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Preparative separation and purification of rosmarinic acid from perilla seed meal via combined column chromatography

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ABSTRACT

In this study, the preparative separation and purification of rosmarinic acid (RA) from perilla seed meal (PSM), which is a by-product of edible oil production, was achieved using combined column chromatography over macroporous and polyamide resins. To optimize the RA enrichment process, the performance and separation characteristics of nine selected macroporous resins with different chemical and physical properties were investigated. SP825 resin was the most effective: the content of RA increased from 0.27% in the original extract to 16.58% in the 50% ethanol fraction (a 61.4-fold increase). During further purification treatment on polyamide resin, 90.23% pure RA could be obtained in the 70% ethanol fraction. RA with a higher purity (>95%) could also be easily obtained using one crystallization operation. The proposed method is simple, easily operated, cost-effective, and environmentally friendly and is suitable for both large-scale RA production and waste management.

1. Introduction

Perilla frutescens (L) Britt is an aromatic herb that is cultivated worldwide and used extensively as a food garnish in some Asian countries, including China and Japan [1]. Perilla seeds possess large amounts of oil rich in alpha-linoleic and omega-3 fatty acids. Perilla seed meal (PSM) is a by-product of perilla seed oil production. Most PSM is used as a protein source for animal feed. However, after the main constituents (proteins) are isolated from the PSM, the residue still contains many bioactive compounds, which makes it a cheap, undervalued material that has been largely ignored until now. Although many reports have been published describing the activities of natural antioxidants and the evaluations of the stability of the different phenolic acids in *P. frutescens* L. [2–5], very few studies have qualitatively or quantitatively analysed the polyphenol and phenolic acids in PSM.

Recently, RA (Fig. 1) has attracted research interest due to its various biological activities, including its antioxidant [6,7], anti-inflammatory [8], anticancer [9], and anti-allergenic activities [10]. In our previous work [11], several phenolic compounds and organic acids were isolated from PSM extracts, and the RA content was determined by HPLC. These results reveal the potential of PSM to provide antioxidant compounds in addition to polysaccharides and proteins.

However, the preparation of RA is not easy because it readily degrades, particularly at high temperatures, under direct sunlight, and in aqueous solutions with high pH. To date, the methods for the preparative separation and purification of high-purity RA include high-speed counter-current chromatography (HSCCC) [12–17], biotechnological production [18], and total synthesis [19]. However, these established methods possess several disadvantages, such as wasted organic solvent, expensive media, time-consuming procedures, and necessary special instruments. Therefore, the broad use of these methods in large-scale industrial production is limited, particularly in some developing areas.

Synthetic adsorbents have been successfully used to adsorb valuable solutes, such as drugs and polyphenols [20]. Recently, macroporous resin chromatography has been used to separate and enrich bioactive components from herbal crude extracts [21–24] because they have several advantages, such as stable chemical and physical properties, inertness toward toxic solvents, simple regeneration, low cost, and long service life [25]. Therefore, various functionally enhanced commercially available macroporous resins are designed and produced in increasing amounts and varieties to improve the separation industry. Moreover, polyamide (Nylon-6) can be used with "green solvents" during the separation and purification process of some secondary metabolites, including

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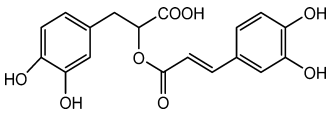

Fig. 1. Chemical structure of RA.

flavonoids, and can be cost-effective and environmentally friendly, which facilitates its extensive application. However, according to previous reports [26–28], it is hard to obtain highly pure RA (>90%) using only macroporous or polyamide resins because the impurities in the crude extracts of herbal raw materials are complex. Therefore, combined column chromatography over macroporous and polyamide resins may be a viable strategy for removing impurities and obtaining highly pure RA.

Additionally, the re-use of agricultural wastes has become more important because of the increasing shortage of natural resources and the development of serious environmental problems. Studies have widely reported the conversion of these waste materials into food ingredients, bio-fuels, and other value-added applications [29]. Therefore, utilising PSM as a source for RA production may help maximise the available resources and solve waste disposal problems. Consequently, this work investigates the adsorption and desorption properties of RA on various macroporous resins with different polarities to maximise its enrichment and to subsequently develop an efficient and inexpensive technology to produce high-purity RA from crude PSM extracts. We disclose the first systematically investigated combined column chromatography procedure using both macroporous and polyamide resins and the first reported use of PSM as a new source for RA.

2. Material and methods

2.1. Samples

The PSM was obtained from Liaoning Jiashi Nutritional Plant Oil Development Corporation Limited (Shenyang, China), and the materials were stored at room temperature in a desiccator until use.

2.2. Chemicals and reagents

RA (purity >98.0%) was purchased from Jianfeng Natural Products Research Co. Ltd. (Tianjin, China). Methanol (chromatography grade) was purchased from Concord Chemical Reagents Co. (Tianjin, China). Orthophosphoric acid was of chromatography grade and was obtained from Shenyang NO. 5 chemical agent factory (Shenyang, China). Ethanol and petroleum ether were of analytical grade and obtained from Bodi Chemical Reagents Co. (Tianjin, China). The water used during the HPLC analysis and for sample preparation was obtained from Wahaha Group Co. Ltd. (Hangzhou, China).

For the standard solution, an appropriate amount of RA was dissolved in methanol to obtain a concentration of 0.2 mg/mL. All of the solutions prepared for HPLC analysis were filtered through 0.45-μm nylon membranes before injection.

2.3. Preparation of crude PSM extract

Dry PSM (100 g) was extracted with 1 L of 50% ethanol (v/v) using ultrasound for 2 h in a sonication water bath (KQ2200B, Kunshan Ultrasonic Equipment Co., Ltd., Kunshan, China) three times. The extracts were combined, filtered, and centrifuged at 3000 rpm for 10 min. The supernatant was concentrated with a rotary evaporator (RE52AA, Yarong Equipment Co., Shanghai, China) under reduced pressure at 50 °C to eliminate any volatile alcohol. This PSM extract (8 g) was used during the following experiments.

2.4. HPLC analysis of RA in PSM

A L2000 series reversed-phase HPLC system (Hitachi, Japan) was employed to determine the RA content. The chromatographic separation was performed with a Kromasil C18 reversed-phase column (250 mm × 4.6 mm, 5 μm). The mobile phases were 0.1% orthophosphoric acid in water (v/v) (eluent A) and methanol (eluent B). A mixture of 50% A and 50% B over 40 min was used for isocratic elution at a flow rate of 1.0 mL/min; the detection wavelength was 330 nm. The injection volume was 10 μL. The quantification was conducted using the RA standard solution. The calibration curve (five data points) was linear with $R^2 = 0.9996$. All of samples used for analysis were filtered through a 0.45-μm membrane before injection.

2.5. Pretreatment of macroporous and polyamide resins

Macroporous resins, including AB-8, ADS-7, ADS-17, HPD100, HPD400A, HPD450, HPD700, and HPD-826, were purchased from Cangzhou Baoen Co. Ltd. (Cangzhou, China), and the SP825 resin was obtained from Mitsubishi Chemical Corporation (Tokyo, Japan). The physical and chemical properties of the resins are summarized in Table 1. The polyamide resin (Nylon-6; particle size, 75–150 μm; surface area, 5–10 m^2/g) was purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China).

The macroporous and polyamide resins were pre-treated by soaking in 95% ethanol for 8 h. After the ethanol was removed, the resins were washed twice with deionized water and subsequently soaked in 1 M NaOH for 5 h. The resins were then washed twice with deionized water. The washed resins were soaked in 1 M HCl for 5 h and then washed thoroughly with deionized water before use. The pre-treated resins were dried in an oven (Taisite Instrument Co. Ltd., Tianjing, China) at 60 °C until their weight was constant [26].

Table 1
Physical and chemical properties of the tested macroporous resins.

Macroporous resin	Surface area (m^2/g)	Average pore diameter (nm)	Particle diameter (mm)	Polarity
AB-8	480–520	130–140	0.3–1.25	Weak-polar
ADS-7	>100	25–30	0.3–1.25	Strong-polar
ADS-17	90–150	25–30	0.3–1.25	Weak-polar
HPD-100	650–700	8–9	0.3–1.2	Non-polar
HPD-400A	500–550	8–9	0.3–1.2	Middle-polar
HPD-450	500–550	9–11	0.3–1.2	Weak-polar
HPD-700	650–700	8–9	0.3–1.2	Nor-polar
HPD-826	500–600	9–10	0.3–1.25	Weak-polar
SP825	1000	5–6	1.25	Weak-polar