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Ultrasound irradiation accelerates the lipase-catalyzed synthesis of methyl caffeate in an ionic liquid

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ABSTRACT

Methyl caffeate is a natural ingredient with several biological activities, but its preparation is generally limited to chemical synthesis. To set up a simple, high-yield and low-cost synthesis process for obtaining methyl caffeate, a novel synthesis method using the lipase-catalyzed esterification of methanol and caffeic acid in an ionic liquid under ultrasound irradiation was established. A maximum yield of 99.79% was obtained using ultrasound irradiation with an ultrasound frequency of 25 kHz and ultrasound power of 150 W under the following optimal conditions: Novozym 435 as a biocatalyst, [Bmim][Tf_2N] as the reaction medium, lipase concentration of 60 g/L, reaction temperature of 75 °C, and reaction time of 9 h. Using the optimal conditions under ultrasound irradiation, the reaction time was reduced 0.75-fold, and the apparent kinetic parameter (V_m/K_m) was increased 2-fold. The lipase could be reused 11 times without significant loss of activity. The results suggest that ultrasound irradiation can accelerate lipase-catalyzed synthesis of methyl caffeate in an ionic liquid.

1. Introduction

Methyl caffeate (methyl-(E)-3-(3,4-dihydroxyphenyl) prop-2-enoate, MC) is one of the alkyl caffeates extracted from *Polygonum amplexicaule* and the fruit of *Solanum torvum*. Alkyl caffeates have obvious biological functions in antimicrobial, antioxidative, antiviral and antineoplastic activities [1,2]. Among the alkyl caffeates, compared with caffeic acid (CA), MC has molecular polarity comparable to that of CA and has appetite suppressant and larval development inhibitory activities [3]. MC also has strong anti-inflammatory, anticancer, antioxidant, antiviral, detoxicant, anti-clotting, and anti-diabetic effects on diabetic rats induced by streptozotocin [4]. Additionally, MC can be used as an intermediate for the production of natural medicines or food additives such as propyl caffeate (PC) and caffeic acid phenethyl ester (CAPE), which are oxidative dimerization products of MC [5].

Currently, preparation strategies for MC typically focus more on chemical synthesis than on enzymatic synthesis. Several reports have been published on the synthesis of MC from CA and methanol using an acidic catalyst. For example, Shin et al. described the esterification of CA and methanol in the presence of sulfuric acid in 10 h with an MC yield of 71.0% [6]. Then, Wang et al. described a similar process catalyzed by *p*-toluenesulfonic acid (PTSA) for the preparation of MC in 4 h, achieving a relatively higher MC yield of 84.0% [7]. However, acidic catalysts are hazardous, requiring special energy-inefficient processes to dispose of the waste acid. Moreover, chemical methods are associated with problems such as high energy consumption, poor reaction selectivity and high cost of manufacturing. In contrast, the lipase-catalyzed synthesis of caffeates has garnered increasing attention due to its relative advantages. Chen et al. [8] used immobilized lipase as a catalyst to synthesize CAPE from CA and 2-phenylethanol (PE), achieving 93.08% molar conversion of CAPE. The results indicated that enzymatic synthesis could offer several advantages, such as mild reaction conditions and reagents, minimization of side products in the reaction, higher activation energy, and better control over

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the esterification reaction. Thus, there is a need to explore more efficient processes for the lipase-catalyzed synthesis of MC via esterification.

Enzymatic catalysis in ionic liquids (ILs) has been extensively studied for its high substrate conversion and product yield [9], high reaction rates, high activity, high stability, and good enantioselectivity [5]. Recently, the lipase-catalyzed synthesis of caffeates has attracted intense interest, with caffeates having higher solubility than CA in hydrophobic media [10]. The synthesis methods tested include esterification and transesterification, the latter having higher reaction efficiency than the former. As we have reported earlier [11], the use of MC as a substrate during the synthesis of propyl caffeate (PC) significantly improves the yield. Thus, biosynthesis of MC could provide greener avenue for the production of the much needed MC to be used in such process. Moreover, the method of lipase-catalyzed esterification synthesis of MC had yet not been tested in an ionic liquid system. Therefore, it was tested in this study for its effectiveness in obtaining better MC yields.

Recently, ultrasound irradiation has seen numerous applications in organic chemistry as an environmentally benign method [12]. Ultrasound irradiation is a useful tool for enhancing mass transfer in liquid–liquid heterogeneous systems and increasing the mass transfer rate of reagents and the active sites of enzymes [13]. Immobilized enzymes are more resistant than native enzymes to thermal deactivation caused by ultrasound irradiation [14]. Although the application of ultrasound irradiation to enzymatic reactions has not been extensively explored, it has been used to accelerate enzymatic reactions [15], such as esterification of phytosterol and different acyl donors [16], enzymatic esterification of rutin and naringinto catalyzed by Novozym 435 [17], transesterification of glycerol and methyl benzoate in the organic solvent [18], and synthesis of sugar esters in ILs [19]. To date, no known studies have investigated the application of ultrasound irradiation to the synthesis of MC.

Hence, the aim of this study was to explore a new method for the synthesis of MC using lipase-catalyzed esterification of CA and methanol in ILs. The effects of the type of IL, the types of lipase, the concentration of substrate, the concentration of biocatalysts in reaction system, the reaction time and reaction temperature on the esterification yield were investigated under incubator shaking. The experiments were subsequently conducted with incubation under ultrasound irradiation. Nuclear magnetic resonance (NMR) and mass spectrometry were used to identify the product.

2. Materials and methods

2.1. Enzymes and materials

Commercial lipases containing Novozyme 435, Lipozyme TL IM (immobilized lipase from *Thermomyces lanuginosus* (previously *Humicola lanuginosa*), immobilized on a granulated silica carrier), and Lipozyme RM IM (*Rhizomucor miehei*, 275 IUN/g, where IUN represents Interesterification Units Novo; carrier: phenol formaldehyde), were provided by Novo Nordisk A/S (Bagsvaerd, Denmark). Eight ILs, including [Hmim][HSO_4], [Bmim][Tf_2N], [Bmim][PF_6], [Bmin][BF_4], [Emim][TfOH], [Toma]Cl, [Toma][Tf_2N], [Omim][BF_4] were obtained from Shanghai Cheng-Jie Chemical Co. Ltd. (Shanghai, China). The residual chloride content in these ILs was less than 50 ppm. CA was from Nanjing Zelang Pharmaceutical Sci. & Tech. Co. Ltd., China. Methanol and acetonitrile were HPLC-grade (Tedia Co., Fairfield, OH, USA), and other reagents were analytical grade (Sinopharm Chemical Reagent Co. Ltd., Shanghai, China).

2.2. Lipase-catalyzed synthesis of MC in an incubator shaker

The esterification reaction was performed in a 5 mL screw-capped vial at 75 °C for 36 h with a constant stirring speed of 120 rpm. CA (100 mg) was dissolved in methanol (10 mL), then different volumes of CA solution were added to IL, making the total reaction system to be 0.5 mL. The reaction was initiated by adding immobilized enzyme. At regular time intervals (3, 6, 12, 24, 36 and 48 h), 20 μL aliquots were taken from the well-stirred reaction mixtures and diluted using 380 μL methanol in preparation for HPLC analysis. The effects of different reaction temperatures (50–80 °C), different concentrations of substrate (0.25, 0.5, 1, 1.5, 2, 4 and 6 g/L), and concentration of Novozym 435 (20, 40, 60, 80, 100, and 120 g/L) on MC yield were investigated. All experiments were carried out in triplicate.

2.3. Lipase-catalyzed synthesis of MC under ultrasound irradiation

The esterification reaction was performed in a 5 mL screw-capped vial at 75 °C for 9 h under ultrasound irradiation using an ultrasound frequency of 25 kHz and ultrasound power of 150 W. CA (100 mg) was dissolved in methanol (10 mL), and different volumes of CA solution were added to IL, making the total reaction system to be 0.5 mL. The reaction was initiated by adding the immobilized enzyme. At regular time intervals (1, 2, 3, 6, 12, 24, 36 and 48 h), 20 μL aliquots were taken from the well-stirred reaction mixture and diluted using 380 μL methanol in preparation for HPLC analysis. The effects of different ultrasound power (90, 120, 150, 180, 210 W), frequency (15, 20, 25, 30, 35 kHz), operation mode (sweep, pulse), reaction temperatures (50–80 °C), different concentrations of substrate (0.25, 0.5, 1, 2, 4 and 6 g/L), and concentration of Novozym 435 (20, 40, 60, 80, and 100 g/L) on the MC yield were investigated. All experiments were performed in triplicate.

2.4. Complexation extraction process of MC

The complexation extraction process was used to extract MC from enzymatic reaction system using trioctylphosphine oxide (TOPO)–cyclohexane as an extractant [20], in which mass fraction of complex agent TOPO in diluter cyclohexane was 100 g/L. MC was extracted from the mixture to extractant with the ratio of 1:1 (v/v). After cyclohexane was volatilized from extractant, the residue was crystallized from methanol, thus MC could be separated from mixtures to obtain white product with the method of vacuum drying.

2.5. Kinetic study of lipase-catalyzed synthesis of MC

The kinetics of the esterification reaction was investigated by studying the effect of the CA concentration on the initial rate of the reaction. The concentration of CA varied within the range of 1.39–33.32 mM. Initial reaction rates, which expressed as mM CA per hour, were determined from the time course of the reaction by using a second order polynomial curve fitting via regression analysis of the product concentration as well as determining the initial slope of the tangent to the curve.

2.6. Analysis of products by LC–MS and HPLC

As in previous test methods [11], LC-PAD-MS was performed using a Thermo Fisher system. The LC equipment comprised a Finnigan MAT Spectra System P4000 pump, a Finnigan AQA mass spectrometer, and an autosampler with a 50 μL loop. LC separation was performed on a Kromasil C_{18} column (150 mm × 4.6 mm, i.d.; 5 μm, W.R.) and monitored using a UV6000 LP diode array detector. Isocratic elution was used to run the mobile phase, which was