Separation and Purification Technology 152 (2015) 155–159

Contents lists available at ScienceDirect

Separation and Purification Technology

journal homepage: www.elsevier.com/locate/seppur

Short Communication

Enhanced separation and purification of curcuminoids on polyamide column via noncovalent interactions

Zujin Yang [a,b], Shen Zheng [c], Zebao Rui [a,b], Yanxiong Fang [c], Hongbing Ji [a,b,*]

[a] School of Chemistry and Chemical Engineering, The Key Laboratory of Low-carbon Chemistry & Energy Conservation of Guangdong Province, Sun Yat-sen University, Guangzhou 510275, China
[b] Huizhou Research Institute, No. 135 Xingang West Road, Haizhu District, Huizhou 516216, China
[c] Faculty of Chemical Engineering and Light Industry, Guangdong University of Technology, Guangzhou 510006, China

ARTICLE INFO

Article history:
Received 30 July 2015
Accepted 31 July 2015
Available online 6 August 2015

Keywords:
Curcumins
Macroporous adsorption resins
Separation and purification
Interaction

ABSTRACT

In this study, a blend of polyamide-6 and poly(styrene–divinyl benzene) (PA–PS–DVB) was prepared and used to produce high-purity individual curcuminoids such as curcumin, demethoxycurcumin (DMC), and bisdemethoxycurcum (BDMC) from turmeric crude extracts. The effects of pH and temperature on the adsorption capacities of the curcuminoids were investigated in details. Dynamic experiments on packed columns of PA–PS–DVB resin were performed, and an optimized process for gradient elution was obtained and the obtained products were further recrystallized in isopropanol. The purities of curcumin, DMC, and BDMC increased up to 99.28%, 98.8%, and 98.2%, respectively. In conclusion, the results in this work provide a cost-effective method for large-scale production of high-purity individual curcuminoids.

1. Introduction

Turmeric (*Curcuma longa* L.), a plant of the *Zingiberaceae* family, has been used as a traditional herb for many ailments because of a variety of pharmacological activities. It is also used for food coloring and preservation as well as functional food in the food industry [1–3]. The major bioactive components found in turmeric are the three curcuminoids, namely curcumin, DMC, and BDMC. The compounds have different methoxy substitution pattern on the aromatic ring (Fig. 1). Scientific studies have shown that the curcuminoids possess diverse pharmacological properties e.g. antioxidant, antitumor, anti-inflammatory, and anti-cancer, resulting in the increased attention of these compounds in recent years [4]. Curcumin, DMC, and BDMC exhibit significantly different pharmacological activities due to different chemical structures. As a result, there has been an increased demand for individual curcuminoids. However, high-purity individual curcuminoids are not available from commercial sources. Therefore, it is necessary to develop an appropriate purification process to obtain these components.

Macroporous resin adsorption is an efficient method to enrich and purify some bioactive substances from plant resources due to its relative inexpensiveness, high adsorption capacity, good stability and mild operating conditions. The resins based on macroporous copolymers of polystyrene are the most widely used materials for the isolation of the curcuminoids [5,6]. However, low adsorption selectivity of the polystyrene resin is not sufficient to obtain high-purity individual curcuminoids from the turmeric extract.

In this study, we incorporated the polyamide-6 (PA-6) into the network of poly(styrene–divinyl benzene) (PS–DVB) (Fig. 2). PA-6 moieties are expected to form hydrogen-bonding interactions between PA-6 and the curcuminoids, which are in theory helpful for their absorption selectivity. In addition, individual curcuminoids could be separated according to the difference in the interactions between the curcuminoids such as curcumin, DMC, and BDMC and the resin. The parameters of adsorption–desorption were studied in detail, further recrystallized in isopropanol, and the obtained curcumin, DMC, and BDMC were quantitatively analyzed by HPLC.

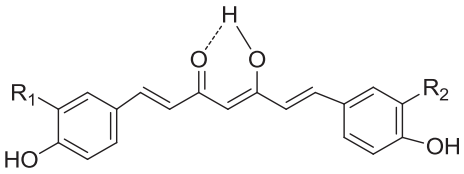
2. Experimental

2.1. Reagents and materials

Standard compounds for the curcuminoids were purchased from Sigma company (St. Louis, MO, USA). HPLC grade acetonitrile was bought from Honeywell, Burdick & Jackson Co. Food-grade of

* Corresponding author at: School of Chemistry and Chemical Engineering, The Key Laboratory of Low-carbon Chemistry & Energy Conservation of Guangdong Province, Sun Yat-sen University, Guangzhou 510275, China.
E-mail address: jihb@mail.sysu.edu.cn (H. Ji).

http://dx.doi.org/10.1016/j.seppur.2015.07.068
1383-5866/



Curcumin: R_1= OCH_3, R_2= OCH_3
Demethoxycurcumin: R_1= OCH_3, R_2= H
Bisdemethoxycurcumin: R_1= H, R_2= H

Fig. 1. Chemical structures of the main curcuminoids such as curcumin, demethoxycurcumin, and bisdemethoxycurcumin.

95% ethanol was used in all extraction and desorption experiments. Ultra-pure water used for analytical and preparative HPLC was produced by Mili-pore Q System (Millipore, USA). PA-6 (M_n = 18,000) and (PS–DVB) were supplied with from Huizhou Shengrong Biotechnology Co., Ltd (Guangdong, China).

Macroporous resins including S-8 and AB-8 were purchased from Chemical plant of Nankai University (Tianjin, China), while D101 and DM301 were purchased from Tianjin Haiguang Chemical Co., Ltd (Tianjin, China). Their physical properties and specification were summarized in Table s1.

2.2. Apparatus

A prism twin screw extruder was used to produce PA–PS–DVB resin. A Mettler Toledo 320-S pH meter was used to determine the pH of the solution. An Shimadzu LC-20AT Series chromatograph with Hypersil ODS C18 column (5 μm, 250 × 4.6 mm) was used to analyze the contents of each curcuminoid.

2.3. Preparation of sample solution

Pulverized turmeric was grounded, sieved at 400 μm, and extracted with 20 volumes petroleum ether (reflux at 60–90 °C for 2 h) for twice to remove essential oil and dried. Then, 10 g of the treated sample was extracted with 100 ml of 75% ethanol solution (v/v) at 80 °C for twice. When the extraction was completed, the solutions combined, and the filtrate was condensed in rotary evaporator (RE52–99, Shanghai Yarong Biochemical Instrument Factory, China) under reduced pressure at 50 °C to a certain volume. Finally, the obtained crude extract was stored in a refrigerator at 4 °C prior to further use.

2.4. Analytical methods

An Shimadzu LC-20AT Series chromatograph was used to analyze the contents of each curcuminoid. HPLC analysis was carried out using an mobile phase of 0.1% potassium dihydrogen phosphate in water/acetonitrile (50/50, v/v) at a flow rate of 1.0 ml/min for 15 min. The detection wavelength was 410 nm. The column temperature was 30 °C. Each sample was filtered through 0.45 μm micro-membrane, and a sample volume of 10 μl was injected. Each run of culture experiments and analysis was triplicated.

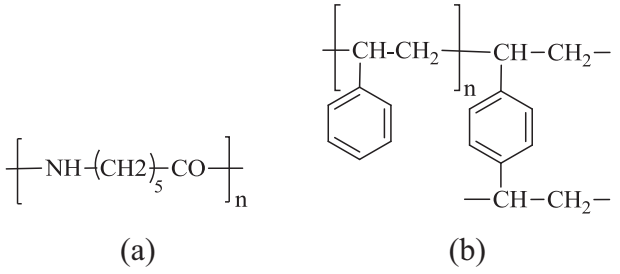


Fig. 2. Chemical structures of polyamide-6 (a) and poly(styrene–divinyl benzene) (b).

2.5. Static adsorption and desorption tests

The static adsorption and desorption tests were carried out in water bath. Adsorbent (2 g) was mixed with 20 ml of turmeric crude extracts, respectively. The initial concentrations (C_0) of the solutions were 2.60, 0.75, and 0.65 mg/ml. The pH of the system started at 6 during the whole adsorption process. The mixture was shaken at 20 °C with 120 rpm until adsorption equilibrium was reached. The concentrations of curcumin, DMC, and BDMC in the residual solutions were analyzed by HPLC. The adsorbent was separated from sample solution by filtration and washed with distilled water, then desorbed with 10 ml of 90% ethanol solution (v/v). After shaking at 20 °C with 120 rpm for 24 h, the contents of curcumin, DMC, and BDMC in the desorption solutions were analyzed by HPLC. The process was repeated for three times. The optimum resin was selected according to its adsorption capacity and desorption ratio.

The adsorption capacity and desorption ratio for each adsorbent were calculated as:

$$q_e = \frac{(C_o - C_e)V_o}{W} \quad (1)$$

$$E(\%) = \frac{C_2}{(C_o - C_1)} \quad (2)$$

where q_e is the equilibrium adsorption capacity (mg/g resin); C_o and C_e were the initial and equilibrium concentrations (mg/ml) of curcumin, DMC, and BDMC in the solution, respectively, (mg/ml); V_o is the volume of the initial solution (ml); and W is the weight of the dry resin (g). E is the desorption radio, C_2 is the concentration of curcumin, DMC, and BDMC in the desorption solution (mg/ml), and C_1 is the concentration of curcumin, DMC, and BDMC in the supernatant.

2.6. Dynamic adsorption and desorption tests

Dynamic adsorption and desorption experiments were carried out in a glass column (Φ 2 mm × 600 mm) packed with 10 g (dry weight) PA–PS–DVB resin. The column was preconditioned for chromatography by pre-equilibrating in 100 ml deionized water and the bed volume (BV) of resin was 20.0 ml. Next, 100 ml solution of the curcuminoids extracts was loaded onto the column at a constant flow rate of 1.5 ml/min. The concentrations of curcumin, DMC, and BDMC in effluent were analyzed by HPLC. After reaching equilibration, 100 ml of deionized water was first used to remove polysaccharides and proteins, and then the column was washed by sequentially passing 80 ml aqueous ethanol of 20%, 40%, 60%, 75%, 85%, and 95% (v/v). The concentrations of curcumin, DMC, and BDMC in each fraction were analyzed by HPLC. The percent recovery of curcumin, DMC, and BDMC was calculated as:

$$\text{Recovery} = \frac{\text{Total curcuminoids eluted}}{\text{Total curcuminoids loaded}} \times 100\% \quad (3)$$

3. Results and discussions

3.1. Blend preparation and characterization

FTIR results indicated that the microsphere was an blend of PA-6 and PS–DVB resin (Fig. s1, supplementary material). SEM