

# Optimization of resin based technology for purification of polysaccharides from *Ziziphus Jujuba* cv. Huizao

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**Abstract:** *Ziziphus Jujuba* cv. Xinzheng Huizao is rich in kinds of nutrients, especially polysaccharides, which possess many biological effects on human bodies. However, the purification of polysaccharides from jujube after a primarily extraction step is still a difficult technology. In present research, enzyme combined with ultrasonic wave extraction, ethanol precipitation and ultrafiltration membrane filtration were employed to extract and preliminarily purify the polysaccharides from *Ziziphus Jujuba* cv. Xinzheng Huizao. The obtained raw polysaccharides were employed for further purification. Through the investigation of static adsorption and desorption of resins, the most suitable macroporous resin for the purification of jujube polysaccharides was selected and its dynamic adsorption and desorption behavior was studied. Furthermore, response surface experiments were conducted to investigate the effects of multifactor on the adsorption properties of macroporous resin for purification of jujube polysaccharide. The optimum adsorption conditions were 1.5 mL/min of in-flux rate, 2.2mg/mL of concentration of the feed liquid, 5.6 of pH value. Under this condition, the maximum dynamic adsorption rate of 19.52 mg/g could be obtained in theory. The optimized technical condition for dynamic desorption was obtained with 0.4 mol/L of NaCl, 60% of ethanol, 0.2 mol/L of HCl and 1.5 mL/min of flow rate. Under this condition, maximum dynamic desorption rate of 85.21% was achieved, and the purity of yield polysaccharides was 88.87%. Taken together, the present optimized technology is a highly effective method for the extraction and purification of polysaccharides from jujube, and has a great application and market potential in the medical and health care industries.

**Key words:** purification, resins, polysaccharides, *Ziziphus Jujuba* cv. Xinzheng Huizao,

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## 0 Introduction

Jujube (*Ziziphus Jujuba* cv. Xinzheng Huizao) is fruit of *Rhamnaceae* tree. Jujube has highly nutritional value as a natural vitamin fruit, which is rich in 18 kinds of necessary amino acid for human bodies, proteins, fats, carbohydrate, organic acid, minerals and vitamins B, C, P, etc.<sup>[1-2]</sup>. Jujube polysaccharides have been proved to possess the biological activities including relieving cough, enhancing immunity, anti-aging, anticancer and so on<sup>[3-8]</sup>.

The most commonly used methods for extraction of polysaccharides from jujube were step-by-step

precipitation, quaternary ammonium salt precipitation, DEAE-cellulose column chromatography and different kinds of gel column chromatography, centrifugal sedimentation chromatography and ion exchange resin method<sup>[9]</sup>.

Macroporous adsorption resin (Model AB-8) is a newly developed organic polymer adsorbent, which possesses big hole mesh structure and specific surface area, and has been widely applied in the areas of environment, food and biomedicine<sup>[10-11]</sup>, as well as the purification of polysaccharides from jujube<sup>[12-15]</sup>. In present research, the performance of AB-8 and the factors of adsorption and desorption were fully considered to establish the technology of dynamic adsorption and desorption of AB-8 for the purification of polysaccharides from jujube.

## 1 Materials and methods

### 1.1 Materials

Jujube fruit cultivar Xinzheng Huizao was purchased from Haoxiangni Jujube Co., Ltd. After

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shipping to the lab, the dried jujube fruits were stored under  $-20^{\circ}\text{C}$ .

### 1.2 Extraction of crude polysaccharides

Clean and de-seed jujube fruits were emulsified with 1:8 of solid-liquid ratio in water by a high shear scattered emulsifying machine, then preliminary extracted by ultrasonic method for 25 min under  $70^{\circ}\text{C}$  and 70 W. After adding 0.15% of papain and adjusting the pH value to 6.0, the extracts was heated at  $60^{\circ}\text{C}$  for 120 min by water bath, then inactivated for 10 min under  $90^{\circ}\text{C}$  before refrigeration. The supernatant of cooling liquid was obtained by centrifugation, and concentrated to 1/6 volume of original by a rotary evaporator. The crude polysaccharides were precipitated by adding 3 times volume of anhydrate ethanol for 10 h, and then dissolved in distilled water<sup>[6]</sup>. After the redundant ethanol was removed by the rotary evaporator, the obtained crude polysaccharides were stored in refrigerator for next step.

### 1.3 Determination of total sugar

Phenol-sulfuric acid method was employed to determine the content of total sugar according to Zhang (1987)<sup>[16]</sup>. Glucose was used as the standard. The obtained regression curve equation is  $Y=0.0066x+0.0067$ , and the regression coefficient ( $R^2$ ) is 0.9995.

### 1.4 Selection and preparation of resin

The characteristics of adsorption and desorption of 6 resins including AB-8, NKA-9, D101, D152, S-8, X-5 were thoroughly compared according to the parameter of adsorption capacity, and the most suitable resin for extraction and purification of polysaccharides from jujube was selected for next optimization of technology. The adsorption capacity was calculated as the following equation:

$$\text{Adsorption rate}(\text{mg/g}) = \frac{(C_0 - C_1) \times V}{M} \quad (1)$$

Where,  $C_0$  is the concentration of polysaccharides of extract before adsorption,  $\text{mg/mL}$ ;  $C_1$  is the concentration of polysaccharides of extract after adsorption,  $\text{mg/mL}$ ;  $V$  is the volume of extract,  $\text{mL}$ ;  $M$  is the wet weight of resin,  $\text{g}$ .

The desorption rate (%) is calculated as follows:

$$\text{Desorption rate}(\text{mg/g}) = \frac{C \times V \times 100}{W} \quad (2)$$

Where  $W$  is total adsorption,  $\text{mg}$ ;  $V$  is the total volume of elution,  $\text{mL}$ ;  $C$  is the concentration of polysaccharides in extracts,  $\text{mg/mL}$ ; Finally, the adsorption kinetic curve is drawn.

The yield of polysaccharides (%) and the purity of polysaccharides (%) are calculated as the following

equations:

$$\text{Yield of polysaccharides}(\%) = \frac{m}{m_1} \times 100 \quad (3)$$

$$\text{Purity of polysaccharides}(\%) = \frac{m_a - m_b}{m} \times 100 \quad (4)$$

Where,  $m_1$  is the mass of raw material,  $\text{g}$ ;  $m$  is the mass of raw polysaccharides,  $\text{g}$ ;  $m_a$  is the mass of total sugars in raw polysaccharides,  $\text{g}$ ;  $m_b$  is the mass of reduced sugars in raw polysaccharides,  $\text{g}$ .

### 1.5 Optimization of adsorption condition of polysaccharides from jujube using AB-8 resin

Based on the results of single factor experiments, sampling flow rate, feed concentrations and filtrate pH value were selected as the independent variables for the response surface design by the software of Design-Expert<sup>[17-18]</sup>. After Box-Behnken design and thoroughly experiments on the three variables, the dynamic adsorption conditions of AB-8 resin were optimized.

### 1.6 Optimization of desorption condition of AB-8 resin

After single factor experiments, hydrochloric acid concentration, ethanol content in eluent, concentration of NaCl and flow rate of elution were investigated by orthogonal experimental design to optimize the desorption condition of AB-8 resin.

### 1.7 Verification test

The theoretical results are testified by optimal adsorption and desorption condition of AB-8 resin.

## 2 Results and analysis

### 2.1 Static adsorption and desorption performance of six resins

Totally six macroporous adsorption resins were compared according to the performance of static adsorption and desorption of polysaccharides from jujube (Table 1). Macroporous resin adsorption separation technology, is a new extraction-purification process to selectively remove the ineffective ingredients using a special solution, and to adsorb the active portion from the adsorbent. The amount of adsorption on the adsorbent depends on the polarity of solute and solvent, different types of resin showed significant adsorption performance differences<sup>[19]</sup>. As shown in Table 1, resin AB-8, D101, X-5 and S-8 showed relatively stronger adsorption capacity of polysaccharides from Xinzhen Huizao, all with more than  $10.0 \text{ mg/g}$  of absorbance. AB-8 resin showed the highest static adsorption, X-5 was the second; NKA-9 and D152 were the minimum. The results showed that D101 had the highest resolution rate of 85.13%. Although AB-8

had a slightly lower desorption rate of 83.87%, it had higher adsorption rate, AB-8 macroporous resin was still selected for the purification of polysaccharides from jujube cv. Xinzheng Huizao.

**Table 1 Static adsorption and desorption performance of 6 resins for polysaccharides**

Resin name	Property	Adsorption capability/ (mg·g <sup>-1</sup> )	Desorption rate/%
AB-8	Nonpolar	15.95±0.35 <sup>c</sup>	83.87±0.26 <sup>c</sup>
D101	Nonpolar	11.64±0.30 <sup>c</sup>	85.13±0.35 <sup>f</sup>
X-5	Nonpolar	13.24±0.38 <sup>d</sup>	79.35±0.41 <sup>d</sup>
S-8	Polar	10.89±0.75 <sup>c</sup>	62.70±0.40 <sup>c</sup>
NKA-9	Polar	6.57±0.32 <sup>b</sup>	48.64±0.78 <sup>b</sup>
D152	Nonpolar	4.87±0.26 <sup>a</sup>	18.38±0.33 <sup>a</sup>

Note: Data superscripted by common letter are not significantly different at  $P < 0.05$ .

## 2.2 Adsorption properties of AB-8 macroporous resin for polysaccharides from Xinzheng Huizao

### 2.2.1 Adsorption kinetics of AB-8 macroporous resin

As shown in Fig. 1, the adsorption rate of AB-8 resin declined sharply with the increase of adsorption time, and then saturate gradually until adsorption equilibrium after 12 h, which suggested physical adsorption was the main manner for polysaccharides adsorption of AB-8 resin, and was considered as a kind of chronic adsorption.

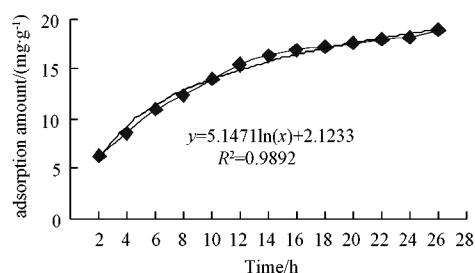


Fig.1 Kinetic curve of static adsorption of AB-8 resin for polysaccharides from jujube cv. Xinzheng Huizao

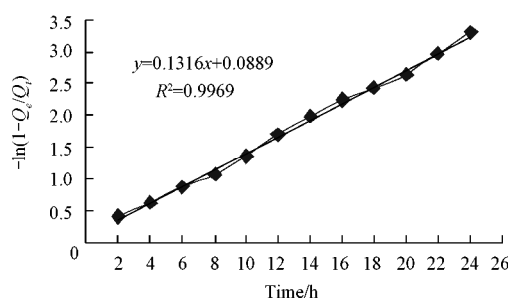


Fig.2 Adsorption balance equation of AP on AB-8 resin for polysaccharides from jujube cv. Xinzheng Huizao

To quantitatively evaluate the adsorption rate of AB-8 resin for purification of polysaccharides, the following Langmuir layer adsorption model was used to fit the equilibrium rate constant of AB-8 resin<sup>[20]</sup>.

$$\ln \frac{Q_e}{Q_e - Q_t} = k \times t \quad \text{or} \quad -\ln \left( 1 - \frac{Q_t}{Q_e} \right) = k \times t \quad (5)$$

Where,  $Q_t$  is the absorbance of resin at time  $t$ ;  $Q_e$  is the absorbance of resin during adsorption equilibrium, and  $K$  is the adsorption rate constant. As the results shown in Fig.2, the Langmuir layer adsorption model was well fit to the purification behavior of the AB-8 resin. Adsorption rate constant  $k$  is an important constant that reflects the adsorption rate of resin, the smaller the constant, the longer period to achieve equilibrium, which was considered as a chronic adsorption.

### 2.2.2 Response surface optimization of AB-8 macroporous adsorption resin dynamic conditions

According to the aforementioned single-factor test, the AB-8 macroporous adsorption resin dynamic conditions were determined and optimized using response surface methodology. Test program and results are shown in Table 2.

**Table 2 Optimization of dynamic adsorption on AB-8 resin**

Test number	In-flux rate/ (mL·min <sup>-1</sup> ) (A)	Feed concentration/ (mg·mL <sup>-1</sup> )(B)	pH value (C)	Adsorption capacity/ (mg·g <sup>-1</sup> )
1	0(1.5)	1(2.5)	1(6.0)	17.25
2	-1(1.0)	1	0(5.0)	17.05
3	0	-1(1.5)	1	15.46
4	0	1	-1(4.0)	17.69
5	-1	0(2.0)	-1	16.13
6	0	0	0	19.25
7	1(2.0)	0	1	18.06
8	1	-1	0	16.31
9	-1	0	1	16.96
10	0	-1	0	16.46
11	0	0	0	18.64
12	0	0	0	18.83
13	0	0	0	18.92
14	0	0	0	19.08
15	1	1	0	19.03
16	1	0	-1	17.39
17	0	-1	-1	15.09

**Table 3 Variance analysis for responses of dynamic adsorption on AB-8 resin**

Variance source	SS	DF	MS	F value	P value
Model	27.54	9	3.06	30.29	<0.0001
Residual	0.71	7	0.10		
Lack of Fit	0.49	3	0.16	2.99	0.1588
Error	0.22	4	0.055		
Total	28.25	16			

Note:  $R^2=0.9750$  Adj. $R^2=0.9428$  Pred.  $R^2=0.7109$  Adeq. Precision=17.240>4.

Using Design-Expert software to fit the experimental data, a quadratic multiple regression model can be obtained as follows:

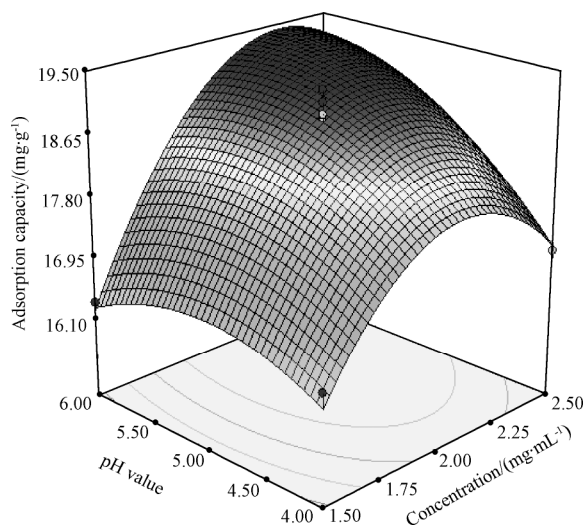
$$Y=18.94+0.18\times A+0.96\times B+0.52\times C-0.20\times A\times B-0.040\times A\times C+0.53\times B\times C-1.32\times A^2-1.25\times B^2-0.48\times C^2$$

Where  $Y$  is the dynamic adsorption capability;  $A$  is in-flux rate,  $B$  is the feed concentration;  $C$  is feed pH value. The significance test results of this model is shown in Table 4, which suggests a strong enough response signal model that can be used to analyze and predict the dynamic polysaccharides adsorption results of AB-8 resin. More interestingly, a nonlinear relationship can also be indicated from different variables as the results shown in Table 4.

**Table 4** Significance of test result for regression coefficient

Variables	Regression coefficient	Mean square	Degree of freedom	$F$ value	$P$ value
In-flux rate ( $A$ )	0.18	0.26	1	2.53	0.1557
feed pH ( $B$ )	0.96	7.41	1	73.36	<0.0001
feed concentration ( $C$ )	0.52	2.19	1	21.72	0.0023
$AB$	-0.20	0.16	1	1.62	0.2432
$AC$	-0.04	$6.400\times 10^{-3}$	1	0.063	0.8085
$BC$	0.53	1.13	1	11.23	0.0122
$A^2$	-1.32	7.39	1	73.12	<0.0001
$B^2$	-1.25	6.55	1	64.81	<0.0001
$C^2$	-0.48	0.99	1	9.78	0.0167

Because no significant effect of the in-flux rate on the dynamic adsorption of AB-8 macroporous resin was determined, a response surface was constructed only according to the influence of feed pH value and feed concentration on the dynamic adsorption of AB-8 resin (Fig.3). As shown, the adsorption capability increased constantly when feed concentration increased from 1.5 mg/mL to 2.0 mg/mL, and then showed a declining trend with the further increase of feed concentration. However, the adsorption capability showed nonobvious change from pH value 4 to 6.



**Fig.3** Effects of pH value and feed concentrations on dynamic adsorption capability

Taken together, the optimized adsorption condition of AB-8 resin by Design-Expert software could be summarized as 1.55 mL/min of in-flux rate, 2.24 mg/mL of feed concentration and 5.58 of pH value, the theoretically maximum adsorption capability could be 19.398 mg/g under these conditions.

### 2.3 Dynamic performance of AB-8 resin for polysaccharides desorption of jujube

According to results of single-factor test, the desorption conditions were optimized using orthogonal test and the results are shown in Table 5 and Table 6. The  $R$  value indicated that the effects of influencing factors were comparative  $B>D>C>A$ , which means the ethanol content played the greatest role in desorption rate, flow rate took the second, and the concentration of NaCl was the last. It was also indicated that the highest extraction efficiency could be obtained when the concentration of ethanol was 60%.

**Table 5** Optimization of dynamic desorption performance of AB-8 resins

Test number	NaCl concentration (mol·L <sup>-1</sup> ) (A)	Ethanol concentration (%) (B)	HCl concentration (mol·L <sup>-1</sup> ) (C)	Flow rate/(mL·min <sup>-1</sup> ) (D)	Desorption rate/%
1	$A_1(0.2)$	$B_1(40)$	$C_1(0.1)$	$D_1(1.0)$	54.26
2	$A_1$	$B_2(50)$	$C_2(0.2)$	$D_2(1.5)$	82.07
3	$A_1$	$B_3(60)$	$C_3(0.3)$	$D_3(2.0)$	73.56
4	$A_2(0.3)$	$B_1$	$C_2$	$D_3$	71.24
5	$A_2$	$B_2$	$C_3$	$D_1$	62.39
6	$A_2$	$B_3$	$C_1$	$D_2$	76.89
7	$A_3(0.4)$	$B_1$	$C_3$	$D_2$	65.63
8	$A_3$	$B_2$	$C_1$	$D_3$	74.66
9	$A_3$	$B_3$	$C_2$	$D_1$	74.99
$K_1$	69.963	63.71	68.603	63.880	
$K_2$	70.173	73.04	76.100	74.863	
$K_3$	71.760	75.147	67.193	73.153	
$R$	1.797	11.437	8.907	10.893	

**Table 6** Orthogonal analysis of variance test

Source of variance	Sum of squared deviations	Freedom	$F$ ratio	$F$ critical value	Significance
NaCl concentration (A)	5.790	2	1.000	19.000	
Ethanol concentration (B)	222.284	2	38.391	19.000	*
HCl concentration (C)	137.517	2	23.751	19.000	*
Flow rate (D)	209.552	2	36.192	19.000	*

Considering all of the test results, the optimized technology for dynamic desorption of data polysaccharides using AB-8 macroporous resin could be concluded as  $A_3B_3C_2D_2$ .

### 2.4 Verification test

The theoretical results were testified and the

results are shown in Table 7. From a workable consideration, adsorption condition of AB-8 resin was verified that the adsorption capability was 19.52 mg/g by 1.5 mL/min of in-flux rate, 2.2 mg/mL of feed concentration and 5.6 pH value. Meanwhile, desorption condition of AB-8 resin was verified by orthogonal optimization results, which represented 0.4 mol/L of NaCl, 60% of ethanol, 0.2 mol/L of HCl and 1.5 mL/min of flow rate. Under these conditions, the best dynamic desorption rate was 85.21 %.

**Table 7 Verification test of adsorption and desorption condition of AB-8 resin**

Test number	Adsorption capacity/ (mg·g <sup>-1</sup> )	Desorption rate/ %
1	20.38	82.87
2	19.14	86.23
3	19.78	85.56
4	18.87	87.91
5	19.42	83.47
Average value	19.52	85.21
RSD/%	3.01	2.42

Note: RSD is the relative standard difference.

## 2.4 Dynamic elution curve of AB-8 resins

The AB-8 resin was saturated with the solution concentration of 2.5 mg/mL and sample flow rate of 1.5 mL/min. The neutral polysaccharide was eluted with 200 mL of distilled water and flow rate of 1.5 mL/min, and then the conditions of elution were 0.4 mol/L of NaCl concentration, 60% of adding ethanol volume, 0.2 mol/L of HCl concentration, and 1.5 mL/min of flow rate. The purified products were collected by automatic collector of 6 min per tube, the high polysaccharide content were collected. The measured purity of polysaccharide was 88.87%. Elution curve is shown in figure 4.

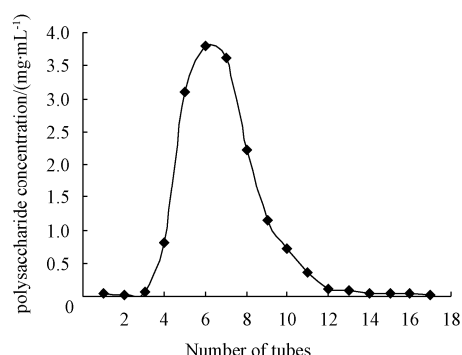


Fig.4 The elution curve of polysaccharide

## 3 Conclusions

After thoroughly comparing the adsorption and desorption characteristic of six kinds of macroporous resins for polysaccharides from jujube, AB-8 resin was declared to hold the best adsorption and

desorption rates, and was used for the following extraction and purification of polysaccharides from jujube cv. Xinzheng Huizao.

The best conditions of adsorption and desorption of AB-8 for the extraction of polysaccharides from jujube were concluded as 1.5 mL/min of in-flux rate, 2.2 mg/mL of feed concentration, 5.6 of pH value. The optimized conditions of elution were 0.4 mol/L of NaCl, 60% of ethanol, 0.2 mol/L of HCl, and 1.5 mL/min of flow rate. The best dynamic desorption rate was 85.21%, and the purity of polysaccharide was 88.87%.

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**摘要:** 新郑灰枣富含各种营养成分, 特别是多糖具有抗氧化抗癌等许多生物效应。为研究新郑灰枣多糖的提纯工艺, 该文在超声波酶法联合提取、乙醇沉淀、超滤膜过滤得到的粗多糖的基础上, 筛选出的 AB-8 大孔树脂纯化多糖, 并对树脂的动态吸附解吸特性进行研究。响应曲面法优化 AB-8 大孔树脂动态吸附工艺条件, 最佳动态吸附条件为: 上样速率 1.5 mL/min, 料液浓度 2.2 mg/mL, pH 值 5.6, 最大动态吸附量为 19.52 mg/g; 正交试验优化 AB-8 大孔树脂动态解吸的最佳工艺条件为: 氯化钠浓度为 0.4 mol/L, 乙醇添加量 60%, 盐酸浓度 0.2 mol/L, 流速 1.5 mL/min, 最优动态解吸率为 85.21%。通过树脂纯化, 多糖纯度可达 88.87%。该红枣多糖提纯技术是一种非常有效的方法, 在医疗及保健行业具有巨大的应用潜力和市场。

**关键词:** 提纯, 树脂, 多糖, 新郑灰枣