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Simultaneous Decoloration and Deproteinization of Crude Polysaccharide from Pumpkin Residues by Cross-Linked Polystyrene Macroporous Resin

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ABSTRACT: A novel method for the purification of crude polysaccharide from fermentation broth of pumpkin residues by macroporous resins was developed. Through static adsorption and desorption and adsorption kinetic tests, six resins (AB-8, S-8, HPH480, HPD100, X-5, and D101) with different polarity, diameter, and surface area were studied for simultaneous decoloration and deproteinization of crude polysaccharide, and S-8 was chosen as the best one. Dynamic breakthrough and desorption tests were performed in a glass column packed with S-8 resin, and the resulting adsorption ratios of pigment and protein were 84.3% and 75.9% (w/w), respectively, with a recovery ratio of polysaccharide 84.7% (w/w). S-8 resin also exhibited higher purification efficiency than the other tested traditional methods. Moreover, UV/vis spectroscopy (200−900 nm) analysis revealed most of the pigment and protein were absorbed by S-8 resin, and HPLC (containing a refractive index detector and a HPSEC column) results indicated that there was no degradation of the polysaccharide. This automated and efficient method via adsorption−desorption strategy could have potential in scale-up purification and preparation of polysaccharide in the future.

KEYWORDS: *purification, adsorption, decoloration, deproteinization, polysaccharide, macroporous resin*

INTRODUCTION

Pumpkins, which belong to the family Cucubitaceae, have been used frequently as a kind of functional food or medicine.[1] They represent important economical species cultivated worldwide and have high production.[2] Nowadays, relevant studies mainly focused on the structure and medicinal activity of their soluble components, such as polysaccharide, sterol, *p*-aminobenzoic acid, protein, and peptide.[1,3,4] In contrast, pumpkin residues, a resultant lignocellulosic material obtained from water extraction of the soluble components, are insufficiently utilized. Recently, pumpkin residues were reused and bioconverted into soluble polysaccharide by *Trichoderma reesei* RUT-C30 and *Phanerochaete chrysosporium* Burdsall in our reported literature.[5] However, the impurities, including the pigment carotenoids,[6] the enzymes secreted by microorganisms and the original component proteins in pumpkin residues, existed in the crude polysaccharide solution, which interferred with determining the structural and functional properties of the polysaccharide. Therefore, a suitable method for purification of the crude polysaccharide is indispensable.

Till now, several purification methods that employ ethanol, H_2O_2, and Sevage reagent have been widely used for the decoloration or deproteinization of crude polysaccharide.[7−10] These liquid−liquid extraction (LLE), precipitation, or chemical approaches are often time and volume consuming, labor intensive, and difficult to automate,[11,12] and they may cause partial hydrolysis of polysaccharide, resulting in variable bioactivities.[13] Solid-phase extraction (SPE) is a popular technique for rapid and selective sample preconcentration and purification of analytical samples.[14] The adsorption mechanism of SPE depends on knowledge of the hydrophobic, polar, and ionogenic properties of both the solute and the sorbent. The most common adsorption mechanisms in SPE are based on van der Waals forces ("nonpolar" interactions), hydrogen bonding, dipole−dipole forces ("polar" interactions), and cation−anion interactions ("ionic" interactions).[14,15] Specifically, the widely used polymeric sorbent polystyrene divinylbenzene (PS-DVB) copolymers belong to hyper-cross-linked macroporous resins, and they have extremely rigid networks and great adsorption capacities with respect to other known organic and inorganic sorbents.[14,16] Moreover, PS-DVB resins are increasing in use because of their excellent properties, such as great analyte retention, low solvent consumption, shorter processing times, easy regeneration, and environmentally friendly features.[12,15]

So far, macroporous resins have been widely used in the separation and purification of targeted component or in the removal of the impurities from the crude samples,[17−19] while little information was available about their application in simultaneous decoloration and deproteinization of crude polysaccharide. Toward this objective, experiments have been carried out in this study to evaluate the purification effect of crude polysaccharide on six macroporous resins (AB-8, S-8, HPH480, HPD100, X-5, and D101) through static tests. Then, the adsorption efficiency of the selected resin was improved through dynamic adsorption and desorption tests. Also, a comparison of the purification effects was made between the selected strategy and three other traditional purification techniques, including use of H_2O_2 (strong oxidant), Sevage reagent (denaturant), and active carbon (absorbent). Moreover, the spectral properties of the polysaccharide before and after

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Table 1. Physical and Chemical Properties of the Macroporous Resins

	polarity	appearance	particle diameter (mm)	surface area (m^2/g)	average pore diameter (nm)
AB-8	weak-polar	milk white	0.3−1.25	480−520	13−14
S-8	polar	slight yellow	0.3−1.25	380−420	28−30
HPH480	polar	brown	0.2−1.25	550−650	100−120
X-5	nonpolar	milk white	0.2−1.25	500−600	29−30
D101	nonpolar	milk white	0.3−1.25	600−700	10−12
HPD100	nonpolar	milk white	0.3−1.25	550−650	90−100

adsorption by the selected resin were measured to test and verify the adsorption effect.

MATERIALS AND METHODS

Adsorbents, Chemicals, and Standards. Four macroporous resins coded D101, S-8, X-5, and AB-8 were provided by the Chemical Plant of Nankai University (Tianjin, China); HPH480 and HPD100 were provided by Cangzhou Bon Adsorber Technology Co. (Hebei, China). Their physical and chemical properties are listed in Table 1. Before the adsorption experiment, each weighed resin (50 g) was pretreated with 1 L of HCl (1 M) and 1 L of NaOH (1 M) solutions sequentially to remove the monomers and porogenic diluents trapped inside the pores. Subsequently, they were washed thoroughly with deionized water (5 L) and dried at 60 °C under reduced pressure for 12 h. Finally, preweighed amounts of dried resins were soaked in 1 L of 95% ethanol and washed thoroughly with 5 L of deionized water.[20,21]

The acetonitrile, methyl alcohol, and dichloromethane purchased from MREDA Technology Inc. (Beijing, China) were of HPLC grade, and the remaining reagents obtained from Sinopharm Chemical Reagent Co., Ltd. (Beijing, China) were of analytical grade. Dextran molecular weight markers (1400, 670, 270, 50, 25, 12, 5, and 0.342 kDa), standard β-carotene, and bovine serum albumin were all obtained from Sigma Chemical Co.

Preparation of Crude Polysaccharide. Crude polysaccharide was obtained from the fermentation broth of pumpkin residues as follows. First, after fermentation of pumpkin residues by *T. reesei* RUT-C30 and *P. chrysosporium* Burdsall in a rotary shaker incubator (180 rpm) at 30 °C for 6 days, the fermentation broth was centrifuged at 8000*g* for 15 min to remove bacterial cells, as our previous literature reported.[5] Then, the resultant supernatant was mixed with three volumes of ethanol (95%), and the mixtures were stored at 4 °C overnight.[22] Finally, the precipitates from the ethanol dispersion were collected by centrifugation at 6000*g* for 15 min, and then they were dissolved in distilled water and dialyzed (cutoff molecular weight ≈ 3.5 kDa) for 3 days against distilled water to separate the crude polysaccharide.

Analysis of Pigment, Protein, and Polysaccharide. Pigment β-carotene was prepared according to Kurz et al.[23] with slight modifications, which involved the addition of ultrasound treatment (60 W, 15 min) after mixing pumpkin residues (3 g) and methanol (50 mL) to enhance the yield. The concentration of β-carotene was determined according to the method established by our laboratory through a Shimadzu model HPLC system (Shimadzu Corp., Kyoto, Japan) consisting of a diode array detector (SPD-M20A) and a column oven (CTO-20A). The chromatography column [Diamonsil C18 (250 mm ×4.6 mm i.d., 5 μm particle size)] was obtained from Bonna-Agela Technologies Inc. (Venusil). Data were collected, processed, and analyzed using Class-VP Chromatography Laboratory Automated software (Shimadzu Corp.). The chromatographic separations were carried out at 445 nm, 30 °C. The mobile phase were acetonitrile, methyl alcohol, and dichloromethane (6:2:2, v/v/v) with a flow of 1.0 mL/min, and the injection volume was 20 μL.

The concentrations of protein and polysaccharide were analyzed by a UV/vis 2802 spectrophotometer (UNICO Instrument Co., Ltd., Shanghai, China). The concentration of protein was determined according to the method of Bradford[24] using bovine serum albumin as standard, and the concentration of polysaccharide was measured by the phenol−sulfuric acid method using fructose as standard.[25]

Static Adsorption and Desorption Tests for Screening of Resins. Static adsorption and desorption tests were performed to evaluate the adsorption and desorption ratios for preliminary screening of the most efficient resin. In the adsorption test, 1 g of resin (dry weight basis) was introduced into a 150 mL airtight conical flask with 50 mL of 1 mg/mL crude polysaccharide solution (The concentrations of polysaccharide, protein, and pigment were 0.728×10^3, 0.057×10^3, and 0.593 μg/mL, respectively). The flasks were shaken (170 rpm) at 20 °C in a water-bath shaker for 10 h until adsorption equilibrium, and the liquid phase was separated from the sample solution by centrifugation at 4000*g* for 10 min. Then, in the desorption test, the adsorbents were desorbed by gradient flushing with 60 mL of NaCl solution (50 mM, pH 7.0) and 60 mL of ethanol−water (70:30, v/v) in a water-bath shaker (170 rpm, 20 °C) until desorption equilibrium. For both static adsorption and desorption tests, the concentration of pigment in the fluid phase was analyzed by HPLC every 20 min, and the concentrations of protein and polysaccharide were analyzed by UV/vis spectrophotometry at equal time intervals (20 min). The equations for quantification of these parameters were expressed as follows:

Absorption ratio

$$A\ (\%) = \frac{(C_o - C_e)}{C_o} \times 100\% \quad (1)$$

Desorption ratio

$$D\ (\%) = C_d \times \frac{V_d}{(C_o - C_e)V_i} \times 100\% \quad (2)$$

Adsorption capacity

$$q_t = \frac{(C_o - C_t)V_i}{W} \times 100\% \quad (3)$$

where A and D are the adsorption ratios (%) and desorption ratio (%), respectively. q_t is the adsorption capacity (mg/g dry resin for protein and polysaccharide and μg/g dry resin for pigment) at time t (min). C_o, C_e, C_t, and C_d are the concentrations of pigment (μg/mL), protein (mg/mL) and polysaccharide (mg/mL) in the solution at the initial adsorption stage, at adsorption equilibrium, at time t (min), and at desorption stage, respectively. V_i and V_d are the volumes of crude polysaccharide solution initially used (mL) and the volumes of the desorption solution (mL) in the study, respectively. W is the weight of the resin used (g).

Adsorption Kinetics of the Selected Resins. The adsorption kinetics of the selected macroporous resins was studied as the static adsorption test described above, except for a change of adsorption time to 3 h until adsorption equilibrium. The optimum resin was finally determined on the basis of the adsorption rates of the resins. Time courses of adsorption were evaluated using the Langmuir adsorption rate equation:

$$\ln \frac{q_e}{q_e - q_t} = Kt + m \quad (4)$$

where q_t and q_e are the adsorption capacities (mg/g dry resin for protein and polysaccharide and μg/g dry resin for pigment) at time t

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(min) and at equilibrium stage, respectively. K is the equilibrium adsorption rate, and m is a constant.

Adsorption Isotherms of the Selected Resin. Absorption isotherm data could give information about the affinity between solutes and adsorbent. Therein Langmuir and the Freundlich isotherms are often used to reveal the linearity fitting and to describe how solutes interact with the resins. Langmuir equation and Freundlich equation[26] used to describe the interaction of solutes with the resin were as follows:

$$\frac{C_e}{q_e} = \frac{C_e}{q_m} + \frac{1}{K_L q_m} \quad (5)$$

$$q_e = aC_e^{1/n} \quad (6)$$

where C_e and q_e represent the same parameters as in formulas 1 and 4. q_m is the theoretically calculated maximum adsorption capacity (mg/g dry resin for protein and polysaccharide and μg/g dry resin for pigment). K_L is the adsorption equilibrium constant. a is the Freundlich constant, an indicator of adsorption capacity. $1/n$ is an empirical constant related to the magnitude of the adsorption driving force.

Dynamic Adsorption and Desorption Tests on the Selected S-8 Resin. Breakthrough volume is very important in solid-phase extraction because it represents the maximum sample volume that can be preconcentrated without loss of analytes during the loading of sample.[27] It was detected by dynamic adsorption and desorption tests in this experiment. Dynamic adsorption and desorption tests were carried out in a glass column (16 mm × 400 mm) wet-packed with pretreated S-8 resin, and the bed volume (BV) of the resin was 20 mL. In the dynamic adsorption test, the crude polysaccharide solution (the same as static adsorption) was loaded continuously on the glass column and then the test was performed with the S-8 resin at a flow rate of 1 mL/min. In dynamic desorption test, the adsorbents were eluted with gradient NaCl solution (50 mM, pH 7.0) and ethanol–water (70:30, v/v) for respective volumes until adsorption equilibrium, at a flow rate of 1 mL/min. For both adsorption and desorption tests, the concentrations of pigment, protein, and polysaccharide in the generating fluid were detected by the same method as static adsorption and desorption tests.

Purification of Crude Polysaccharide by H_2O_2, Sevage, and Active Carbon. The crude polysaccharide used in the following experiments was the same as in the dynamic adsorption test described above. Purification of crude polysaccharide by H_2O_2 was developed according to Yu et al.[28] with some modifications, which reflected in the changed volume of H_2O_2 used as follows: 50 mL of crude polysaccharide solution and 50 mL of 30% H_2O_2 were mixed in a 250 mL Erlenmeyer flask, which was shaken (170 rpm) at 20 °C for 5 h. The fluid after reaction was centrifuged at 6000g for 15 min to remove insoluble impurities. Purification of crude polysaccharide by Sevage reagent (n-BuOH/$CHCl_3$, v/v = 1:4) was developed according to Staub.[7] Purification of crude polysaccharide by active carbon was performed according to the optimized method established by our laboratory previously. Particularly, 50 mL of crude polysaccharide solution flowed past a separating funnel packed with 20 g of dried active carbon five times. The fluid after reaction was centrifuged at 6000g for 15 min to remove residual active carbon and insoluble impurities. For all these three traditional purification methods, the concentrations of pigment, protein, and polysaccharide were detected by the same method as in the dynamic adsorption tests. The following equations are used to make comparison of purification efficiencies of the four methods.

$$\text{decoloration/deproteinization ratio (\%)} = \frac{(M_b - M_a)}{M_b} \times 100\% \quad (7)$$

$$\text{polysaccharide recovery ratio (\%)} = \frac{P_a}{P_b} \times 100\% \quad (8)$$

where M_b and M_a are the concentrations of pigment (μg/mL) or protein (mg/mL) before and after treatment, respectively. P_b and P_a are the concentrations of polysaccharide (mg/mL) before and after treatment, respectively.

Characterization of Polysaccharide before and after Adsorption. In order to validate the adsorption effect of column packed S-8 resin, the polysaccharide solution before and after treatment was characterized by UV/vis spectroscopy and HPLC. Specially, the UV/vis full-wave spectra (200–900 nm) of the crude polysaccharide before and after adsorption were determined by a UV/vis 2802 spectrophotometer from UNICO Instrument Co., Ltd. (Shanghai, China); the molecular weights of polysaccharide before and after adsorption were determined by high-performance size exclusion chromatography (HPSEC) on a HPLC system (KNAUER series, Germany) with a refractive index detector and a Shodex SB 805 HQ HPSEC column (8 mm × 300 mm, separation ranges 0.10–4000 kDa). The column oven temperature was 35 °C. The mobile phase was sodium nitrate (0.1 M) and the flow rate was 0.5 mL/min. Solutions of sample and standards (2 mg/mL) were filtered through 0.45 μm membranes, and the injection volume was 20 μL. The regression lines between molecular weight and retention time was $y = -0.6515x + 16.4465$ ($R^2 = 0.9239$, $n = 9$), where y is the logarithm of the molecular weight (Da) and x is the retention time (min).

RESULTS AND DISCUSSION

Static Adsorption and Desorption Tests of Six Resins. As shown in Figure 1A, both S-8 and AB-8 resins exhibited higher adsorption ratios toward pigment and protein than those of the other four resins. On the other hand, all adsorption ratios of polysaccharide on six resins were lower than that of pigment and protein, and they had no significant differences except for a much lower value on HPD100 resin.

In general, the selection of proper resins should be in accordance with their physical and chemical properties. Basically, polarity, surface area, and average pore diameter, etc. are important factors for selecting a proper resin.[29,30] All the tested resins are types of PS-DVB polymers, excluding the possibility that different resin types resulted in different adsorption capacities. The hydrophily and adsorption properties of PS-DVB polymers can also be increased by light surface modifications with a polar substituent, such as ester (AB-8) and methylamine (S-8). Both hydrophilic resins S-8 (polar) and AB-8 (weakly polar) exhibited relatively better adsorption ratios than those of the three nonpolar resins, indicating that polarity played an important role in adsorbing the pigment and protein. The mechanism could be explained by the polar groups reducing the interfacial tension between the polymer surface and sample and therefore increasing the contact between analytes and polymeric sorbent.[11] Another polar resin, HPH480, exhibited lower adsorption ratios of pigment and protein, perhaps due to its large average pore diameter, whose distribution is another important factor affecting adsorption. The low adsorption ratios of polysaccharide on all of the tested six resins perhaps were as a result of the high molecular weight and the high space structure of polysaccharide.

It was also observed that only S-8, AB-8, and HPH 480 showed higher desorption ratios than the other three resins in term of pigment and protein, as illustrated in Figure 1B. The different mechanisms of adsorption (retention) or desorption (elution) are due to intermolecular forces between the analyte, the active sites on the surface of the adsorbent, and the liquid phase.[15] The reason why three polar resins had higher desorption ratios toward pigment and protein was that they possessed a lower affinity for solute than the solvent; consequently, the desorption performances of pigment and

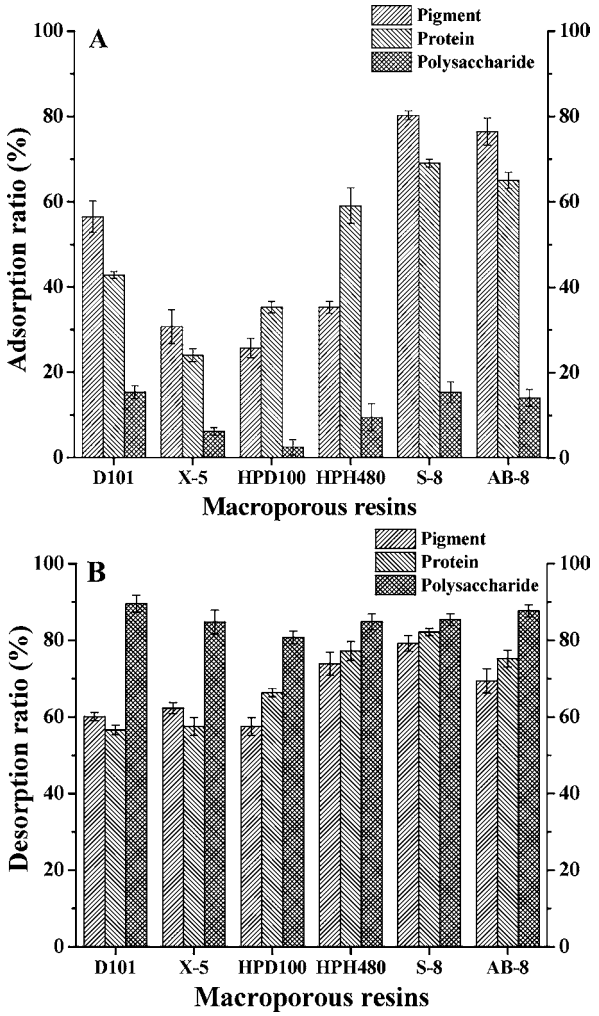

Figure 1. Adsorption ratios (A) and desorption ratios (B) of pigment, protein, and polysaccharide on different resins.

protein were notable. Nevertheless, all six resins exhibited almost equally high desorption ratios toward polysaccharide, which showed that the polysaccharide had a higher affinity for solvent than the adsorbent. In this case, the surface area, diameter, and the polarity may have unremarkable effects on desorption of the polysaccharide.

Thus, only S-8 and AB-8 resins possessed better adsorption as well as desorption performances in terms of pigment and protein. Absorption kinetics and Langmuir adsorption rate curves could reflect the rate of adsorption process, and they are important parameters for selecting a favorite resin. For this reason, further selection of the resins would be performed by adsorption kinetic tests and Langmuir adsorption rate tests to assess the performances of the two adsorbents.

Adsorption Kinetics on S-8 and AB-8 Resins. The adsorption kinetics curves obtained on S-8 and AB-8 resins were shown in Figure 2. For both two resins, the adsorption capacities of pigment, protein, and polysaccharide increased rapidly in the first 60 min and then increased slightly until adsorption equilibrium after 100 min. At the adsorption equilibrium stage, the adsorption capacities toward pigment, protein, and polysaccharide on S-8 and AB-8 resin were 23.79 μg/mL, 2.00 mg/mL, and 5.46 mg/mL, and 21.70 μg/mL, 1.66 mg/mL, and 5.09 mg/mL, respectively.

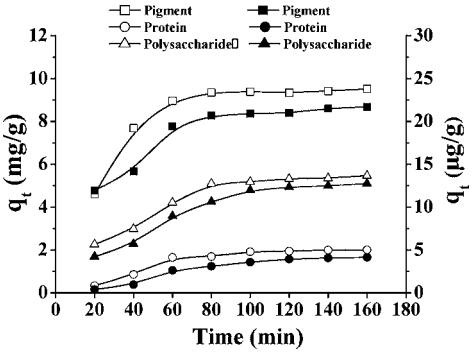

Figure 2. Adsorption kinetics curves of pigment, protein, and polysaccharide on S-8 (hollow) and AB-8 (solid) resin. Units for q_t are μg/g for pigment and mg/g for protein and polysaccharide.

Although static adsorption and desorption ratios are important parameters for screening the best resin, they are not enough for assessing the performance of an adsorbent, as a high adsorption rate is also a must.[29] The equilibrium adsorption rate constants K, which related to adsorption kinetics of pigment, protein, and polysaccharide on S-8 and AB-8 resins, are calculated according to the Langmuir adsorption rate equation, as shown in Figure 3. It showed that both S-8 and AB-8 resins had roughly the same adsorption rate toward polysaccharide ($K_{S\text{-}8}$ = 0.0337, $K_{AB\text{-}8}$ = 0.0300); however, S-8 showed relatively faster adsorption rates toward pigment ($K_{S\text{-}8}$

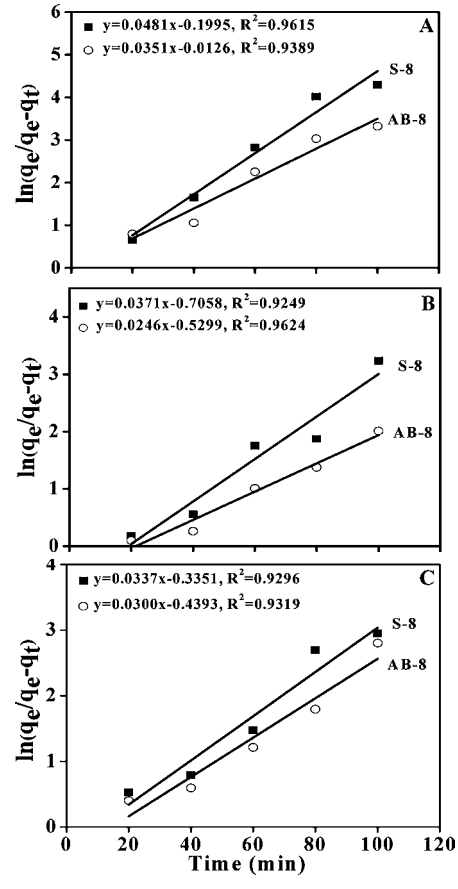

Figure 3. Langmuir adsorption rate curves of pigment (A), protein (B), and polysaccharide (C) on S-8 and AB-8 resins.

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= 0.0481, K_{AB-8} = 0.0351) and protein (K_{S-8} = 0.0371, K_{AB-8} = 0.0246).

Upon full consideration the performance of the six resins, S-8 was selected as the most suitable resin for purification of the crude polysaccharide owing to its high adsorption and desorption ratios, as well as the high adsorption rates toward pigment and protein. Hence, S-8 resin may consume less time to achieve the same adsorption capacity relative to the other resins, and it is more likely to be efficient in large-scale purification of the crude polysaccharide.

Adsorption Isotherms. Equilibrium adsorption isotherms of S-8 resin were investigated at 20 °C, as shown in Figure 4.

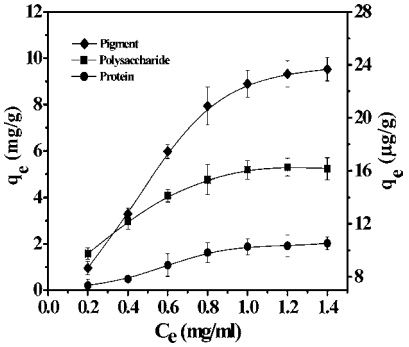

Figure 4. Adsorption isotherms of pigment, protein, and polysaccharide on column packed with S-8 resin. Units for q_e are μg/g for pigment and mg/g for protein and polysaccharide.

The adsorption capacities of S-8 toward pigment, protein, and polysaccharide gradually increased with the increase of initial adsorbate concentrations, and they reached saturation (22.57 μg/g resin and 5.19 and 1.87 mg/g resin, respectively) when the initial concentration of crude polysaccharide was 1.49 mg/mL.

The Langmuir and Freundlich parameters used to describe the relationship between solute and liquid phase at equilibrium are summarized in Table 2. Upon comparison of the features of

Table 2. Langmuir and Freundlich Parameters of Pigment, Protein and Polysaccharide on a Column Packed with S-8 Resin

	Langmuir equation			Freundlich equation		
absorbate	q_{max}	K_L	R^2	a	$1/n$	R^2
pigment	24.60	11.30	0.9324	22.64	0.36	0.9278
protein	2.01	13.36	0.9218	1.86	0.44	0.9356
polysaccharide	5.31	47.27	0.9289	5.15	0.62	0.9434

the two types of isotherms, both Langmuir equation and Freundlich equation showed high correlation coefficients in the cases of pigment, protein, and polysaccharide. It indicated that the adsorption behaviors were of monomolecule layer adsorption with a homogeneous distribution of adsorption energies, and there was no mutual interaction between adsorbed molecules.

Moreover, the extent of favorability of adsorption can be described by the Freundlich characteristic constant $1/n$, the empirical constant related to the magnitude of the adsorption driving force, and the adsorption process will be favorable when the $1/n$ value is between 0.1 and 1.0.[31,32] In general, within the ranges from 0.1 to 1.0, the affinity decreases with the increase of the $1/n$ value. In the present case, it was calculated that the $1/n$ value for pigment, protein, and polysaccharide was 0.36, 0.44, and 0.62, respectively, which indicated that the S-8 resin is applicable to separate pigment and protein from polysaccharide.

Dynamic Breakthrough Curves on S-8 Resin. The dynamic breakthrough curves, on the basis of which the sample volume without loss of analytes can be calculated, were obtained on the basis of the volumes of effluent liquid and the concentration of solute. The breakthrough point was defined as the concentration at which the exit solute concentration reached 1−5% of the inlet concentration.[29] As shown in Figure 5A, pigment, protein, and polysaccharide in the solution were

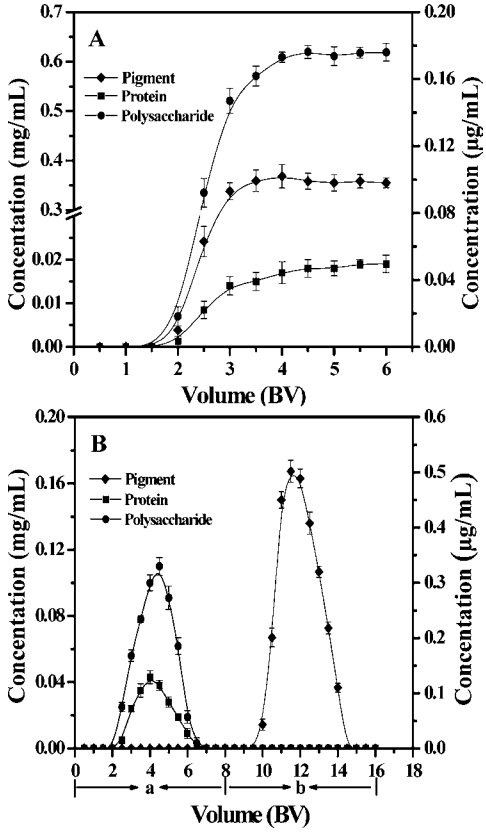

Figure 5. Dynamic leakage curves (A) and dynamic desorption curves (B) of pigment, protein, and polysaccharide on a column packed with S-8 resin. The column packed with adsorbed S-8 resin was gradually flushed with (a) NaCl solution (50 mM) and (b) 70% ethanol−water (70:30, v/v) for respective volumes. Units for concentration are μg/mL for pigment and mg/mL for protein and polysaccharide.

almost completely absorbed by S-8 resin before 2 BV (breakthrough point), and then the concentrations of pigment, protein, and polysaccharide in leak solution increased rapidly until they reached a steady plateau in 3.5 BV. So the breakthrough point in the present study appeared at a processing volume of sample solution around 2 BV.

Dynamic Desorption Curve on S-8 Resin. As shown in Figure 5B, the dynamic desorption curves on S-8 resin were obtained on the basis of the volumes of desorption solution and the concentration of solute in eluent. The protein and polysaccharide could be desorbed using approximately 8 BV of NaCl solution (50 mM, pH 7.0). Subsequently, about 8 BV of ethanol−water (70:30, v/v) could fully desorb the pigment. Therefore, gradient elution by NaCl solution and ethanol−

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water could be an effective process for desorption of loaded S-8 resin.

In summary, the optimum parameters for the decoloration and deproteinization of the crude polysaccharide on a column packed with S-8 resin were conducted as follows. Adsorption conditions: concentration of crude polysaccharide solution, 1 mg/mL; processing volume, 2 BV; flow rate, 1 mL/min; temperature, 20 °C. Desorption conditions: 8 BV of NaCl solution (50 mM, pH 7.0) and 8 BV of ethanol−water (70:30, v/v) as the eluent; flow rate, 1 mL/min. In this case, the optimized resulting adsorption ratios of pigment and protein were 84.3% and 75.9% (w/w), respectively, with a recovery ratio of polysaccharide being 84.7% (w/w). The reason why macroporous resin absorbed less polysaccharide was probably because the solute molecules pigment and protein were prone to reach and be adsorbed by the resin by a strong dipole−dipole force in the adsorption process. Besides, macroporous resin S-8 had a weak adsorption ratio for polysaccharide, which only accounted for 15.3% of the total quantity; some adsorbed polysaccharide may be further desorbed from the resin in the continuous sample loading process.[13]

Comparison of the Adsorption Efficiency by S-8 Resin with Three Traditional Methods. The comparison of purification effects of the crude polysaccharide by a column packed with S-8 resin with the methods of H_2O_2, Sevage reagent, and active carbon are shown in Table 3. It was

Table 3. Different Purification Methods Influencing Decoloration Ratio, Deproteinization Ratio, and Recovery Ratio of the Polysaccharide[a]

methods	decoloration ratio (%)	deproteinization ratio (%)	recovery ratio (%)
S-8 resin	84.3 ± 1.2 A	75.9 ± 0.9 A	84.7 ± 1.2 A
H_2O_2	66.7 ± 2.2 B	33.9 ± 3.2 C	62.9 ± 0.9 B
Sevage reagent	25.2 ± 2.3 C	78.2 ± 1.1 A	81.7 ± 0.3 A
active carbon	78.4 ± 2.0 A	63.8 ± 1.1 B	64.1 ± 1.1 B

[a]Mean of three replications ± standard deviation. Values in the same row with different letters (A—C) are significantly different ($P < 0.05$).

observed that S-8 resin showed (1) a higher decoloration ratio than that of H_2O_2 and Sevage reagent, (2) a higher deproteinization ratio than that of H_2O_2 and active carbon, and (3) a higher polysaccharide recovery ratio than that of H_2O_2 and active carbon.

Macroporous resin S-8 is a hyper-cross-linked polar resin with extremely rigid networks. Its adsorption mechanism is based on dipole−dipole forces because of the polar derivation compounds.[14] This synthetic resin is a polymeric adsorbent with large internal surface areas; meanwhile, it has a much more consistent structure compared to activated carbon.[16] Besides, the polar group of S-8 resin played an important role in adsorbing the polar pigment and protein relative to active carbon, contributing to the higher decoloration and deproteinization ratios. Purification of the crude polysaccharide by Sevage reagent is based on the precipitation of protein with chloroform and *n*-butanol,[33] while it more weakly decolored the sample compared to S-8 resin. Different from the adsorption mechanisms by S-8 resin and active carbon, decoloration of crude polysaccharide by H_2O_2 results from its strong oxidation ability. However, aside from adsorbing pigment, H_2O_2 may meanwhile oxidize the protein and polysaccharide, leading to decreased deproteinization ratio and polysaccharide recovery ratio. Consequently, purification by S-8 resin was much more efficient in simultaneous decoloration and deproteinization of the crude polysaccharide.

Characterization of Polysaccharide before and after Adsorption. As shown in Figure 6A, the absorbance in the

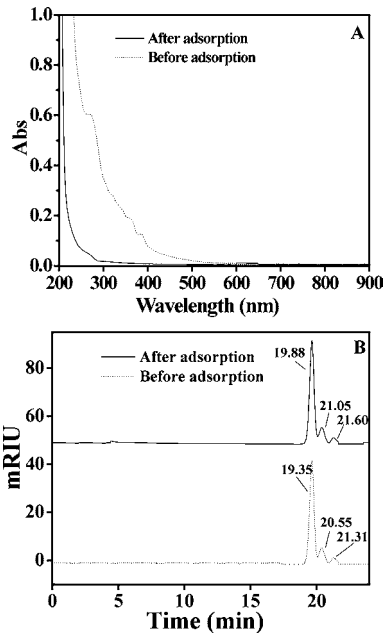

Figure 6. The UV/vis spectra (A) and HPSEC chromatograms (B) of the polysaccharide before and after adsorption by a column packed with S-8 resin.

region of 200−900 nm almost disappeared, indicating that most of the pigment and protein was absorbed by S-8 resin. The molecular weight distributions of polysaccharide before and after adsorption by column-packed S-8 resin are shown in Figure 6B. It was observed that there were no obvious changes in distribution and proportion of the three portions, demonstrating that nearly no degradation of polysaccharide occurred during the treating process. Thus, purification by S-8 resin could be a promising strategy for retaining the majority of the polysaccharide without destroying its original molecular weights while simultaneously decoloring and deproteinizing the crude polysaccharide.

This study developed a highly automated and promising approach for purification of the crude polysaccharide extracted from fermentation broth of pumpkin residues by column-packed S-8 resin. Results obtained in this work indicated that this new attempt would be a cost-effective, easily regenerated, environmentally friendly, and easily upscaled method to purify the polysaccharide extracted from the degradation product of lignocellulosic agricultural wastes, such as pumpkin residue, bagasse, and pomace. This achievement may also be helpful for further structural and pharmacological research of the purified polysaccharide.

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Notes

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■ ABBREVIATIONS USED

HPLC, high-performance lipid chromatography; HPSEC, high-performance size exclusion chromatography; SPE, solid-phase extraction; LLE, liquid−liquid extraction; PS-DVB, polystyrene divinylbenzene; BV, bed volume

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