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Separation and purification of epigallocatechin-3-gallate (EGCG) from green tea using combined macroporous resin and polyamide column chromatography

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ABSTRACT

Epigallocatechin-3-gallate (EGCG) is a major bioactive ingredient of green tea that produces beneficial neuroprotective effects. In this paper, to optimize the EGCG enrichment, thirteen macroporous resins with different chemical and physical properties were systemically evaluated. Among the thirteen tested resins, the H-bond resin HPD826 exhibited best adsorption/desorption capabilities and desorption ratio, as well as weakest affinity for caffeine. The absorption of EGCG on the HPD826 resin followed the pseudo-second-order kinetics and Langmuir isotherm model. The separation parameters of EGCG were optimized by dynamic adsorption/desorption experiments with the HPD826 resin column. Under the optimal condition, the content of EGCG in the 30% ethanol eluent increased by 5.8-fold from 7.7% to 44.6%, with the recovery yield of 72.1%. After further purification on a polyamide column, EGCG with 74.8% purity was obtained in the 40–50% ethanol fraction with a recovery rate of 88.4%. In addition, EGCG with 95.1% purity could be easily obtained after one-step crystallization in distilled water. Our study suggests that the combined macroporous resin and polyamide column chromatography is a simple method for large-scale separation and purification of EGCG from natural plants for food and pharmaceutical applications.

1. Introduction

Green tea (*Camellia sinensis*) produced by steaming without fermentation is commonly consumed as a health-beneficial beverage in East Aisa [1]. EGCG, a member of the catechin family, is the most abundant polyphenolic constituent, representing approximately 2–13% in the dry green tea. EGCG (Fig. 1) is an ester derivative of epigallocatechin (EGC) that can be synthesized through esterification of EGC with a gallic acid at 3-hydroxyl position of the C-ring. EGCG has multiple phenolic hydroxyl groups in the structure, and is widely used as a natural antioxidant in the healthcare, food and cosmetics products [2]. EGCG exhibits a variety of biological activities, including antioxidant [3], antiinflammatory [4], antimutagenic [5], anticarcinogenic [6] and antiviral effects [7]. We have previously found that EGCG inhibits β-amyloid (Aβ) toxicity, reduces oxidative stress, and decreases apoptosis inhibition in a mouse model of Alzheimer's disease (AD) [8], suggesting that EGCG may be a promising candidate for the treatment of AD. However, due to small quantities and high cost of commercial EGCG products, it is necessary to develop an industry-operational and effective preparative method to purify EGCG from its natural sources.

Conventional methods such as inorganic ion precipitation and solvent extraction are often used to purify EGCG from green tea [9]. Unfortunately, these established methods have several disadvantages such as low efficiency and requirement of a large amount of organic solvents and inorganic salts. Despite the high efficiency of chemical synthesis of EGCG [10], the difficulty to remove possible residues of poisonous chemical reagents prevents its use as a safe food or drug. In addition, column chromatography of Sephadex LH-20 [11], β-cyclodextrin (β-CD) bonded silica [12] and reversed-phase ODS [13] are not appropriate for industrial separation due to their small sample loading volume. Currently, supercritical CO_2 extraction [14], high-speed counter-current chromatography [15]

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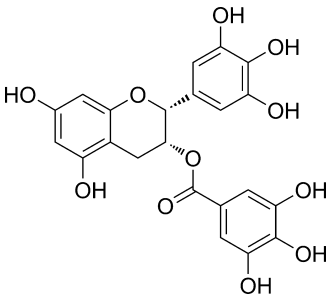

Fig. 1. Molecular structure of EGCG.

and simulated moving bed chromatography [16] have been developed to obtain high-purity EGCG. However, the high cost of these special pharmaceutical instruments prevents their application.

Macroporous resins display distinguishing features such as procedural simplicity, high purification efficiency, good stability, and easy regeneration [17], and have emerged in the fields of enrichment and separation of secondary metabolites from medicinal plant extracts [18–21]. Macroporous resins interact with absorbents through multiple interactions involving Van der Waals force, electrostatic interaction, and molecular sieve action. Polyamide containing functional acylamino and terminal amino groups has specific selectivity for phenolic compounds through strong hydrogen bond interactions, and thus can be used to isolate phenolic compounds. Most importantly, both macroporous resin and polyamide generally use water and food grade ethanol as solvents to desorb target compounds, and thus cost less and are environmental friendly. Recently, it has been reported that tea polyphenols are successfully purified from green tea using macroporous resin or polyamide [22,23]. However, due to the structural similarity among catechins and the chemical complexity of green tea extracts, it is more challenging to separate high pure EGCG from green tea. Therefore, combined column chromatography with macroporous resins and polyamide is a rational strategy for preparing high-purity EGCG.

In this work, we investigated the feasibility of purifying EGCG from green tea, using combined macroporous resin and polyamide column chromatography. This study aimed to investigate the adsorption/desorption properties of EGCG on different macroporous resins, and to develop an efficient method to separate and purify EGCG with the optimal resin and polyamide column.

2. Materials and methods

2.1. Samples and reagents

The green tea used as the crude extracts was obtained from Rongbida Tea Market. (Shenyang, China) in November 2013. EGCG and caffeine standards (purity ≥98%) were purchased from Sigma–Aldrich (St. Louis, MA, USA). HPLC grade methanol was purchased from Sigma–Aldrich (St. Louis, MA, USA). The water used in the HPLC was obtained from Wahaha Group Co., Ltd. (Hangzhou, China).

Acetic acid of analytical grade was purchased from Beijing Chemical Works (Beijing, China). Ethanol and other reagents of analytical grade were obtained from Damao Chemical Reagent Factory (Tianjin, China). Distilled water was purified by a Milli-Q water purification system (Millipore Co., Billerica, MA, USA).

2.2. Adsorbents

Thirteen macroporous resins, including AB8, ADS7, ADS17, D101, DM130, HPD200, HPD400, HPD600, HPD722, HPD750, HPD826, NKA9 and X5 were purchased from Bonchem Co., Ltd. (Cangzhou, China). Their physical properties are listed in Table 1. Polyamide (Nylon-6, particle size: 80–100 mesh) was purchased from SI-JIA Biochemical Plastic Company (Zhejiang, China). Briefly, the resins and polyamide were pre-treated by soaking in ethanol for 24 h. They were then rewashed with fresh ethanol adequately, distilled until no oligomers, porogenic agents and other ethanol-dissolved substances were left, and dried in a drying oven (DGX-9243, Fuma Laboratory Instrument Co., Ltd., Shanghai, China) at 70 °C. Prior to use, the resins and polyamide were soaked in ethanol, and washed thoroughly with distilled water.

2.3. Preparation of green tea extracts and sample solutions

Green tea (1.0 kg) was extracted with 10 L distilled water in an ultrasonic bath (PS-80A Jeken Ultrasonic Cleaner Co., Ltd., Shenzhen, China) at 70 °C for 1 h, twice. The extracted liquids were combined and filtered by membrane filters. The filtrates were evaporated to remove the solvent at 50 °C in a rotary evaporator (N1100, Tokyo Rikakikai Co., Ltd., Tokyo, Japan), and subsequently lyophilized by a freeze dry system (Scientz-18SN, Scientz Biotechnology Co., Ltd., Ningbo, China). The obtained dried green tea extracts contained 7.7% EGCG. Distilled water was added to the extracts to get appropriate EGCG solutions at initial concentrations of 231, 308, 385, 462, 539, 616 and 694 μg/mL for EGCG. The sample solutions were stored in the dark at 4 °C, and used for the following experiments.

2.4. HPLC analysis of EGCG and caffeine

The quantitative analysis of EGCG was determined using the Waters HPLC systems (Waters, USA) equipped with Empower Pro system software, a Waters 600 pump and 600 controller, a Waters 2487 dual λ absorbance detector and a vacuum degasser. The liquid chromatographic separation was performed on a reversed-phase Diamonsil C_{18} column (150 mm × 4.6 mm, i.d., 5 μm, Dikma) at the oven temperature of 30 °C. The detection wavelength was 278 nm, the flow rate was 1.0 mL/min, and the injection volume was 20 μL for each run. The mobile phase consisted of methanol (A) and 0.5% aqueous acetic acid (B). The gradient elution of mobile phase was 20% (A) in 0–15 min, 20–50% (A) in 15–35 min, and 50–80% (A) in 35–50 min. The tested samples and standards were dissolved with distilled water and filtered through 0.45 μm filters before use.

The chromatographic peaks of EGCG and caffeine in the sample were identified by their retention times. The retention time of caffeine and EGCG was 12.5 min and 16.1 min, respectively. Six experimental points were used to plot a calibration curve. The regression equations for EGCG and caffeine were $Y = 28{,}988X - 272.72$ ($R^2 = 0.9997$) and $Y = 49{,}179X + 132.09$ ($R^2 = 0.9995$), respectively, where Y is the peak area, X is the concentration (mg/mL).

2.5. Static adsorption/desorption tests

2.5.1. Adsorption/desorption properties of resins

Static adsorption/desorption tests were performed to select the proper macroporous resin for EGCG separation. Pre-treated hydrated resins (1.0 g, dry weight) were put into 250 mL Erlenmeyer flasks, followed by addition of 200 mL sample solutions with the initial EGCG concentration of 462 μg/mL. Subsequently, the flasks were sealed tightly with a stopper and continually shaken in an incubator shaker (QYC-211, Fuma Laboratory Instrument Co., Ltd., Shanghai, China) at 120 rpm for 12 h at 25 °C. The contents of EGCG and caffeine in the solutions after adsorption were analyzed by HPLC.