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Synthesis of benzyl cinnamate by enzymatic esterification of cinnamic acid

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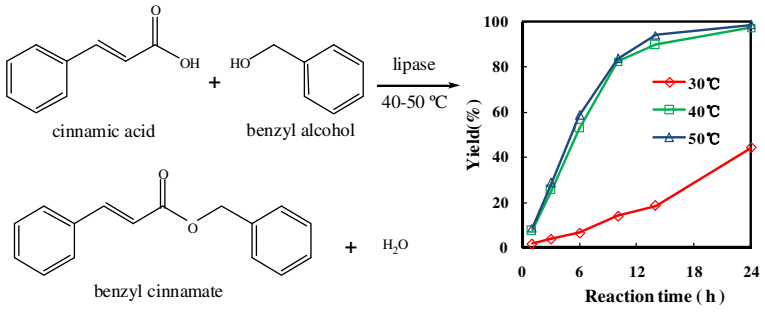
HIGHLIGHTS

- Benzyl cinnamate was synthesized by enzymatic esterification of cinnamic acid.
- High yield of benzyl cinnamate was obtained using isooctane as media.
- The effects of several parameters on this reaction were analyzed.

GRAPHICAL ABSTRACT



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ABSTRACT

In this study, lipase catalysis was successfully applied in synthesis of benzyl cinnamate through esterification of cinnamic acid with benzyl alcohol. Lipozyme TLIM was found to be more efficient for catalyzing this reaction than Novozym 435. In order to increase the yield of benzyl cinnamate, several media, including acetone, trichloromethane, methylbenzene, and isooctane, were used in this reaction. The reaction showed a high yield using isooctane as medium. Furthermore, the effects of several parameters such as water activity, reaction temperature, etc, on this reaction were analyzed. It was pointed out that too much benzyl alcohol would inhibit lipase activity. Under the optimum conditions, lipase-catalyzed synthesis of benzyl cinnamate gave a maximum yield of 97.3%. Besides, reusable experiment of enzyme demonstrated that Lipozyme TLIM retained 63% of its initial activity after three cycles. These results were of general interest for developing industrial processes for the preparation of benzyl cinnamate.

1. Introduction

Lipase (EC 3.1.1.3) make up a diverse group of enzymes that have the ability to catalyze ester hydrolysis and the reverse esterification (Compton et al., 2000). Lipase-catalyzed esterification in organic media offers numerous possibilities for biotechnological production of useful chemicals (Badgujar and Bhanage, 2014; Chen et al., 2011). Particularly, this technology can be used to product natural substance relevant to various industrial scopes such as cosmetics, fine chemical, pharmaceuticals, and food ingredients (Wang et al., 2016).

Benzyl cinnamate, as one of the cinnamic acid esters, is widely found throughout the nature kingdom, e.g. in leaves and twigs of Bermuda buttercup, lignin, balsam, and propolis (DellaGreca et al., 2007; Aliboni et al., 2011). There has been continuing research interest in benzyl cinnamate in recent years due to its useful biological properties and physico-chemical properties. For

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instance, benzyl cinnamate has been approved as a flavor agent by the US Food and Drug Administration (FDA) (Bhatia et al., 2007). Moreover, benzyl cinnamate can be used as antioxidant owing to its redox properties (Lee et al., 2006). More importantly, benzyl cinnamate has been reported to have anti-inflammatory, anti-cancer, anti-ultraviolet radiation and anti-fungal activities, to a greater or lesser extent (DellaGreca et al., 2007; Compton et al., 2000). Therefore, benzyl cinnamate is a strong candidate as ingredient in food, health, cosmetics and pharmaceutical industries (Bhatia et al., 2007).

At present, benzyl cinnamate could be extracted from natural materials or manufactured by chemical synthesis (DellaGreca et al., 2007). However, these ways have many drawbacks such as low productivity and tedious purification steps, use of hazardous chemicals, and costly equipment. As a promising alternative, enzymatic approach is often preferred because of its high catalytic efficiency, mild reaction conditions, and green and environmentally friendly process (Nasaruddin et al., 2014; Zhang et al., 2014). Recently, there have been several reports on enzymatic synthesis of ethyl cinnamate (Wang et al., 2016), butyl cinnamate (Jakovetic et al., 2013), oleyl cinnamate (Lue et al., 2005), L-ascorbic acid cinnamate (Yang et al., 2012), and monoleyl-L-cinnamate or dioleyl-2-cinnamate esterification (Karboune et al., 2005), by esterification/transesterification of cinnamic acid in either solvent-free systems or organic solvents. However, to the knowledge, the use of enzymes to synthesize benzyl cinnamate has so far remained unexplored, which inspired the researchers to try to synthesize benzyl cinnamate via enzymatic approach.

Here, a high-yield approach to synthesis of benzyl cinnamate by employing biocatalysts was reported. In order to develop a competitive strategy for enzymatic high-efficiency synthesis of benzyl cinnamate, Lipozyme TLIM-catalyzed esterification of cinnamic acid with benzyl alcohol was carried out in various organic solvents. Moreover, several parameters such as water activity, reaction temperature, substrate molar ratio, enzyme loading, and enzyme reusability were examined systematically.

2. Methods

2.1. Materials

Lipozyme TLIM (lipase from *Thermomyces lanuginosus* immobilized on silica gel, 0.25 U/mg) and Novozym 435 (lipase from *Candida antarctica* immobilized on macroporous resin, 10 U/mg) were from Novo. Industries (Bagsvaerd, Denmark). Cinnamic acid (99%) and benzyl cinnamate (99%) was provided by Hubei Yuancheng Pharmaceutical Co., Ltd. (Hubei, China). Benzyl alcohol was of analytic grade and purchased from Beijing Yili Fine Chemicals Co., Ltd. (Beijing, China). Isooctane was from Kemiou Chemical Company (Tianjin, China). Chromatographic grade methanol was obtained from Xingke Chemical Company (Shanghai, China). All other reagents were of analytic grade and obtained from local sources.

2.2. Enzymatic esterification

For the typical reaction, 30 mg of Lipozyme TLIM (or 6 mg Novozym 435) was added to a reaction mixture containing 10 μmol of cinnamic acid and 30 μmol of benzyl alcohol in 1 mL of isooctane. The reaction mixture was incubated in a temperature-controlled incubator shaker at 150 rpm at 40 °C for the time appointed in the paper. Here, 150 rpm of shaking rate was chosen because the reaction rate and the yield of benzyl cinnamate reached the optimum at 150 rpm (date not shown). At various time intervals, 10 μL of the reaction mixture was withdrawn, and then the solvent was evaporated. Subsequently, the above sample was dissolved in 1 mL of methanol and then analyzed by high-performance liquid chromatography (HPLC) (Villa et al., 2007). All experiments were analyzed in triplicate, and the mean values were calculated.

To investigate the reusability, after each reaction cycle, Lipozyme TLIM was filtered and washed with isooctane for three times to remove any substrate or product. Then, the lipase was dried and consecutively reused in the next reaction batch.

2.3. HPLC analysis

The samples were monitored via HPLC (ChuangXinTongHeng Science and Technology Co., Ltd., China) with a C-18 column (ZORBAX 300SB-C18 4.6 mm ID × 250 mm (5 μm), Agilent Technologies, Palo Alto, CA) and a UV detector at 280 nm. A 20 μL of the sample was injected, and the methanol was served as eluent with a flow rate at 0.5 mL min^{-1}. The retention time were found to be 3.632 and 6.373 min for cinnamic acid and benzyl cinnamate, respectively. The percentages of the products were calculated from areas of their respective peaks. The yield of the reaction was quantified in terms of the mole percentage of esterification based on the ratio of consumed cinnamic acid to the total amount of cinnamic acid before the reaction.

2.4. Setting initial water activity

Initial water activity (a_w) of the reaction system was adjusted by pre-equilibration of the reaction components prior to starting the reaction, and the method was as follows: Lipozyme TLIM, cinnamic acid, isooctane and several saturated aqueous salt solutions, including LiCl (a_w = 0.11), K_2CO_3 (a_w = 0.43), NaCl (a_w = 0.75) and KCl (a_w = 0.86), were placed in sealed containers, separately, and pre-equilibration of the reaction system was achieved after 3 days at room temperature. Molecular sieves were used to prepare the nearly anhydrous condition ($a_w \approx 0$).

3. Results and discussion

3.1. Lipase selection and reaction media screening

Two commercially available immobilized lipases, including Lipozyme TLIM and Novozym 435, were tried to perform lipase-catalyzed esterification of cinnamic acid with benzyl alcohol. This reaction was carried out for 24 h and the results were listed in Table 1. Because the nominal activity of Lipozyme TLIM (0.25 U/mg) is far lower than the nominal activity of Novozym 435 (10 U/mg) in propyl laurate hydrolysis, Lipozyme TLIM (30 mg/mL) is added more than Novozym 435 (6 mg/mL) in enzymatic esterification of cinnamic acid. As can be seen, Lipozyme TLIM lipase showed much higher activity (79.88% yield) in isooctane than Novozym 435 lipase, which suggested that Lipozyme TLIM (lipase from *T. lanuginosus*) was efficient for catalyzing the esterification of cinnamic acid. As known, the lipase from *Thermomyces laguginosus* (formerly *Humicola laguginosa*) (TLL) is a basophilic and noticeably thermostable enzyme, and it has found applications in many different industrial areas, from biodiesel production to fine chemicals (Fernandez-Lafuente, 2010).

Reaction media have significant impacts not only on the activity and stability of enzymes but also on the solubility of substrates (Pohnlein et al., 2015). Additional aspects like water content of the reaction medium, its boiling point and possible post-processing have to be considered as well. Here, four kinds of solvent, including acetone, trichloromethane, toluene and isooctane, were used as reaction media to carry out this reaction (Table 1). As shown, Lipozyme TLIM exhibited the best performance