Food Chemistry 151 (2014) 380–384

Contents lists available at ScienceDirect

Food Chemistry

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

Short communication

Simultaneous preparation of naturally abundant and rare catechins by tannase-mediated biotransformation combining high speed counter current chromatography

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ARTICLE INFO

Article history:
Received 15 June 2013
Received in revised form 6 November 2013
Accepted 18 November 2013
Available online 26 November 2013

Keywords:
Catechin
Tannase
Preparation
High speed counter current chromatography
HPLC
MS

ABSTRACT

Simultaneous preparation of naturally rare catechins, EGC and EC, has been realized by tannase-mediated biotransformation combining high speed counter current chromatography. In addition, simultaneous preparation of the four catechins, EGCG, ECG, EGC, and EC in green tea extract has also been achieved by HSCCC under the normal phase and the reversed phase modes. The identity of the catechins was determined by HPLC-DAD-ESI-MS and quantification of the catechins was performed by HPLC-DAD. In a typical HSCCC separation, 27.2 mg 98.8% EGCG, 14.1 mg 94.7% EGC, and 9.3 mg 97.5% EC were obtained. This new method is efficient, time-saving and valuable for biological studies.

1. Introduction

Tea has gained a worldwide popularity as a health-beneficial drink. The nutritional value of tea is mostly attributed to the polyphenols that possess a broad spectrum of biological activities (Yang, Wang, Lu, & Picinich, 2009). The flavan-3-ols and their gallates, named as catechins, are the most abundant components in green tea. Epigallocatechin gallate (EGCG) and epicatechin gallate (ECG) account for around 95% of the catechins, and the content of epigallocatechin (EGC) and epicatechin (EC) usually less than 5% (El-Shahawi, Hamza, Bahaffi, Al-Sibaai, & Abduljabbar, 2012). Due to increasing interest in the beneficial effects of catechins, ready availability of analytically pure specific catechins, particularly naturally rare EGC and EC, is essential to explore the detail biological mechanisms.

Tannase can specifically hydrolyze the galloyl ester bond and is widely applied in degradation of complex galloyl polyphenols such as tannic acid and chlorogenic acid (Chávez-González et al., 2011). Treatment by tannase readily converts EGCG and ECG to the corresponding degalloyl EGC and EC (Battestin, Macedo, & De Freitas, 2008; Lu & Chen, 2008; Macedo et al., 2012; Zhong, Zhao, Jönsson, & Hong, 2008). Therefore, enzymatic conversion of naturally abundant EGCG and ECG to naturally rare EGC and EC is a very efficient way to produce EGC and EC.

Another challenge to be addressed to afford analytically pure specific catechins is efficient preparative separation of catechin mixtures. Recently high speed countercurrent chromatography (HSCCC) has attracted much attention for its capacity in preparative separation of complex natural mixtures. Although the combination of tannase-mediated biotransformation and HSCCC separation has been practiced by other scientists (Cao & Ito, 2004; Du et al., 2013; Kumar & Rajapaksha, 2005; Si et al., 2006; Yanagida et al., 2006), simultaneous preparation of naturally rare catechins, EGC and EC, have not been accomplished, and the efficacy and the conditions have to be optimized to achieve practical application.

In current study, a simultaneous preparation of naturally rare catechins, EGC and EC, by tannase-mediated biotransformation combining high speed counter current chromatography was attempted. To the best of our knowledge, it is the first time to report simultaneous preparation of EGC and EC by tannase-mediated biotransformation and HSCCC separation that can readily provide requisite materials for biological studies.

2. Materials and methods

2.1. Materials and reagents

The green tea was obtained from Xuancheng City Jingting Mountain tea plantation (Anhui, China). Tannase with an

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http://dx.doi.org/10.1016/j.foodchem.2013.11.090

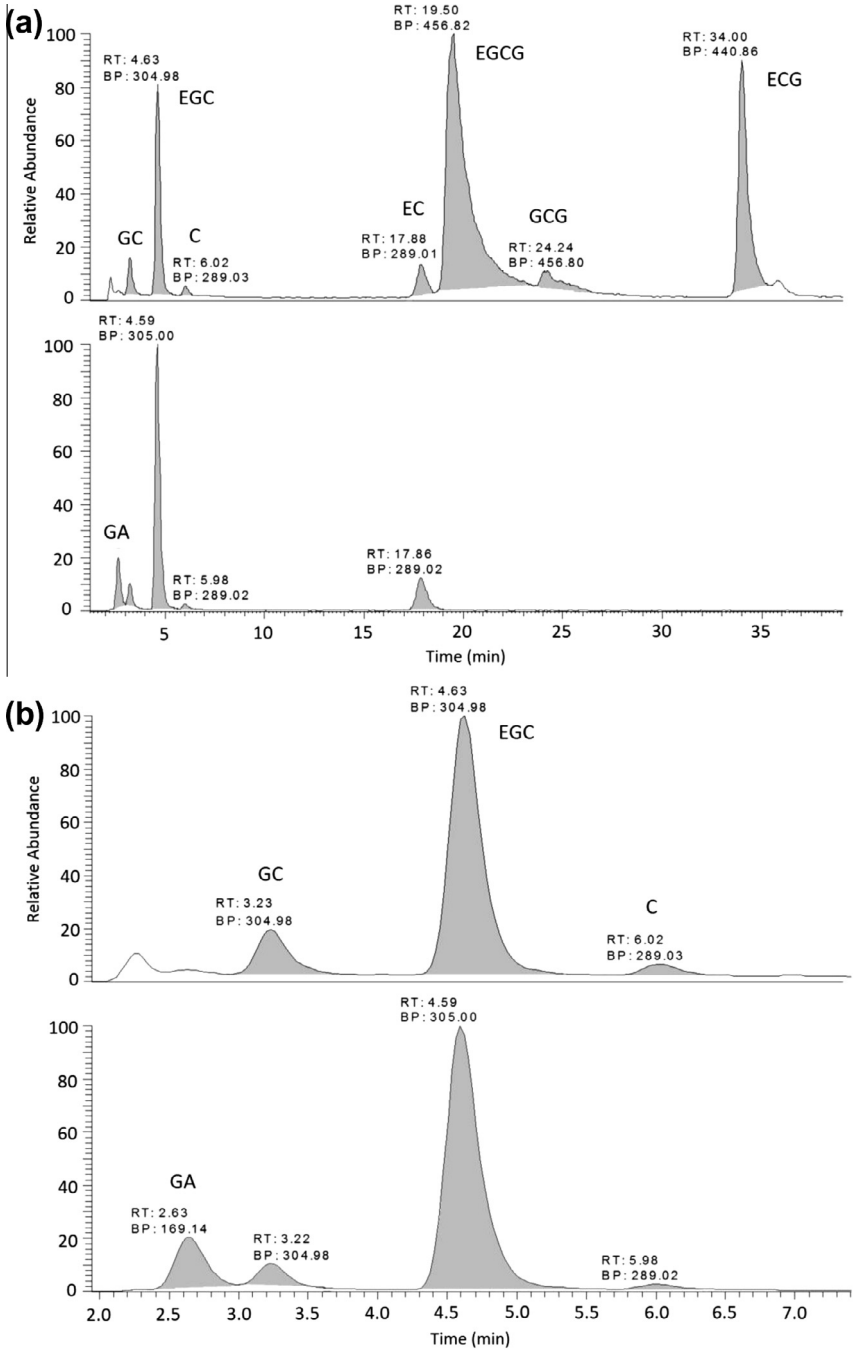

Fig. 1. HPLC-MS analysis of TE (tea extract) and TBP (tannase-mediated biotransformation product) labelled with characteristic mass ion fragments of catechins. (a) Full RT (retention time); (b) Expanded view from RT 1.42 to 7.71 min. BP represents the corresponding base peak (molecular ion) in the mass spectrum.

enzymatic activity of 250 U/mg isolated from *Aspergillus niger* was purchased from Nanning Dong-Higher Bi-Tech (Guangxi, China). All reagents are of analytical grade. Catechin standards including (−)-epicatechin (EC) (⩾98%), (−)-epigallocatechin (EGC) (⩾98%), (−)-epicatechin gallate (ECG) (⩾98%), and (−)-epigallocatechin gallate (EGCG) (⩾98%) were purchased from Aladdin-Reagent (Shanghai, China).

2.2. Green tea decaffeination and catechin extraction

Extraction of catechins from green tea was carried out according to the modified literature procedure (Vuong, Golding, Nguyen, & Roach, 2013). Ten grams of green tea were precisely weighted and extracted with 500 mL of purified water in an ultrasonic water bath at 90 °C for 30 min. Then the tea infusion was centrifuged at 5000 rpm (3773 g) for 15 min. The supernatant was immediately filtrated and partitioned twice with an equal volume of chloroform to eliminate impurities and caffeine. Then the resulting aqueous layer was partitioned twice with equal volume of ethyl acetate. The ethyl acetate phase containing tea catechins was evaporated under reduced pressure. Approximately 970 mg (mean value of duplicate) of green tea extract was obtained as a pale brown powder.

2.3. Tannase-mediated biotransformation of tea extract

The catechin gallates in tea extract were transformed into the corresponding degalloyl catechins by the treatment with tannase following a modified literature procedure (Cao & Ito, 2004; Lu &