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A novel continuous flow biosynthesis of caffeic acid phenethyl ester from alkyl caffeate and phenethanol in a packed bed microreactor

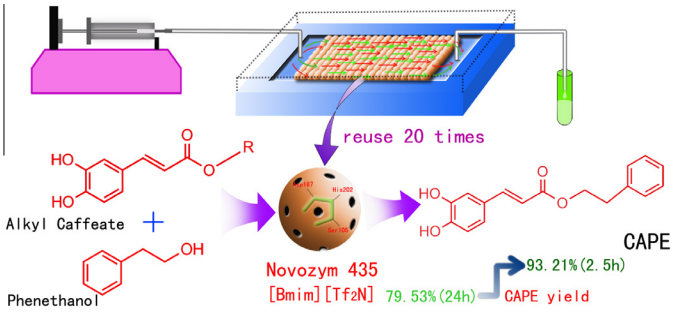
Jun Wang [a,b,*], Shuang-Shuang Gu [a], Hong-Sheng Cui [a], Xiang-Yang Wu [b,*], Fu-An Wu [a,c]

[a] School of Biology and Chemical Engineering, Jiangsu University of Science and Technology, Zhenjiang 212018, PR China
[b] School of the Environment, Jiangsu University, Zhenjiang 212013, PR China
[c] Sericultural Research Institute, Chinese Academy of Agricultural Sciences, Zhenjiang 212018, PR China

HIGHLIGHTS

- Ten alkyl caffeates were screened as suitable substrates via transesterification.
- MC was the best substrate for enzymatic synthesis of CAPE at a lower temperature.
- A packed bed microreactor was firstly used for the continuous synthesis of CAPE.
- The highest CAPE yield of 93.21% was obtained in 2.5 h using RSM optimization.
- Novozym 435 was reused without a loss of activity for 20 cycles or 9 days.

GRAPHICAL ABSTRACT



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ABSTRACT

Caffeic acid phenethyl ester (CAPE) is a rare natural ingredient with several biological activity, but the industrial production of CAPE using lipase-catalyzed esterification of caffeic acid (CA) and 2-phenylethanol (PE) in ionic liquids is hindered by low substrate concentrations and a long reaction time. To establish a high-efficiency bioprocess for obtaining CAPE, a novel continuous flow biosynthesis of CAPE from alkyl caffeate and PE in [Bmim][Tf_2N] using a packed bed microreactor was successfully carried out. Among the tested alkyl caffeates and lipases, methyl caffeate and Novozym 435, respectively, were selected as the suitable substrate and biocatalyst. Under the optimum conditions selected using response surface methodology, a 93.21% CAPE yield was achieved in 2.5 h using a packed bed microreactor, compared to 24 h using a batch reactor. The reuse of Novozym 435 for 20 cycles and continuous reaction for 9 days did not result in any decrease in activity.

1. Introduction

Caffeic acid phenethyl ester (CAPE), a natural flavonoid-like compound, is one of the main active components of propolis (Suzuki et al., 2006). This compound has strong anticancer (Ozturk et al., 2012), antiviral (Noelker et al., 2005), antioxidant (Gocer and Gulcin, 2011), and immunomodulatory properties in a diverse set of systems (Lee et al., 2009). However, the isolation of this highly valuable CAPE from natural product extracts is inefficient, time-consuming and uneconomical. Therefore, to produce CAPE at a reasonable price, biosynthesis is becoming increasingly attractive due to its economic benefits when compared to extraction from natural sources and greater ecological acceptability compared to chemical synthesis (Kim et al., 2011).

* Corresponding authors. Address: School of Biology and Chemical Engineering, Jiangsu University of Science and Technology, Zhenjiang 212018, PR China. Tel.: +86 511 85616571; fax: +86 511 85635850 (J. Wang). Tel.: +86 511 85038750; fax: +86 511 85038451 (X.-Y. Wu).

E-mail addresses: jimwang_js@hotmail.com, wangjun@just.edu.cn (J. Wang), wuxy@ujs.edu.cn (X.-Y. Wu).

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In recent years, the lipase-catalyzed esterification of CAPE from caffeic acid (CA) and 2-phenylethanol (PE) in organic solvents and ionic liquids (ILs) has been successively established. In most cases, CAPE could be obtained from CA and PE catalyzed by Novozym 435 in isooctane (Widjaja et al., 2008) or [Emim][Tf_2N] (Wang et al., 2013a) and the conversion of CA was nearly 100% in 48 h. Although the reaction time was shortened to 9.6 h when ultrasound-accelerated enzymatic technology was used to synthesize CAPE (Chen et al., 2011a,b), this procedure is not suitable for industrial production due to the required special equipment and energy consumption. However, above all, the CAPE yield is still not high enough (only approximately 64.55%) (Wang et al., 2013a). A moderate yield in the esterification of CA with PE to afford CAPE is observed because CA possesses two hydroxyl groups on its aromatic ring and a double bond on the side chain that cause it to inhibit lipase (Tan and Shahidi, 2012). In addition, water is formed as one of the products of the esterification of CA and PE. The water concentration increases during the reaction and has a negative influence not only on substrate conversion but also on lipase activity.

In our previous work, lipase-catalyzed esterification of propyl caffeate was performed using methyl caffeate (MC) and 1-propanol in [Bmim][CF_3SO_3] with a maximum yield of 98.5% in 24 h, which indicated that the transesterification process of alkyl caffeate and the corresponding alcohol provided an efficient pathway for the synthesis of some caffeate esters (Pang et al., 2013). [Bmim][Tf_2N] was chosen as the suitable reaction medium to enzymatic transesterification synthesis of CAPE analogues (Kurata et al., 2010). It shows environmentally friendly and can enhance enzyme activity, selectivity, and stability. In addition, the synthesis of CAPE by the lipase-catalyzed transesterification of alkyl caffeate and the corresponding alcohol in [Bmim][Tf_2N] has been proven a feasible procedure because alkyl caffeates are much more easily prepared and have cost less. However, there are still some drawbacks for industrial-scale production in a traditional batch reactor, such as long reaction times. Thus, there is a need to explore more efficient processes for the lipase-catalyzed synthesis of CAPE from alkyl caffeate and PE via transesterification.

Nowadays, microreactor technology has shown promise as a novel method in biocatalysis, in which the reactions generally produce the desired product with a higher yield, in a shorter time, and more efficiently, safely, and selectively than traditional batch-scale reactions (Chen et al., 2013; Woodcock et al., 2008). These microreactors take advantage of rapid heat and mass transfer rates that cannot be achieved by conventional batch systems (Lévesque and Seeberger, 2012; Seo et al., 2012). In addition, microreactors contribute to the rationalization of process development with significant reductions in manpower, the quantity of reagents required, the amount of waste solvent generated and cost (Wen et al., 2009; Marques et al., 2012). For industrial-scale production, the use of packed bed reactors with immobilized enzymes is more cost effective than the use of reactors operated in batch mode (Chen et al., 2011a,b). The advantages of packed bed reactors include continuous operation, effective reuse of enzyme without prior separation, reduction of labor costs, and protection of enzymes from mechanical shear stress (Kundu et al., 2011; Martin-Rapun et al., 2013). Recent publications in the field of miniaturization of packed bed reactors have shown promising results (Wang et al., 2013b), including improved mass transport rates leading to higher yields and shorter reaction times; for example, a relatively cheap and reusable miniaturized packed bed reactor was used for the lipase-catalyzed synthesis of anhydride and isoamyl alcohol (Cvjetko et al., 2012). Thus, lipase-catalyzed synthesis of CAPE in a miniaturized packed bed reactor is an effective strategy to obtain a greater yield in a shorter amount of time. However, to the best of our knowledge, no report has been published concerning the biosynthesis of CAPE from alkyl caffeate and PE using lipase-catalyzed transesterification in a packed bed microreactor.

The aim of this work was to develop a simple and efficient approach to the enzymatic transesterification synthesis of CAPE from alkyl caffeate and PE in [Bmim][Tf_2N] using a novel miniaturized packed bed reactor. For this purpose, a series of alkyl caffeates were synthesized, and the optimal substrate for lipase-catalyzed transesterification of CAPE was chosen by screening. Furthermore, the effect of substrate concentration, substrate molar ratio, reaction temperature, and flow rates on the CAPE yield as well as the stability of the system were investigated in a continuous flow packed bed microreactor. Additionally, kinetic models for the biosynthesis of CAPE in batch reactors and microreactors were proposed and compared.

2. Methods

2.1. Materials

[Bmim][Tf_2N] was obtained from Shanghai Cheng-Jie Chemical Co., Ltd. (Shanghai, China). Lipases including Novozym 435, Lipozyme TL IM and Lipozyme RM IM were purchased from Novozymes (Bagsvaerd, Denmark). Caffeic acid was purchased from Nanjing Zelang Pharmaceutical Sci. & Tech. Co., Ltd. (Nanjing, China). Methanol and acetonitrile were HPLC grade (Tedia Co., Fairfield, OH, USA), and all other reagents and solvents were analytical grade (Sinopharm Chemical Reagent Co., Ltd., Shanghai, China).

2.2. Batch reaction

Initial screening of the suitable alkyl caffeate and lipase for enzymatic transesterification from alkyl caffeate and PE to obtain CAPE was performed in 5 mL glass vials with screw caps (Pang et al., 2013). Table 1 shows the abbreviation of the alkyl caffeates used as substrates in the present study. Alkyl caffeate and PE were added to 1 mL [Bmim][Tf_2N]. The reaction was initiated by the addition of lipase. All reactions were performed at temperatures of 55 °C, 70 °C, and 85 °C with a constant stirring speed of 120 rpm. After 3 days, 20 μL aliquots were taken from the reaction mixture and diluted using 980 μL of methanol for HPLC analysis. All experiments were performed in triplicate.

2.3. Continuous flow microreactor reaction

Microchannels with dimensions of 1 cm, 500 μm, and 75 mm in width (W), height (H), and length (L), respectively, were milled on a PDMS plate. The designed packed-bed microreactor was easily assembled, operated and cleaned. 90 mg of the Novozym 435 beads was uniformly put in the 75 mm long channel. Then the channel was covered with another PDMS plate. Due to the PDMS material is elastic and two PDMS plates can be sealed by using

Table 1
The abbreviation of the alkyl caffeates used as substrates in the present study.

Antioxidant	Abbreviation	−R
Methyl caffeate	MC	CH_3
Ethyl caffeate	EC	CH_2CH_3
Propyl caffeate	PC	$CH_2CH_2CH_3$
Isopropyl caffeate	IpC	$CH_2CH_2CH_3$
Butyl caffeate	BuC	$(CH_2)_3CH_3$
Amyl caffeate	AC	$(CH_2)_4CH_3$
Isoamyl caffeate	IaC	$(CH_2)_4CH_3$
Hexyl caffeate	HexC	$(CH_2)_5CH_3$
Heptyl caffeate	HepC	$(CH_2)_6CH_3$
Octyl caffeate	OC	$(CH_2)_7CH_3$