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A novel method for the synthesis of glyceryl monocaffeate by the enzymatic transesterification and kinetic analysis

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ABSTRACT

A novel enzymatic method for glyceryl monocaffeate (GMC) preparation by the transesterification of ethyl caffeate (EC) was investigated. The effects of reaction variables (reaction pressure, temperature, reaction time, enzyme load, and substrate ratio) on the enzymatic transesterification were studied and optimized using response surface methodology. HPLC-ESI-MS and HPLC-UV were used to monitor the transesterification. Thermodynamics, kinetic analyses and reaction mechanism were also evaluated. Results showed that, GMC can be successfully prepared by the enzymatic transesterification of EC with glycerol. Under the optimal conditions (enzyme load 22.54%, EC:glycerol = 1:12.75 (mol/mol), 72.5 °C, and 10.5 h), EC conversion and GMC yield were 97.9 ± 0.7% and 95.8 ± 1.0%, respectively. The activation energies (Ea) for EC conversion and GMC formation were 44.23 and 46.51 kJ/mol, respectively. The kinetic values for V_{max}, K'_m and K_{IA} were 2.18 × 10^{-3} mol/(L min), 0.086 mol/L, and 0.52 mol/L, respectively. The transesterification mechanism with EC inhibition was also proposed.

1. Introduction

Caffeic acid (CA, 3,4-Dihydroxycinnamic acid) is a naturally widespread phenolic compound in many agricultural products such as fruits, grains, vegetables, wine, olive oil, coffee and some Chinese medicinal herbs (Jiang et al., 2005; Medina et al., 2012). CA has many biological activities, for examples, antiviral (de Campos Buzzi et al., 2009), antimicrobial, antitumor, free radical-scavenging (Son & Lewis, 2002; Wu et al., 2007), UV-absorption (Prasad, Jeyanthimala, & Ramachandran, 2009), anti-carcinogenic (Zhang et al., 2014), anti-inflammatory (Teixeira et al., 2013), anti-HIV (Bailly & Cotelle, 2005; Jiang et al., 2005) and antioxidant activities (Aleman et al., 2015; Chen & Ho, 1997; Damasceno et al., 2013; Gülçin, 2006; Medina et al., 2012), which have made CA and its derivatives attract considerable attention in recent years. However, the unsatisfactory solubility of CA in polar/non-polar media limits its effectiveness in water/oil systems. Therefore, modifications of CA are necessary to widen its application in the food, pharmaceutical, and cosmetics industries.

Several chemical and enzymatic methods have been developed for the preparation of caffeate derivatives. For examples, chemical, chemo-enzymatic, or enzymatic methods in solvent-free or ionic liquids to prepare alkyl caffeate (Chen, Twu, Chang, Liu, & Shieh, 2010; de Campos Buzzi et al., 2009; Pang et al., 2013; Wang, Gu, Pang, Wang, & Wu, 2013; Yang, Guo, & Xu, 2012), caffeic acid phenethyl ester (Chen, Chen, Chang, & Shieh, 2011; Ha, Anh, Lee, & Koo, 2012; Kurata et al., 2010; Wang, Gu, Cui, Wu, & Wu, 2014), phytosteryl caffeates (Tan & Shahidi, 2012), caffeic acid amides (Rajan et al., 2001; Xiao et al., 2013), dihydrocaffoylated glycerides (Feddern, Yang, Xu, Badiale-Furlong, & de Souza-Soares, 2011; Yang, Feddern, Glasius, Guo, & Xu, 2011), and dicaffeoyltartaric acids (King et al., 1999) have been reported. Glyceryl monocaffeate (GMC) is a kind of hydrophilic derivatives of CA, and the solubility of GMC in water is about 3 times that of CA (1.76 mg/mL in water at 20 °C). However, to the best of our knowledge, enzymatic synthesis of GMC has not yet been reported in the literature.

In the work, a novel enzymatic preparation of GMC using lipase-catalyzed transesterification of ethyl caffeate (EC) with glycerol was investigated (Fig. 1). The effects of reaction variables (reaction pressure, temperature, reaction time, enzyme load, and substrate ratio) on the transesterification were studied. High Performance Liquid Chromatography-Electro Spray Ionization-Mass Spectroscopy (HPLC-ESI-MS) and HPLC-UV were used to monitor the transesterification. Thermodynamic and kinetic analyses of the transesterification were also performed to investigate the reaction mechanism. Response surface methodology (RSM) was used to evaluate the relationship between reaction variables and GMC yield (or EC conversion).

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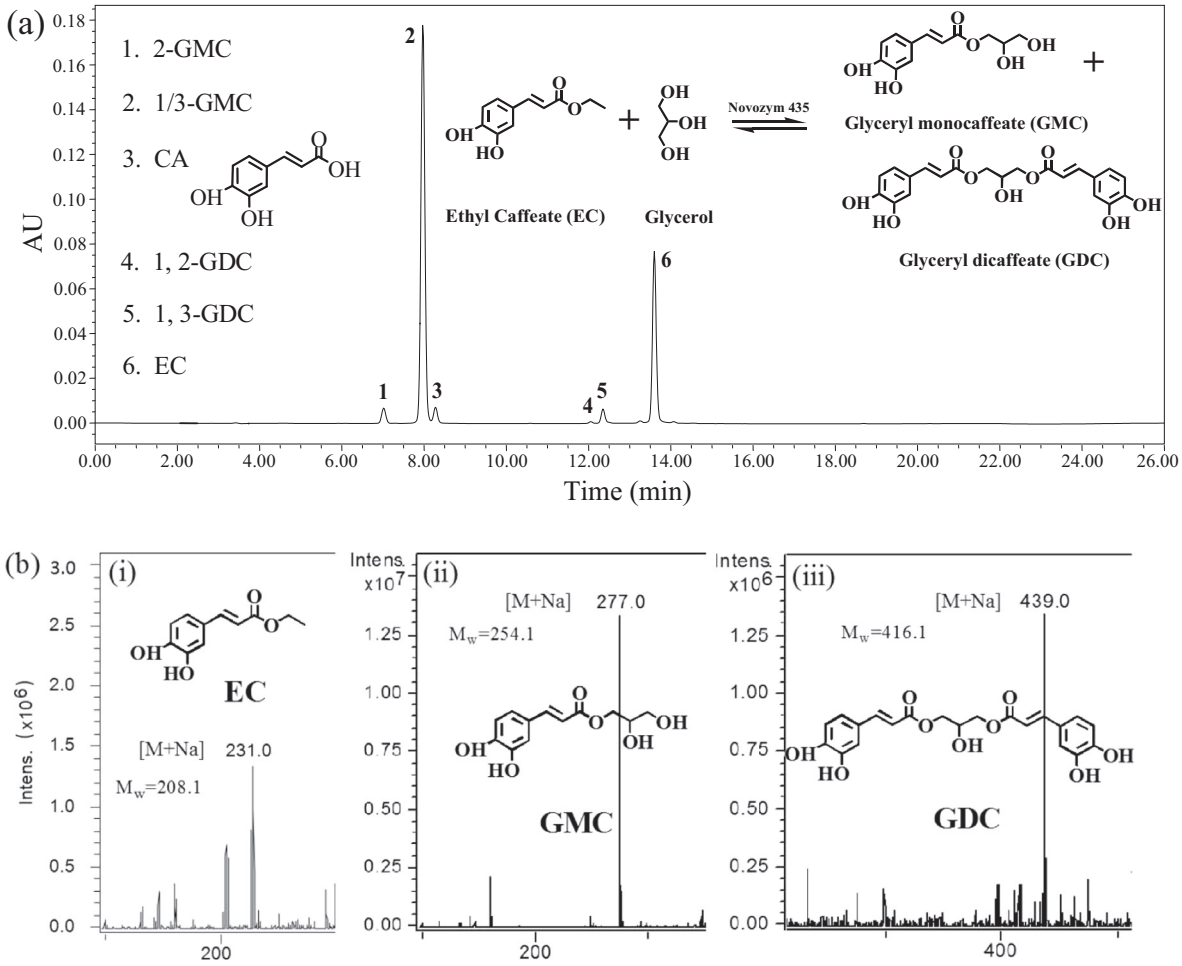

Fig. 1. (a) Reaction scheme for enzymatic transesterification of ethyl caffeate (EC) with glycerol and the HPLC chromatogram of reaction mixture at 325 nm. Reaction conditions: EC:glycerol = 1:20 (mol:mol), 90 kPa, enzyme load 20% (relative to the weight of total substrates), 70 °C, 3 h. (b) Electro Spray Ionization-Mass Spectra (ESI-MS) analysis of ethyl caffeate (EC), glyceryl monocaffeate (GMC), and glyceryl dicaffeate (GDC).

2. Materials and methods

2.1. Materials

Ethyl caffeate (EC, purity > 99%) was purchased from Nanjing Zelang Chemical Co., Ltd. (Nanjing, China). Glycerol (purity > 99%, dehydration using an activated molecular sieve before used) was purchased from Tianjin Kermel Chemical Co., Ltd. (Tianjin, China). Novozym 435 (*Candida antarctica* lipase immobilized on polyacrylic resin by adsorption, 7000 PLU/g solid enzyme) was from Novozymes A/S (Bagsvaerd, Denmark). Methanol (HPLC grade), Ethanol (HPLC grade) and glacial acetic acid (HPLC grade) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). All other reagents were of analytical grade.

2.2. Enzymatic transesterification

The transesterification of EC with glycerol was carried out in 25 mL round-bottom flasks. Reaction mixtures were incubated at various temperatures using an oil bath with a magnetic stirrer under 90 kPa, and then the biocatalyst Novozym 435 was added to the reaction mixtures. Samples (5 μL) were withdrawn at specific time intervals.

2.3. HPLC analysis

The reactants were analyzed by HPLC (waters 1525) with a C18 reverse phase column (5 μm, 250 mm × 4.6 mm) fitted with a dual absorbance detector (waters 2489) at 325 nm. The mobile phases were solvent A (400 mL methanol) and solvent B (water, 0.5% v/v glacial acetic acid) at 1 mL/min. The elution sequence consisted consecutively of a linear gradient from 80% (v/v) A to 0% A (v/v) in 18 min, then to 80% A in 4 min, followed by 80% A for 4 min at 35 °C.

2.4. HPLC-Electro Spray Ionization-Mass Spectroscopy (HPLC-ESI-MS) analysis

Mass spectroscopic analyses were performed using Agilent 6310 mass spectrometer with a direct ESI interface, using positive ion (PI) mode. Components of the samples were identified with regard to the relevant major ions detected by HPLC-ESI-MS: GMC (required M 254, $[M+Na]^+$ 277), glyceryl dicaffeate (GDC, 416, 439), ethyl caffeate (208, 231). The operating conditions used were 3.5 kV for the capillary voltage, 24 eV for the cone voltage, 120 °C for the ion source temperature, 350 °C for the desolvation temperature, 700 V for the photomultiplier voltage, $2.6e^{-5}$ mBar for analyzer vacuum, and 120 L/min for the gas flow.