Running title: Melanoidins extracted from heat-induced *Lycium barbarum*

Preliminary study on physicochemical properties and antioxidant activities of Melanoidins extracted from low thermal induced black *Lycium barbarum* L.

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Supplementary file

1. Screening of product purification conditions

The effects of six macroporous resins with different polarities (D101, D312, X-5, HPD100, AB-8, CAD-40) on the purification of melanoidin were compared, and the optimal resin and eluent concentration suitable for the purification of Lycium barbarum melanoidin were determined by static adsorption-desorption experiments and dynamic desorption experiments.

(1) Static adsorption

The specific process was as follows: 6 macroporous adsorbent resins were expanded with absolute ethanol and then washed with deionized water until no ethanol odor was present. They were then soaked in 5% HCl and 5% NaOH for 24 hours, then washed with deionized water until neutral, and placed in deionized water for later use. 5g of the resin was placed in a 100mL Erlenmeyer flask and 30mL of a 5mg/mL sample was added. The mixture was shaken in a shaker for 24 hours, completely adsorbed and filtered. The content of melanoidin in the solution was determined and the adsorption rate was calculated as shown in (1) and (2):

$$Q = \frac{(\omega_0 - \omega_1) \times V}{m} \qquad (1)$$

$$E/\% = \frac{(\omega_0 - \omega_1) \times V}{\omega_0 \times V} \times 100 \qquad (2)$$

Note: Q: adsorption capacity(mg/g); ω_0: mass fraction of melanoidins in the sample solution (mg/g); ω_1: mass fraction of melanoidin in the adsorption solution (mg/g); *V:* adsorption liquid volume (mL); *m*: quality of resin (g); E: adsorption rate (%).

(2) Static desorption

The adsorbed resin was placed in an erlenmeyer flask with a specific concentration of 30 mL ethanol solution for desorption. The erlenmeyer flask was placed in a shaker and shaken for 24 hours. After complete desorption, it was filtered, the absorbance value of the desorption solution was determined and the resolution was calculated. As shown in equation (3):

$$B/\% = \frac{\omega_2 V_1}{(\omega_0 - \omega_1) \times V} \qquad (3)$$

Note: B: resolution rate (%); ω_2: analyze the mass fraction of melanoidins in the solution (mg/g); V_1: analyze liquid volume (mL); ω_0: mass fraction of melanoidins in the sample solution (mg/g); ω_1: mass fraction of melanoidin in the adsorption solution (mg/g); *V*: adsorbent volume(mL).

(3) Eluent concentration and dynamic desorption

5 g of saturated AB-8 resin and add ethanol solutions of 20%, 40%, 60%, 80% and 100% by volume as eluent for thorough elution. The sample was added to a column filled with AB-8 adsorption resin and the eluent was passed through the adsorption resin column at a flow rate of 4 BV/h. Every 2 minutes, 1 sample tube was removed for elution using an automatic pressure divider and the melanoidin content was determined and the resolution calculated.

2. Result and discussion

2.1 Static adsorption effect of different types of resins on melanoidin

As can be seen in Figure 1, there is a significant difference in the adsorption rate of the sample between different resins. Effects are as follows: AB-8 > D101 > D312 > X-5 > CAD-40 > HPD-100. The adsorption rate of AB-8 resin to the sample is 70. 24%, and the adsorption capacity was 6.322, which was significantly higher than that of other groups.

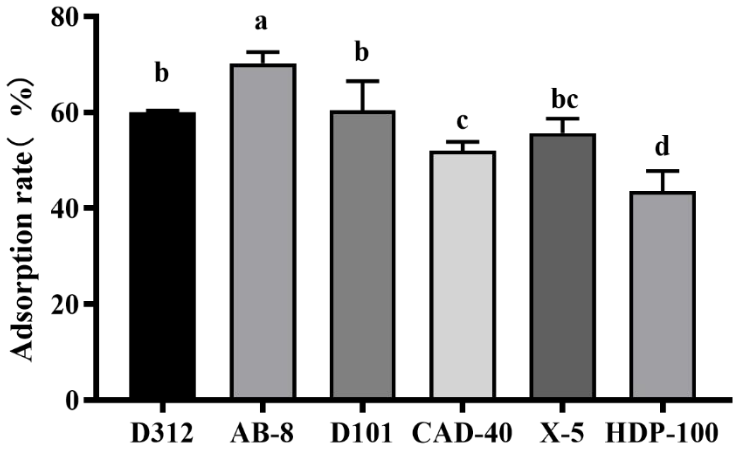

Fig. 1. Effect of six types of resins on the adsorption rate of melanoidin

2.2 Static desorption effect of different types of resins on melanoidin

There was no significant difference in desorption rate between the six resins ($P < 0.05$), the resolution rate of each type of resin is more than 80 % (Fig. 2). AB-8 is slightly higher than other resins, and its resolution rate is 88.84%. AB-8 resin was selected for the purification and separation of melanoidin, because AB-8 has high adsorption and resolution effects.

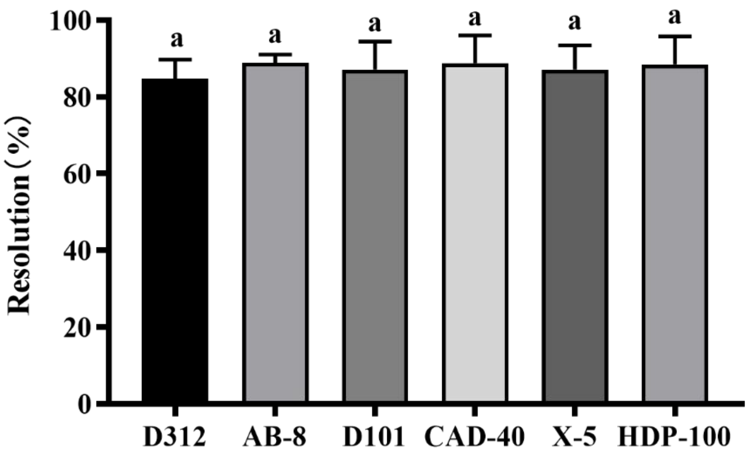

Fig. 2. Desorption rate of the six types of resins of melanoidin

2.3Effect of different ethanol volume fractions on the resolution of melanoidin

The resolution of melanoidin increased gradually with the volume fraction of the ethanol solution, with the trend first increasing and then decreasing. At 40%, 60% and 80% ethanol solution, the resolution rate of melanoidin was more than 95%. The 40 % ethanol solution was selected as the resolving agent considering the cost.

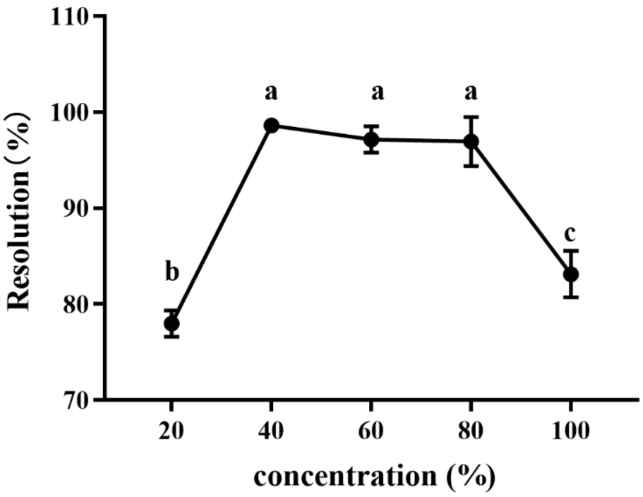

Fig. 3. Effects of different ethanol concentrations on static desorption capacity of AB-8 resin

2.4 Effect of AB-8 resin on dynamic resolution rate of melanoidin

Melanoidin essence adsorbed on AB-8 resin was eluted with 40% ethanol solution at an elution rate of 1.5 mL/min. As can be observed in Fig. 4, the peak was reached at tube 8 and is eluted by tube 18. Therefore, melanoid essence is eluted with 40% ethanol solution, and the peak shape is symmetrical and there is no tailing.

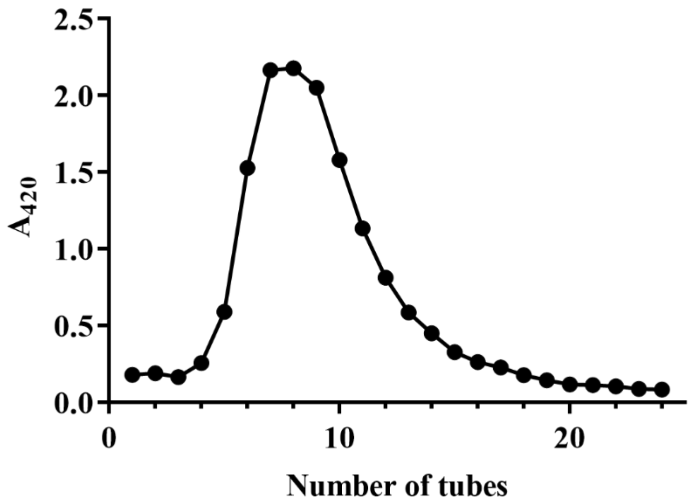

Fig. 4. Dynamic desorption curve of 40% ethanol concentration

3.Conclusion

In summary, 40% ethanol was selected as the elution solution, and the AB-8 macroporous adsorption resin was used to separate and purify melanoidin.