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Adsorption properties of macroporous adsorbent resins for separation of anthocyanins from mulberry

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ABSTRACT

In this study, the adsorption/desorption characteristics of mulberry anthocyanins (MA) on five types of macroporous resins (XAD-7HP, AB-8, HP-20, D-101 and X-5) were evaluated, XAD-7HP and AB-8 showed higher adsorption/desorption capacities. On the basis of static adsorption test, XAD-7HP and AB-8 resins were selected for kinetics, isotherms and thermodynamics. The adsorption mechanism indicated that the process was better explained by pseudo-first-order kinetics and the Langmuir isotherm model, and the thermodynamics tests showed that the processes were exothermic, spontaneous and thermodynamically feasible. Dynamic tests were performed on a column packed with XAD-7HP and AB-8, and breakthrough volume was reached at 15 and 14 bed volumes of MA solution, respectively. The purity of the fraction by 40% ethanol elution on XAD-7HP reached 93.6%, from which cyanidin-3-glucoside and cyanidin-3-rutinoside were identified by HPLC–ESI-MS/MS. The method could be used to prepare high purity anthocyanins from mulberry fruits as well as other plants.

1. Introduction

In the past decades, there has been increased interest in diets containing berries because of the potential health benefits derived from the biological effects of the anthocyanins they contain (Gardana, Ciappellano, Marinoni, Fachechi, & Simonetti, 2014). Anthocyanins have been proven to be good antioxidant compounds due to their effective free radical scavenging properties and their potential use as dietary modulators for various diseases (Grigoras, Destandau, Zubrzycki, & Elfakir, 2012; He & Giusti, 2010).

Mulberry (*Morus alba* L.) is a traditional Chinese fruit; it serves as a rich source of anthocyanins and has been widely consumed in food processed products and effectively applied in folk medicines (Chang et al., 2013). As a bioactive phytochemical, anthocyanins extracted from mulberries are typically known as nutraceuticals due to their excellent antioxidant capacity. In virtue of such characteristics, anthocyanins provide protective effects against chronic

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diseases such as diabetes, cancer and cardiovascular diseases (Liang, Wu, Zhu, et al., 2012; Yin & Chao, 2008). Moreover, they have been shown to have health-promoting properties in neuroprotective, analgesic and anti-inflammatory activities (Chen et al., 2006; Zhao, Sheng, Zheng, & Liu, 2011). Our previous study also showed that mulberry anthocyanins (MA) possess good antioxidant activity *in vivo* (Liang, Wu, Zhao, et al., 2012) and neuroprotective effects on mice (Chen, Li, et al., 2014). Other studies have also demonstrated that MA can exhibit inhibitory effects on the migration and invasion of human lung cancer cell lines (Oki et al., 2006). The mulberry fruit extract, by its antioxidant and anti-apoptotic effects, significantly protected neurons against neurotoxins *in vitro* and *in vivo* Parkinson's disease (PD) models (Kim et al., 2010). Therefore, the mulberry fruit could be a type of potential functional food to protect against many types of diseases.

Despite their significant potential, mulberries have been rarely used in the medicine industry. They are usually made into juices and fruit wines, or even consumed directly. As a result of the low yields achieved during extraction and the instability of anthocyanins, high-purity anthocyanin products are still unavailable in the market. Hence, the preparation of pure anthocyanins from mulberries is a very promising endeavor, if technologically challenging (Santos, Albarelli, Beppu, & Meireles, 2013).

The separation of anthocyanins from plants has been studied using high-speed counter-current chromatography (HSCCC) (Sheng et al., 2014), preparative high-performance liquid chromatography (HPLC) (Wang, Yin, Xu, & Liu, 2014), solid-phase extraction (SPF) (Santos et al., 2014) and so on. However, these methods are fraught with several disadvantages, in that they are time-consuming, laborious, expensive with poor recovery, and unsuitable for large-scale industrial production. Accordingly, macroporous resin emerges as an alternative candidate for the separation of anthocyanins from plant materials. First, the material is easily obtained and lost cost. In addition, the utilization of macroporous resin always reveals outstanding properties, including good selectivity, high mechanical strength, and fast adsorption speed. Moreover, macroporous resin as an efficient adsorbent has received fruitful advancements in academia, including cyaniding-3-O-glucoside from orange juice (Scordino, Di Mauro, Passerini, & Maccarone, 2004), anthocyanins from citrus-processing by-products (Di Mauro, Arena, Fallico, Passerini, & Maccarone, 2002), anthocyanins from blueberries (Wang et al., 2014), and anthocyanins from blood oranges (Cao, Pan, Yao, & Fu, 2010).

In this study, five macroporous resins were selected according to their polarity and separation effects in previous studies (Chandrasekhar, Madhusudhan, & Raghavarao, 2012; Jampani, Naik, & Raghavarao, 2014). In these studies, the resins were used to systematically investigate the adsorption and desorption of anthocyanins from mulberry extract and develop a simple, efficient, and eco-friendly process for the separation and purification of MA with the optimal resin.

2. Materials and methods

2.1. Chemicals, reagents and adsorbents

Standard of cyaniding-3-O-glucosid was purchased from J&K Chemical Co., Ltd. (Shanghai, China). All solvent and chemicals used were of analytical grade or HPLC grade.

2.2. Pretreatment of macroporous resins

Macroporous resins including AB-8, D101, and X-5 were provided by the Chemical Plant of Naikai University (Tianjin, China). Amberlite XAD-7HP and HP 20 were purchased from Sigma–Aldrich Chemical Co. (St. Louis, MO, USA) and Mitsubishi Chemical Co. (Tokyo, Japan), respectively. The physical properties of these resins are summarized in Table 1a. Prior to the study, adsorbent was activated by overnight treatment with 2 bed volumes (BV) of 95% ethanol for the removal of monomers and porogenic agents trapped inside the pores during the synthesis process. This was done until there was no residue after distillation, and further rinsed by 4–5 BV distilled water until neutral.

2.3. Preparation of crude anthocyanins extracts from mulberry

2.3.1. Pretreatment of mulberry fruits

Mulberry fruits, were manually picked at the ripening stage on the farm of Sericultural Research Institute (Jiangsu University of Science and Technology, Zhenjiang, Jiangsu, China), and then transported to laboratory immediately, and stored in polyethylene bags at −18 °C in a refrigerator until analysis.

2.3.2. Extraction of crude mulberry anthocyanins

Aqueous two-phase extraction was applied to extract MA according to the method published by our previous studies (Wu et al., 2011). The defrosted mulberry fruit (10 g) was triturated, homogenized and treated with an aqueous two-phase system consisting of 30% (w/w) ethanol, and 20% (w/w) ammonium sulfate. The residue was dissolved with 1 L of 0.1% HCl acidified 70% (v/v) ethanol and extracted for 24 h. This process was repeated until the filtrate became light-colored. The supernatant was then concentrated to a volume by a rotary evaporator to remove the ethanol at temperatures not exceeding 40 °C for 1 h, and then freeze-dried.

2.3.3. Determination of anthocyanin content

The total anthocyanins content in extracts was directly determined using the pH differential method (Hosseinian, Li, & Beta, 2008). The absorbance of anthocyanin was measured at 513 nm and 700 nm, employing Eq. (1):

$$\text{Anthocyanins concentrations (mg/L)} = \frac{A \times M\omega \times DF}{\varepsilon \times L} \quad (1)$$

where $A = \left[(A_{513} - A_{700})_{\text{pH1.0}} - (A_{513} - A_{700})_{\text{pH4.5}}\right]$, $M\omega$ is the molecular weight of anthocyanins (449.2 g/mol); DF is the dilution factor; ε is the molar extinction coefficient (26,900 L/cm mol); L is the path length (1 cm).

2.4. Static adsorption and desorption tests

2.4.1. Static adsorption and desorption properties of the resins

Activated adsorbent (2.0 g dry weight) was added to 10 mL of anthocyanins extract in 100 mL flask while agitating on a vibratory shaker at 25 °C for 12 h to reach the adsorption equilibrium. After adsorption, filtration was completed, and the filtrate was subjected to further analysis. After reaching the adsorption equilibrium, the resin was washed with deionized water for 2–3 times and then desorbed with 20 mL 80% ethanol in a 100 mL flask while agitating on a vibratory shaker at 25 °C for 30 min to reach the desorption equilibrium. The content of anthocyanins was then measured using the pH differential method.

The adsorption capacity was quantified as follows:

$$Q_e = \frac{(C_0 - C_e) \times V_i}{W} \quad (2)$$

$$Q_{re} = (C_0 - C_e)/C_o \times 100\% \quad (3)$$

where C_0 and C_e are the initial and equilibrium concentrations of anthocyanins in the solution, respectively (mg/mL); Q_e represents the adsorption capacity at adsorption equilibrium (mg/g dry resin);