiments[35]. A full second-order polynomial model of the design was developed to evaluate the adsorption yield (response variable, Y_1) as a function of independent variables including adsorption time, pH, and adsorption temperature and their significant interactive terms. The factors and their levels were shown in Table 1.

Table 1 Factors and levels in adsorption stage

Levels	Adsorption time/h	pH	Adsorption temperature/°C
1	3	3	30
0	2	2	20
-1	1	1	10

2.4.2 Desorption combination design

Value range of each factor was taken based on the results from the single factor experimental data. A full second-order polynomial model was constructed to evaluate the desorption yield (response variable, Y_2) as a

function of independent variables, namely desorption time, ethanol concentration, and desorption temperature and significant interactive terms. The factors and levels were shown in Table 2.

Table 2 Factors and levels in desorption stage

Levels	Desorption time /min	Ethanol concentration /%	Desorption temperature /°C
1	30	50	40
0	20	40	30
-1	10	30	20

2.4.3 Design determination of adsorption rate and desorption rate

1) Adsorption experiments

Taking 2.0000±0.0005 g pretreatment AB-8 macroporous adsorbent resin into 100 mL conical flask, and then added 50 mL condensed anthocyanins extraction liquid. This liquid was diluted by citric acid-citric acid sodium buffer solution in setting pH value, and then sealed and placed in a constant temperature water-bath oscillator with the oscillation speed of 110 r/min. Anthocyanin extraction rate was calculated by Equation (1):

$$\alpha = (A_0 - A_1) / A_0 \times 100\% \quad (1)$$

where, A_0 is anthocyanins mass concentration before adsorption, mg/mL; A_1 is anthocyanins mass concentration after adsorption, mg/mL; α is adsorption rate, %.

2) Desorption experiments

Taking 2.0000±0.0005 g pretreatment AB-8 macroporous adsorbent resin into 100 mL conical flask, and then added 50 mL fixed the concentration of ethanol solution. This liquid covered with sealing membrane was place in constant temperature water-bath oscillator with the oscillation speed of 110 r/min. Anthocyanin extraction rate was calculated by Equation (2):

$$\beta = A_2 V_2 / (A_0 - A_1) V_1 \times 100\% \quad (2)$$

where, A_2 is anthocyanins mass concentration in desorption solution; mg/mL; V_2 is volume of desorption solution; β is desorption rate, %.

2.5 Data analysis

Statistical analysis and equation construction of experimental data were performed by Design Expert software (ver6.0.10, Stat-Ease, Inc., Minneapolis, MN, US). The optimal parameters of anthocyanin purification followed by microwave extraction were obtained by using the embodied procedure of software.

3 Results and discussion

3.1 Interactions of factors on anthocyanin adsorption rate

The experiments were performed according to a central composite design (shown at left half part in Table 3), and the response surface methodology method was employed to determine the relative contributions of input parameters for the desorption ratio of anthocyanin.

Table 3 Experimental procedure and results in adsorption and desorption stages

Run	Adsorption stages				Desorption stages			
	time x_1	pH x_2	Temp x_3	Ad rate/%	De time x_4	Ec x_5	De temp x_6	De rate/%
1	0	0	0	87.10	0	0	0	83.73
2	0	0	0	90.65	0	0	0	89.29
3	0	0	0	92.33	0	0	0	86.61
4	0	0	0	89.78	0	0	0	86.39
5	0	0	0	89.99	0	0	0	82.39
6	1	1	0	82.75	1	1	0	76.15
7	-1	1	0	70.74	-1	1	0	69.34
8	1	-1	0	87.16	1	-1	0	73.22
9	-1	-1	0	73.18	-1	-1	0	63.10
10	1	0	-1	76.01	1	0	-1	68.27
11	-1	0	-1	69.19	-1	0	-1	59.59
12	0	1	-1	82.39	0	1	-1	76.70
13	0	-1	-1	81.20	0	-1	-1	60.61
14	0	1	1	75.50	0	1	1	67.28
15	0	-1	1	85.08	0	-1	1	60.89
16	-1	0	1	70.05	-1	0	1	68.48
17	1	0	1	77.85	1	0	1	78.72

Note: Ad = Adsorption; temp=temperature; EC = Ethanol Concentration; De = Desorption.

The analysis of variance (ANOVA) results from Table 4 indicated the regression equation was in extremely significant level at p level of 0.0029 and fit degree was high due to the lack of fit in insignificant level. Variation of 92.89% for the desorption rate of anthocyanin onto AB-8 macroporous resin was dependent upon the selected variables in terms of absorption time, ethanol concentration and temperature according to determination coefficient of 0.9289. Considering the interaction of these factors, the sequence that influencing the adsorption rate within the experimental model was as follows: adsorption time (F=17.77) > pH (F=3.25) >

dsorption temperature (F=0.31). Excluded non-significant terms in Table 4, a multiple regression equation (Equation (3)) was established to describe the relationship of absorption time, pH value and temperature on the adsorption ratio.

$$y_1 = 89.97 + 4.45x_1 - 9.02x_1^2 - 6.43x_3^2 \quad (3)$$

where, y_1 is adsorption rate, %; x_1 is adsorption time, h; x_3 is adsorption temperature, °C.

Table 4 Variance analysis of RSM in absorption stage

Source of variance	Sum of square	*df*	Mean square	*F* value	*P* value
Model	815.46	9	90.61	10.16	0.0029**
X_1	158.51	1	158.51	17.77	0.0040**
X_2	29.03	1	29.03	3.25	0.1142
X_3	2.75	1	2.75	0.31	0.5960
X_1^2	342.19	1	342.19	38.36	0.0004**
X_2^2	26.26	1	26.26	2.94	0.1299
X_3^2	174.08	1	174.08	19.52	0.0031**
X_1X_2	0.97	1	0.97	0.19	0.6721
X_1X_3	4.04	1	4.04	0.45	0.5225
X_2X_3	29.00	1	29.00	3.25	0.1144
Residual Error	62.44	7	8.92	4.49	0.0906
Lack-of-fit	48.13	3	16.04	6.32	0.3212
Error	14.31	4	3.58		
Total	877.90	16			

Note: ** extremly significant at (p<0.01), *significant at (p<0.05).

As shown in Figure 1, the anthocyanins adsorption rate was notably changed with the adsorption time and temperature. For pH level and adsorption time in fixed level, anthocyanidian adsorption rate first increased up to certain level, and then decreased with the increase of adsorption temperature. In the process of adsorption, rising temperature may improve transport rate of adsorbate across the external boundary layer and within the pores of adsorbent due to the decreased solution viscosity and surface tension[30]. The peak level of anthocyanin adsorption rate occurred at adsorption temperature of 20°C. When adsorption temperature was kept at fixed level, anthocyanins adsorption rate tended to the sharp rise. This was attributed to thermal accumulation, determined by the interaction of temperature and time, promotes the anthocyanin diffusion to adhere to the AB-8 macroporous adsorbent resin in the temperature of 10-20°C and time of 1.0-2.5 h, then a decline trend of anthocyanins adsorption rate. This may be resulted from the anthocyanin degradation caused by the thermal promotion in the temperature of 20-30°C and time of 2.5-3.0 h, and/or rising temperatures may promote irreversible interactions[36] to weaken the capacity of the adsorbent. According to F-value of variance analysis of X_1 and X_3 in Table 4, the adsorption time has greater effect than the adsorption temperature on the anthocyanin adsorption rate.

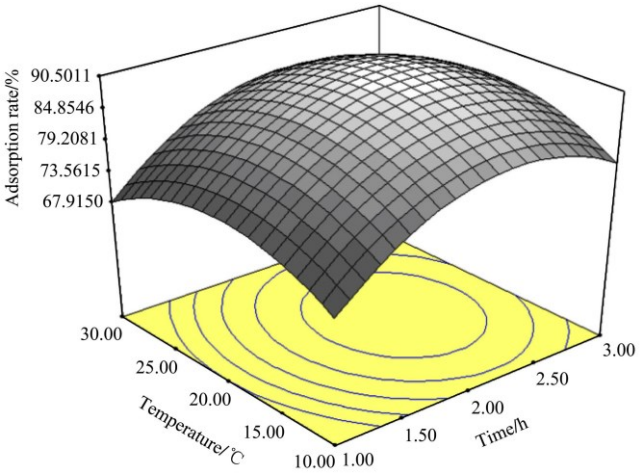

Figure 1 Interactions of temperature and time on adsorption rate of anthocyanin (at pH of 2)

The response surface in Figure 2 indicated the obvious interactions of adsorption time and pH value on anthocyanidian adsorption rate. When adsorption temperature was kept at 20°C and adsorption time was in fixed level, non-significant change of anthocyanidian adsorption rate with pH value was found due to anthocyanin keep high stability in acid solution. When pH was held at fixed level, adsorption rate was first significantly increased with the increase of adsorption time in the range of 1-2.5 h due to the promotion of thermal accumulation, and then turn on a slow decrease till end due to thermal degradation on the anthocanyin. According to the F-value of variance analysis of X_1 and X_2, the adsorption time had the higher influence on the anthocyanin adsorption rate than pH.

The response surface plot in Figure 3 presented the interactions of pH value and adsorption temperature on anthocyanin adsorption rate. In the conditions of adsorption time of 2 h and pH value, anthocyanin adsorption rate was obviously then slightly increased with the adsorption temperature. Rising dsorption temperature enhanced the diffusion of anthocaynin molecule toward AB-8 macroporous resin. However, in absorption

temperature of 20-30°C, the decline of concentration difference between the anthocyanin in solution and AB-8 macroporous resin powder due to absorption obstruction of the other bioactive components in extract liquid weakened the driving force of anthocyanin diffusion. At pH value in acidic case, the undissociated and dispersion interactions of phenols improve the capacity of adsorbents attracting phenolics[37]. AB-8 macroporous resin easily adsorbs anthocyanin in acid conditions. According to the *F*-value in Table 4, the influence of pH value on the anthocyanin adsorption rate was the more important than the adsorption temperature. This result was attributed to the susceptible nature of anthocyanin to acid solvent[38].

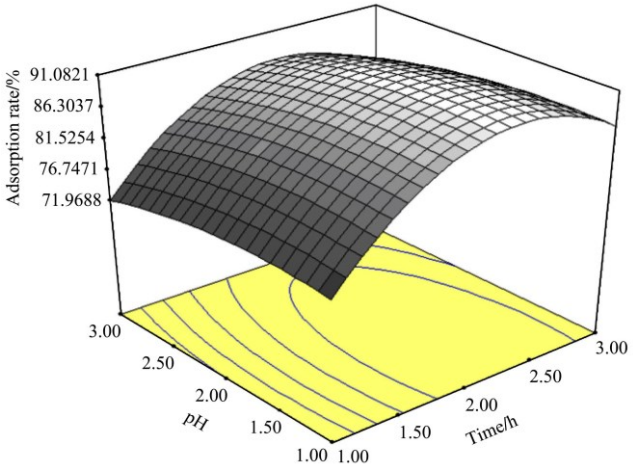

Figure 2 Interactions of time and pH value on adsorption rate of anthocyanin (temperature of 20°C)

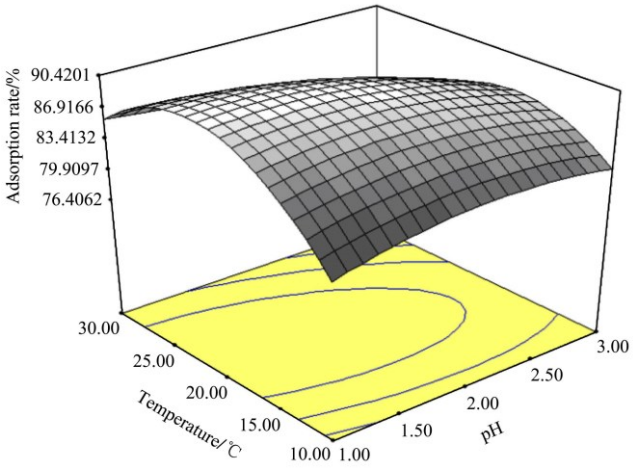

Figure 3 Interactions of pH value and temperature on adsorption rate of anthocyanin (at adorption time of 2 h)

3.2 Interaction of factors on the desorption rate of anthocyanin

The experiments were performed according to a central composite design (as shown in Table 3), and the response surface methodology was employed to determine the relative contributions of input parameters for the desorption ratio of anthocyanin. ANOVA results from Table 5 indicated the regression equation was in extremely significant level at *p* level of 0.0082 and fit degree was high due to the lack of fit in insignificant level. Variation of 90.25% for the desorption rate of anthocyanin on to AB-8 macroporous resin was dependent upon the selected variables in terms of absorption time, ethanol concentration and temperature according to determination coefficient of 0.9025. Excluded Non-significant terms in Table 5, a multiple regression equation (Equation (4)) was established to describe the relationship of desorption time, ethanol concentration, and desorption temperature on the desorption ratio.

$$y_2 = 85.69 + 4.44x_4 + 3.96x_5 - 6.38x_4^2 - 8.86x_6^2 \quad (4)$$

where, y_2 is desorption rate, %; x_4 is desorption time, min; x_5 is ethanol concentration, %; x_6 is adsorption temperature, ºC.

The analysis of variance (ANOVA) results (in Table 5) presented the influences on desorption ratio of blueberry anthocyanin that were desorption time (F=7.34) > ethanol concentration (F=5.84) > desorption temperature (F=0.56).

Table 5 Variance analysis of RSM in desorption stage

Source of variance	Sum of square	*df*	Mean square	*F* value	*P* value
Model	1391.31	9	154.59	7.20	0.0082**
X_1	157.53	1	157.53	7.34	0.0302*
X_2	125.37	1	125.37	5.84	0.0463*
X_3	12.08	1	12.08	0.56	0.4776
X_1^2	171.15	1	171.15	7.97	0.0256**
X_2^2	330.38	1	330.38	15.39	0.0057**
X_3^2	460.50	1	460.50	21.45	0.0024**
X_1X_2	2.74	1	2.74	0.13	0.7314
X_1X_3	0.93	1	0.93	0.043	0.8409
X_2X_3	23.43	1	23.43	1.09	0.3309
Residual Error	150.25	7	21.46		
Lack-of-fit	121.32	3	40.44	5.59	0.0649
Error	28.93	4	7.23		
Total	1541.56	16			

Note: ** extremly significant at (p<0.01), *significant at (p<0.05).

In Figure 4, the interaction of desorption time and ethanol concentration on desorption ratio of anthocyanin was presented when desorption temperature was kept at 30°C. The desorption ratio increases significantly with

the increase of ethanol concentration in desorption duration of 10-20 min. This was explained that the driving force caused by polarity difference between ethanol and anthocyanin was higher than that of affinity of anthocyanin and macroporous adsorbent resin. Whereas with the further recovery proceeding, the desorption rate kept stable with the increase of ethanol concentration due to desorption rate limiting controlled by intra-particle mass transfer step. According to the *F*-value in Table 5, desorption time has more obviously influence on the desorption rate of anthocyanin than the ethanol concentration.

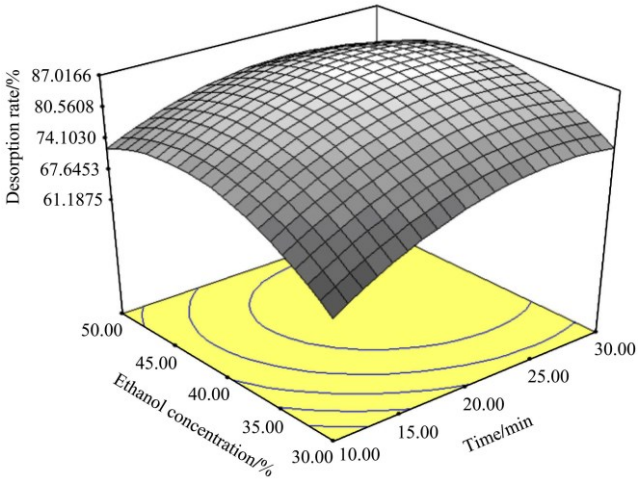

Figure 4 Interactions of time and ethanol concentration on desorption rate of anthocyanin (at temperature of 30°C)

Temperature influences desorption process by two simultaneous ways: (i) acceleration way: increasing the transport capacity of adsorbate by overcoming the affinity onto absorbent across the external boundary layer, and (ii) degradation way: induce the degradation of anthocyanin due to the thermal accumulations from rising temperature. Based on the interactions between desorption time and temperature on the desorption ratio of anthocyanin using AB-8 microporous resin shown in Figure 5, the desorption ratio increased with the temperature up to 30°C due to the predomination of acceleration way, and then decreased with further rising temperature due to the predomination of degradation way. Under the same temperature, the desorption rate of anthocyanin sharply increased with the time up to 25 min, followed by a constant stage. According to *F*-value of variation factors in Table 5, desorption time has more obviously influence on the desorption rate of anthocyanin than the temperature.

As shown in Figure 6, temperature and ethanol concentration had distinct impacts on the desorption rate of anthocyanin. The changes of desorption rate tended to climb up then decline with the increase of temperature and ethanol concentration, and the highest level of was achieved at temperature of 30°C and ethanol concentration of 40% since the amount of water in the alcoholic solution influences polarity[39]. No significant change of anthocyanin content was found under ethanol concentration from 40% to 50%. According to the *F*-value in Table 5, the ethanol concentration had greater effect on desorption rate of anthocyanin than the temperature.

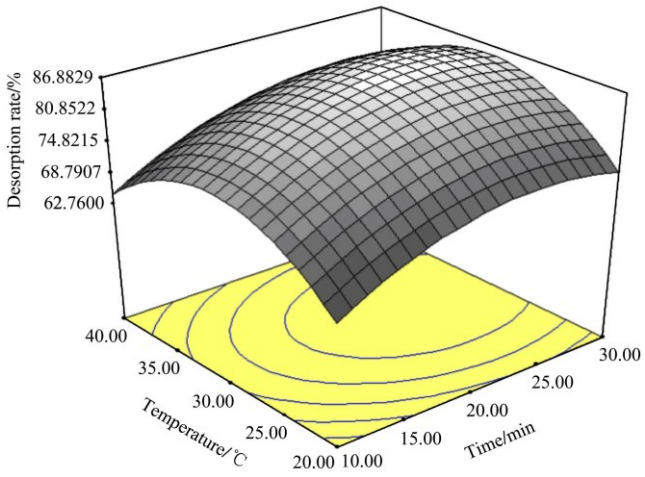

Figure 5 Interactions of time and temperature on desorption rate of anthocyanin (in ethanol concentration of 40%)

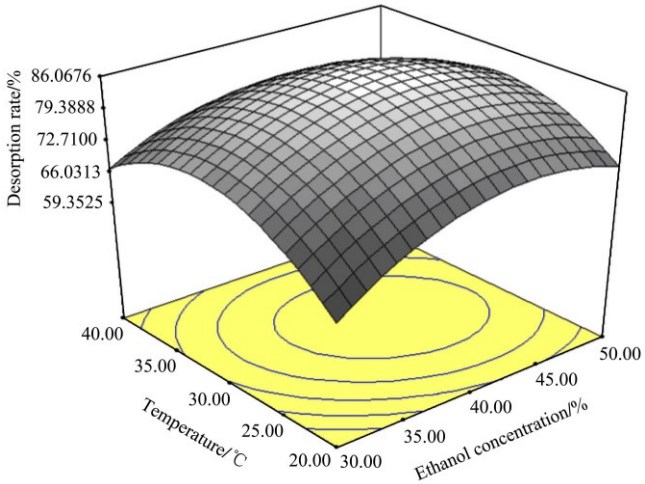

Figure 6 Interactions of ethanol concentration and temperature on desorption rate of anthocyanin (desorption time of 20 min)

Desorption parameters in terms of desorption time, ethanol concentration and temperature had significant influence on the desorption rate of anthocyanin from

microporous resin. The desorption rate of anthocyanin tended to a sharp increase to the highest value of 86.89% at 20 min, and followed by a slow drop with desorption time attributed to the re-adsorption of anthocyanin[35]. Desorption rate markedly increases from 34.43% to 87.63% with the elevation of ethanol concentration from 30% to 40%. Temperature had obviously negative effects on anthocyanin content in desorption solvent when it exceed 30°C because of thermal degradation caused by the predomination of temperature elevation[40].

3.3 Optimizations of parameters for adsorption of anthocyanin

Both adsorption and desorption parameters of blueberry anthocyanin were optimized and combined using Design Expert Software (ver 6.0.10). The variation ranges for each parameter were determined, and then selected "Numerical" option in the optimization column and set maximum value for the adsorption rate and desorption rate of blueberry anthocyanin. The optimized parameters were obtained as shown in Table 6 and Table 7.

Table 6 The optimization results and validation values of adsorption stage

Factors	Adsorption time/h	pH value	Adsorption Temperature/°C	Prediction value/%	Validation value/%
Optimization value	2.0	2.03	19.7	91.97	—
Validation value	2.0	2.0	20.0	—	90.65

Table 7 The optimization results and validation values of desorption test

Factors	Desorption time /min	Ethanol Concentration /%	Desorption Temperature /°C	Prediction value /%	Validation value /%
Optimization value	20.2	39.50	30.1	85.69	—
Validation value	20	40.0	30	—	84.98

Due to the limitation of instrument, the experimental condition and the environmental factors during the test, there exists certain error between theoretic value and the experimental one. The optimal conditions were generated by validation in Table 6 and Table 7. The test was repeated for three times to obtain the relative error between optimum value and predictive value in absorption and desorption stage as 1.44% and 0.83%, respectively.

4 Conclusions

AB-8 macroporous resin is a feasible adsorbent for the purification of crude anthocyanin extracted by using microwave extraction method from blueberry powder. The adsorption and desorption characteristics of AB-8 macroporous resin determine the purification yield of the anthocyanin. In adsorption stage, adsorption time has the most significant effect on the adsorption rate of blueberry anthocyanin, followed by pH value, and adsorption temperature is the least; in desorption stage, the influences of factors on desorption rate of blueberry anthocyanin from high to low are as desorption time, ethanol concentration, desorption temperature. Temperature in least effect makes for the avoidance of anthocyanin degradation in both adsorption and desorption stages. An optimal technological processing of adsorption and desorption for anthocyanin purification were obtained by using AB-8 macroporous resin as purification media in adsorption time of 2 h under pH 2.0 and adsorption temperature of 20°C, followed by desorption time of 20 min under ethanol concentration of 40% and desorption temperature of 30°C, and the anthocyanin yield may achieve 78.81%. Optimal purification process and parameters of anthocyanin improve and perfect the application of the microwave technology in extraction of bioactive components from plant-materials.

Acknowledgments

The authors acknowledge that the financial support provided by the Key Project of Science and Technology Research of Heilongjiang Provincial Education Department of China (No: 12521z003) for this research project.

[References]

[1] Mauro B, Loredana C, Elisa E. An innovative method for the purification of anthocyanins from grape skin extracts by using liquid and sub-critical carbon dioxide. Separation and Purification Technology, 2008; 64(2): 192–197. doi: 10.1016/j.seppur.2008.10.012.

[2] Pu J, Si Y Y, Ying D, Zhang X G, Jin Y, Kan J Q, et al. Optimization of purification conditions of radish (*Raphanus*

sativus L.) anthocyanin-rich extracts using chitosan. LWT–Food Science Technology, 2011; 44(10): 1–7. doi: 10.1016/j.lwt.2011.06.003.

[3] Li R, Wang P, Guo Q Q, Wang Z Y. Anthocyanin composition and content of the vaccinium uliginosum berry. Food Chemistry, 2011; 125(1): 116–120. doi: 10.1016/j.foodchem.2010.08.046.

[4] Dawidowicz A L, Wianowska D, Baraniak B. The antioxidant properties of alcoholic extracts from *Sambucus nigra* L. LWT–Food Science Technology, 2006; 39(3): 308–315. doi: 10.1016/j.lwt.2005.01.005.

[5] Thomasset S, Teller N, Cai H, Marko D, Berry D P, Steward W P, et al. Do anthocyanins and anthocyanidins, cancer chemopreventive pigments in the diet, merit development as potential drugs? Cancer Chemotherapy and Pharmacology, 2009; 64(1): 201–211. doi: 10.1007/ s00280-009-0976-y.

[6] Marczylo T H, Cooke D, Brown K, Steward W P, Gescher A J. Pharmacokinetics and metabolism of the putative cancer chemopreventive agent cyanidin-3-glucoside in mice. Cancer Chemotherapy and Pharmacology, 2009; 64(6): 1261–1268. doi: 10.1007/s00280-009-0996-7.

[7] Bonerz D, Wurth K, Dietrich H. Analytical characterization and the impact of ageing in anthocyanin composition and degradation in juices from five sour cherry cultivars. European Food Research and Technology, 2006; 224(3): 355–364. doi: 10.1007/s00217-006-0328-7.

[8] Greenspan P, Bauer J D, Pollock S H, Gangemi J D, Mayer E P, Ghaffar A. Antiinflammatory properties of the muscadine grape (*Vitis rotundifolia*). Journal of Agricultural and Food Chemistry, 2005; 53(11): 8481–8484. doi: 10.1021/jf058015.

[9] Prior R L, Wilkes S E, Rogers T R, Khanal R C, Wu X L, Howard L R. Purified blueberry anthocyanins and blueberry juice alter development of obesity in mice fed an obesogenic high-fat diet. Food Chemistry, 2010; 58(7): 3970–3976. doi: 10.1021/jf902852d.

[10] Giongo L, Vrhovsek U, Gasperi F, Endrizzi I, Palmieri L, Saviane A, et al. Three-year highbush blueberry survey in different European locations for the fresh and the processing markets. Acta Horticulturae, 2009; 810(8): 887–894.

[11] You Q, Wang B W, Chen F, Huang Z L, Wang X, Luo P G. Comparison of anthocyanins and phenolics in organically and conventionally grown blueberries in selected cultivars. Food Chemistry, 2011; 125(1): 201–208. doi: 10.1016/j. foodchem.2010.08.063.

[12] Barnes J S, Nguyen H P, Shen S J, Schug K A. General method for extraction of blueberry anthocyanins and identification using high performance liquid chromatography–electrospray ionization-ion trap-time of flight-mass spectrometry. Journal of Chromatography A, 2009; 1216(23): 4728–4735. doi: 10.1016/j.chroma.2009. 04.032.

[13] Nicoué E E, Savard S, Belkacemi K. Anthocyanins in wild blueberries of Quebec: Extraction and Identification. Food Chemistry, 2007; 55(14): 5626–5635. doi: 10.1021/ jf0703304.

[14] Yao X M, Lu S W, Zheng X Z, Sun J K, Ren C Y, Zhang Y L, et al. Effect of cooking conditions on quality of sweetened adzuki *an*. Int J Agric & Biol Eng, 2014; 7(4): 134–142. doi: 10.3965/j.ijabe.20140704.015.

[15] Mu Y Q, Zhao X H, Liu B X, Liu C H, Zheng X Z. Influences of microwave vacuum puffing conditions on anthocyanin content of raspberry snack. Int J Agric & Biol Eng, 2013; 6(3): 80－87. doi: 10.3965/j.ijabe.20130603. 0010.

[16] Liu Y F, Liu J X, Chen X F, Liu Y W, Di D L. Preparative separation and purification of lycopene from tomato skins extracts by macroporous adsorption resins. Food Chemistry, 2010; 123(4): 1027–1034. doi: 10.1016/j.foodchem.2010. 05.055.

[17] Engelberth A S, Clausen E C, Carrier D J. Comparing extraction methods to recover ginseng saponins from American ginseng (Panax quinquefolium), followed by purification using fast centrifugal partition chromatography with HPLC verification. Separation and Purification Technology, 2010; 72(1): 1–6. doi: 10.1016/j.seppur.2009. 12.002.

[18] Zheng X Z, Xu X W, Liu C H, Sun Y, Lin Z, Liu H J. Extraction characteristics and optimal parameters of anthocyanin from blueberry powder under microwave-assisted extraction conditions. Separation and Purification Technology, 2013; 104: 17–25. doi: 10.1016/j.seppur. 2012.11.011.

[19] Zhang B, Yang R Y, Zhao Y, Liu C Z. Separation of chlorogenic acid from honeysuckle crude extracts by macroporous resins. Journal of Chromatography B, 2008; 867(2): 253–258. doi: 10.1016/j.jchromb.2008.04.016.

[20] Ma C Y, Tao G J, Tang J, Lou Z X, Wang H X, Gu X H, et al. Preparative separation and purification of rosavin in Rhodiola rosea by macroporous adsorption resins. Separation and Purification Technology, 2009; 69(1): 22–28. doi: 10.1016/j.seppur.2009.06.002.

[21] Chandrasekhar J, Madhusudhan M C, Raghavarao K S M S. Extraction of anthocyanins from red cabbage and purification using adsorption. Food and Bioproducts Processing, 2012; 90(4): 615–623. doi: 10.1016/j.fbp.2012.07.004.

[22] You Q H, Yin X L, Zhang S N, Jiang Z H. Extraction, purification, and antioxidant activities of polysaccharides from Tricholoma mongolicum Imai. Carbohydrate Polymers, 2014; 99: 1–10. doi: 10.1016/j.carbpol.2013.

07.088.

[23] Hu Z Q, Jia L L, Yue T L. Microwave-low-pressure process (MWLP): An effective technology applied in extraction of total polyphenols. Int J Agric & Biol Eng, 2013; 6(4): 84–95. doi: 10.3965/j.ijabe.20130604.010.

[24] Oh H J, Hye R J, Kyeong Y J, Kim J H. Evaluation of adsorbents for separation and purification of paclitaxel from plant cell cultures. Process Biochemistry, 2012; 47(2): 331–334. doi: 10.1016/j.procbio.2011.11.004.

[25] Lee J Y, Kim J H. Development and optimization of a novel simultaneous microwave-assisted extraction and adsorbent treatment process for separation and recovery of paclitaxel from plant cell cultures. Separation and Purification Technology, 2011; 80(2): 240–245. doi: 10.1016/j.seppur.2011.05.001.

[26] Geng X T, Ren P, Pi G P, Shi R F, Yuan Z, Wang C H. High selective purification of flavonoids from natural plants based on polymeric adsorbent with hydrogen-bonding interaction. Journal of Chromatography A, 2009; 1216(47): 8331–8338. doi: 10.1016/j.chroma. 2009.09.015.

[27] Wu Y C, Wang Y, Zhang W L, Han J, Liu Y, Hu Y T, et al. Extraction and preliminary purification of anthocyanins from grape juice in aqueous two-phase system. Separation and Purification Technology, 2014; 124:170–178. doi: 10.1016/j.seppur.2014.01.025.

[28] Jing P, Ruan S Y, Dong Y, Zhang X G, Yue J, Kan J Q, et al. Optimization of purification conditions of radish (*Raphanus sativus* L.) anthocyanin-rich extracts using chitosan. *LWT*–Food Science Technology, 2011; 44(10): 2097–2103. doi: 10.1016/j.lwt.2011.06.003.

[29] Jampani C, Naik A, Raghavarao K S M S. Purification of anthocyanins from jamun (*Syzygium cumini* L.) employing adsorption. Separation and Purification Technology, 2014; 125: 170–178. doi: 10.1016/j.seppur.2014.01.047.

[30] Soto M L, Moure A, Dominguez H, Parajo J C. Recovery, concentration and purification of phenolic compounds by adsorption: A review. Journal of Food Engineering, 2011; 105(1): 1–27. doi: 10.1016/j.jfoodeng.2011.02.010.

[31] Li X Y, Song H, Yao S, Jia C M, Yang Y Y, Zhu W X. Quantitative analysis and recovery optimisation of flavonoids and anthocyanins in sugar-making process of sugarcane industry. Food Chemistry, 2011; 125(1): 150–157. doi: 10.1016/j.foodchem.2010.08.053.

[32] Ma C Y, Tao G J, Tang J, Lou Z X, Wang H X, Gu X H, et al. Preparative separation and purification of rosavin in Rhodiola rosea by macroporous adsorption resins. Separation and Purification Technology, 2009; 69(1): 22–28. doi: 10.1016/ j.seppur.2009.06.002.

[33] Zhao Z Y, Dong L L, Wu Y L, Lin F. Preliminary separation and purification of rutin and quercetin from Euonymus alatus (Thunb.) Siebold extracts by macroporous resins. Food and Bioproducts Processing, 2011; 89(4): 266–272. doi: 10.1016/j.fbp.2010.11.001.

[34] Dong A Y, A G, Zhao G J, Wang X D, Yan L N. Purification of anthocyanins from red-core radish by macroporous resin. Journal of Food Science, 2011; 32: 21–24. (in Chinese with English abstract).

[35] Xu X W. Studies on anthocyanin extraction and purification from blueberry by microwave method. Master thesis 2012, Northeast Agricultural University, Harbin, China. (in Chinese with English abstract).

[36] Qiu N, Guo S, Chang Y. Study upon kinetic process of apple juice adsorption decoloration by using adsorbent resin. Journal of Food Engineering, 2007; 81(1): 243–249. doi: 10.1016/j.jfoodeng.2006.10.030.

[37] Caqueret V, Bostyn S, Cagnon B, Fauduet H. Purification of sugar beet vinasse – adsorption of polyphenolic and dark colored compounds on different commercial activated carbons. Bioresource Technology, 2008; 99(13): 5814–5821. doi: 10.1016/j.biortech.2007.10.009.

[38] Liu S B, Fu Y Q, Nian S. Buffering colour fluctuation of purple sweet potato anthocyanins to acidity variation by surfactants. Food Chemistry, 2014; 162: 16–21. doi: 10.1016/j.foodchem.2014.04.029.

[39] Caetano M, Valderrama C, Farran A, Cortina J L. Phenol removal from aqueous solution by adsorption and ion exchange mechanisms onto polymeric resins. Journal of Colloid and Interface Science, 2009; 338(2): 402–409. doi: 10.1016/j.jcis.2009.06.062.

[40] Kara Ş, Erçelebi E A. Thermal degradation kinetics of anthocyanins and visual colour of Urmu mulberry (Morus nigra L.). Journal of Food Engineering, 2013; 116(2): 541–547. doi: 10.1016/j.jfoodeng.2012.12.030.