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Enrichment of erucic acid from crambe oil in a recirculated packed bed reactor via lipase-catalyzed ethanolysis

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ABSTRACT

Erucic acid enrichment from crambe oil was accomplished in a recirculated packed bed (RPBR) reactor via ethanolysis using Novozym 435 lipase from *Candida antarctica* as a biocatalyst. The content of erucic acid of the crambe oil used was 59.8 mol% The erucic acid was located predominantly in the *sn*-1,3 position of the triacylglycerol of the crambe oil. Novozym 435 used in this study has selectivity toward fatty acids in *sn*-1,3 position of triglyceride in excess ethanol. The effects of reaction temperature, molar ratio, and residence time of the substrate in RPBR on the enrichment of erucic acid as a function of reaction time were studied. The optimal temperature, molar ratio (crambe oil to ethanol), and residence time of the substrate in RPBR were 45 °C, 1:60, and 4 min, respectively.

1. Introduction

Erucic acid (*cis*-13-docosenoic acid) is a naturally occurring fatty acid in seed storage triglycerides (TAG) of the *Cruciferae* family, which includes rapeseed, wallflower, mustard, and crambe seed. Specifically, seeds containing high level erucic acid are crambe seed and rapeseed. Erucic acid has been reported to cause heart lesions and disrupt other physiological processes in animal models if consumed. However, erucic acid also has useful qualities. For example, erucic acid and its derivatives are employed as an additive in plastics and as a component in biodegradable lubrication oils [1]. Pure erucic acid can be used to treat the symptoms of generic disorders known as adrenoleukodystrothy (ALD). In particular, behenic acid is produced simply by full hydrogenation of erucic acid [2]. A structured lipid (SL) called Caprenin, composed of behenic acid and medium chain fatty acids, has been shown to contain approximately half the calories of natural TAG due to incomplete absorption during digestion [3,4]. Another type of SL called BOB (B: behenic acid, O: oleic acid) is a symmetrical TAG with behenic acid at *sn*-1,3 positions and oleic acid at the *sn*-2 of glycerol backbone. The BOB is used as an anti-blooming agent in the preparation of chocolate.

Lipases have been widely used in many oil modification processes, including alcoholysis, hydrolysis, interesterification, and transesterification [5]. The ultimate purpose of using lipase is based on whether the selectivity of a given lipase for or against a particular fatty acid/acyl moiety enables the selective enrichment of industrially or nutritionally important fatty acids from fats and oils. In particular, lipase-catalyzed alcoholysis is considered as an effective reaction for the production of biodiesel and value-added products, such as 2-monoacylglycerol (MAG), and high purity polyunsaturated fatty acids such as EPA, DHA, and GLA [6–8]. Piyatheerawong et al. [9] demonstrated that Novozym 435 from *Candida antarctica* exhibited the *sn*-1,3-regiospecificity for ethanolysis of TAG with excess amounts (>20 molar amounts for TAG) of ethanol.

Crambe oil is an excellent source of erucic acid because it has a high content of erucic acid and erucic acid is located predominantly in *sn*-1,3 position of TAG (Scheme 1). Hence erucic acid can be efficiently enriched from crambe oil if the regiospecifity of Novozym 435 from *C. antarctica* is applied. In our study, erucic acid was enriched from crambe oil via lipase-catalyzed ethanolysis in an RPBR system using Novozym 435 as a biocatalyst. The effects

Abbreviations: RPBR, recirculated packed bed reactor; TAG, triacylglycerol; BOB, behenyl-oleyl-behenyl glycerol; MAG, monoacylglycerol; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; GLA, γ-linolenic acid; FID, flame ionization detector; FAEE, fatty acid ethyl ester; GC, gas chromatography; M.W., molecular weight.

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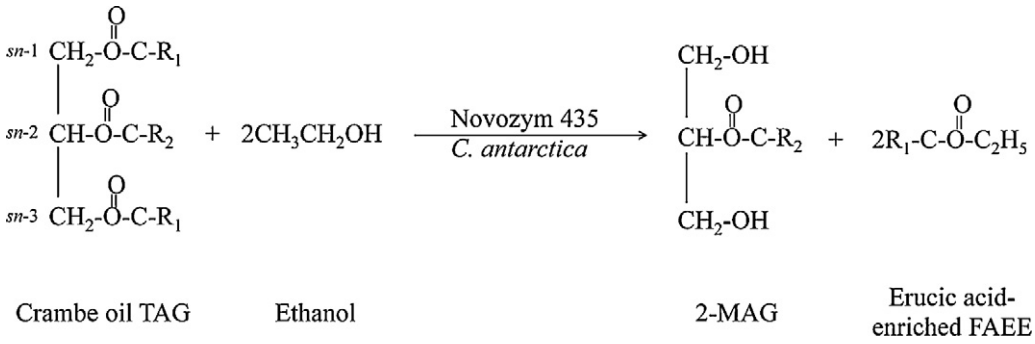

Scheme 1. Selective ethanolysis of crambe oil TAG by Novozym 435; erucic acid is mainly located in *sn*-1,3 positions in crambe oil TAG.

of temperature, the molar ratio of crambe oil to ethanol, and the residence time of the substrate in the reactor were investigated in an RPBR.

2. Materials and methods

2.1. Materials

Crambe oil was generously donated by Technology Crops International (Kensington, PE, Canada). Novozym 435 from *C. antarctica* was purchased from Novo Nordisk Bioindustry Ltd. (Seoul, Korea). Absolute ethanol (>99.9%) was used in this study. Other chemicals used in this study were of analytical grade unless otherwise noted.

2.2. Lipase-catalyzed ethanolysis in RPBR

Lipase-catalyzed ethanolysis of crambe oil was carried out in an RPBR system. Fig. 1 shows diagram of the RPBR used for the ethanolysis. The RPBR consisted of a 300 mm long glass column (10 mm inner diameter) with a water jacket. The glass column was manually packed with the dry immobilized enzyme (5 g), which was then fixed in place using a stainless steel sieve (50 mesh). The substrate mixture (100 g) was kept in a reservoir, which consisted of a glass vessel (270 mm height/120 mm inner diameter) with a water jacket, and was agitated at 400 rpm using a magnetic stirrer during the reaction. Prior to starting the reaction, the RPBR and reservoir were heated or cooled to the desired temperature with a water circulator (Model CW-05G, Jeio Tech, Seoul, Korea). This reaction mixture was pumped upward into the column by a metering pump (Model QG 150, FMI, Syosset, NY, USA). All trials were conducted in duplicate.

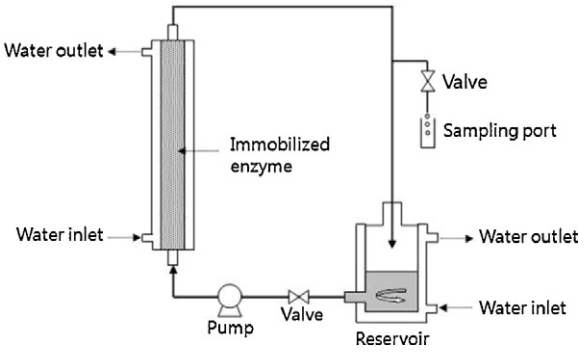

Fig. 1. Diagram of the recirculated packed bed reactor (RPBR) used for the ethanolysis.

2.3. Positional distribution of fatty acids in the crambe oil

Pancreatic hydrolysis was used to determine the positional distribution of the fatty acid residues in the TAG of crambe oil. Five milligrams of TAG were mixed with 2 mL of 1 M Tris–HCl buffer (pH 7.6), 0.5 mL of 0.05% bile salts, 0.2 mL of 2.2% $CaCl_2$, and 3 mg of pancreatic lipase. The mixture was incubated in a water bath at 37 °C for 2 min, vortexed vigorously, extracted with diethyl ether, and dried using anhydrous sodium sulfate. The mixture was then placed on a silica gel G TLC plate (Alltech Associates, Inc., Deerfield, IL, USA) and developed with hexane/diethyl ether/acetic acid (50:50:1, v/v/v). The band corresponding to the 2-MAG was scraped off, extracted with diethyl ether, methylated, and analyzed by GC. The fatty acid composition of the *sn*-1,3 position in the TAG of crambe oil was calculated from the *sn*-2 position and the total fatty acid composition in the TAG of crambe oil.

2.4. Analysis of products

Individual reaction mixtures were withdrawn from the sampling port at selected times during the ethanolysis reaction. To determine the content of erucic acid and the yield of erucic acid in FAEE produced by lipase-catalyzed ethanolysis 120 μL samples corresponding to the different reaction conditions were dissolved in 880 μL of chloroform. A gas chromatograph (Model 3800; Varian, Palo Alto, CA, USA) equipped with a DB-1ht column (15 m × 0.25 mm i.d.; J&W Scientific, Folsom, CA, USA) and FID was used for analysis. Initially, the column was held at 150 °C for 1 min and programmed to rise to 365 °C at a rate of 10 °C min-1. The column was then held at 365 °C for 20 min. The carrier gas was helium, and the total gas flow rate was 50 mL min^{-1}. The injector and detector temperatures were 375 and 380 °C, respectively.

The content of erucic acid and the yield of erucic acid in FAEE produced by ethanolysis were calculated as follows:

$$\text{The content of erucic acid in FAEE (mol\%)} = \frac{\text{mole of erucic acid ethyl ester}}{\text{mole of total FAEE}} \times 100$$

$$\text{The yield of erucic acid (mol\%)} = \frac{\text{mole of erucic acid ethyl ester}}{\text{mole of erucic acid in initial crambe oil}} \times 100$$

3. Results and discussion

3.1. Positional distribution of fatty acids in the TAG of crambe oil

The positional distribution of the fatty acids in the TAG of crambe oil employed in this study and the composition of erucic acid enrichment from the lipase-catalyzed crambe oil ethanolysis reaction are presented in Table 1. The primary fatty acids in crambe oil are oleic acid (C18:1*n*-9, 17.5 mol%), linoleic acid (C18:2*n*-6, 9.3 mol%), and erucic acid (C22:1*n*-9, 59.8 mol%). Erucic acid was