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Synthesis of jatropha oil based wax esters using an immobilized lipase from *Burkholderia* sp. EQ3 and Lipozyme RM IM

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ABSTRACT

A comparison of the synthesis of wax esters by transesterification of jatropha oil and oleyl alcohol using the immobilized lipase EQ3 (*Burkholderia* sp. EQ3) and Lipozyme RM IM (*Rhizomucor miehei*) was carried out. The predominant fatty acids in jatropha oil were palmitic acid (14%), stearic acid (7%), oleic acid (41%) and linoleic acid (35%). The optimum conditions for the synthesis of wax esters by the immobilized lipase EQ3 were 10 U of enzyme, a molar ratio of jatropha oil and oleyl alcohol of 1:4 in isooctane at 30 °C for 12 h to achieve an 89% conversion. The optimum conditions for wax ester synthesis by the Lipozyme RM IM were 10 U of enzyme, a molar ratio of jatropha oil and oleyl alcohol of 1:3 in hexane at 45 °C for 12 h to achieve an 86% conversion. For the reusability test, the immobilized lipase EQ3 provided a higher percentage of wax esters (88%) than the Lipozyme RM IM (24%) at the 5th batch. The wax ester composition of the jatropha oil was oleyl palmitate (18%), oleyl stearate (10%), oleyl oleate and oleyl linoleate (67%). The wax esters had melting points of −14.83, 8.50 and 13.17 °C, and were completely decomposed at 380 °C.

1. Introduction

Wax esters are long-chain esters of fatty acids and fatty alcohols. The liquid wax esters have a high degree of unsaturated esters that help liquefaction at room temperature. Wax esters are important ingredients in the formulations of cosmetics, such as their use as cleansers, conditioners and moisturizers [1–3]. They are also used as coating materials in the pharmaceutical and food industries [4], lubricants for machinery [5], and contribute to the properties of polishes and plasticizers [6,7]. Wax esters are found in a variety of natural resources, including honeycombs, jojoba seeds, carnauba, sperm whale, skin lipids, sheep wool and seafowl feathers [6] and are also synthesized using either chemical or enzymatic methods [8]. The disadvantages of chemical catalyzed methods include the use of corrosive acids that can become hazards, and can degrade any synthesized esters and the need for high energy sources [9,10]. On the other hand, an enzyme catalyzed synthesis could produce many kinds of products through the specificity of the lipase enzymes used [11] and also the requirement for mild reaction conditions. The lipase-catalyzed alcoholysis of alkyl esters of fatty acids with long-chain alcohols has been carried out successfully to prepare wax esters [1,12,13]. However, wax ester production using lipase has a bottleneck for an industrial application due to the high cost of obtaining the enzyme. Enzyme immobilization technology may be an effective means to allow for enzyme reuse which will reduce the total cost of the reaction as well as the ability to improve its activity and stability. Thus, the reuse of immobilized enzymes is very important as it minimizes the costs and allows for economic viability.

Jatropha curcas is widely cultivated in America, Asia and Africa. It has been promoted for planting in Thailand [14]. It is a plant with many attributes, so it is widely used and has considerable potential [15]. The seeds of *J. curcas* contain about a 30–40% oil content that consists of 21% saturated fatty acids and 79% unsaturated fatty acids [16]. The jatropha seed oil also contains non-edible oil. Therefore, it has a higher priority over edible oils as a feedstock for bio-energy production. In addition, the application of jatropha oil for wax ester synthesis will lead to an even wider variety of uses.

An energy saving and environmentally friendly process to produce wax esters has been studied for several years. Steinke et al. [17] studied a high yielding preparation of wax esters from crambe and camelina oil using Novozyme 435 and obtained ≥95% conversion after 4–6 h of reaction. Radzi et al. [18] studied the synthesis of oleyl oleate using the immobilized lipase from Novozyme 435, and produced >90% conversion of wax esters for up to 9 cycles. Gunawan and Suhendra [19] investigated the synthesis of wax esters from

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palm kernel oil catalyzed by Lipozyme RM IM, and obtained an 84% yield after 7–10 h.

Recently, a crude lipase from *Burkholderia* sp. EQ3 had been produced and used in the enzymatic production of wax esters by esterification of crude fish fat and cetyl alcohol [8]. There has been no report on wax ester synthesis from jatropha oil. The present paper was related to the enzymatic synthesis of wax esters by a transesterification process using jatropha oil and oleyl alcohol with an immobilized lipase from *Burkholderia* sp. EQ3 and compared with production by the Lipozyme RM IM enzyme. The parameters affecting wax ester synthesis were optimized. The physical and chemical properties of the oleyl esters produced were also examined.

2. Materials and methods

2.1. Bacterial strain and immobilized lipases

The lipase producing *Burkholderia* sp. EQ3 isolated from a wastewater treatment system of a tuna canning factory in southern Thailand was cultivated in a basal medium containing 1% fish oil at 37 °C for 12 h [8,20]. For the enzyme preparation, the crude lipase EQ3 powder from the acetone precipitation was dissolved in 20 mM Tris–HCl (5 mL, 75 U). For the carrier preparation, 0.5 g of Accurel MP-100 (size 200–400 μm) (Membrana GmbH, Obernburg, Germany) was pretreated with isopropanol (1.5 mL). After that, the crude lipase was added to the Accurel MP-100 and immobilized at 25 °C for 30 min (95% activity yield and 98% immobilized yield) [21,22]. The commercial immobilized lipase, Lipozyme RM IM from *Rhizomucor miehei* was immobilized on an anionic resin (Duolite A568) by the adsorption technique (Novozymes, Bagsvaerd, Denmark). The immobilized lipases were stored at 4 °C until used.

2.2. Chemicals

Jatropha oil (molecular weight: 1,200 g/mole) was obtained from the Center of Excellence for Jatropha, Kasetsart University (Bangkok, Thailand). Oleyl alcohol was supplied by Sigma–Aldrich (St. Louis, MO, USA). All other chemicals used were of analytical grade.

2.3. Analyses

2.3.1. Lipase activity

The lipase activity was assayed in a two-phase system according to Lee and Rhee [23] using 10% (w/v) palm olein as a substrate and the reaction was incubated at 37 °C, while shaking at 300 rpm for 30 min. One unit of enzyme activity was defined as the enzyme necessary to release 1 μmol of palmitic acid per minute during the specified conditions.

2.3.2. Water activity (a_w)

The water activity of the immobilized lipase EQ3 and Lipozyme RM IM were measured at 25 °C by a water activity meter (AquaLab Series3, Decagon Devices, USA).

2.3.3. Analysis of the fatty acid content

The fatty acid composition of the jatropha oil was determined by gas chromatography [24]. The sample was injected into the gas chromatograph (Hewlett Packard model 6850A, USA) equipped with a flame ionization detector (FID). The Select™ Biodiesel for FAME capillary column (30 m length, 0.32 mm internal diameter, 0.25 μm film thickness) was used. Helium was used as the carrier gas (1.0 mL/min). An initial column temperature was programmed at 210 °C for 12 min, then raised from 210 to 250 °C at a rate of 20 °C/min, and held constant at 250 °C for 8 min. The relative percentage (area%) of the fatty acids was determined using a reference mixture of methyl esters of fatty acids.

2.3.4. Wax ester analysis

Wax esters were analyzed using a thin layer chromatography-flame ionization detection system (TLC-FID) (IATROSCAN MK5, Iatron Laboratories Inc., Tokyo, Japan). Triplicate samples of each experiment were separated on Chromarods S III (silica gel coated rods) (Mitsubishi Chemical Medicine Corporation, Tokyo, Japan) and developed in the solvent mixture of hexane:diethyl ether:formic acid (64:6.4:0.4, v/v/v) [25] to a distance of 10 cm from the origin. The rods were analyzed for wax esters and other compositions by the Iatroscan operated using a 160 mL/min flow of hydrogen, a flow of air at 2 L/min and 30 sec scan speed. After that, the rod was scanned with the FID to detect and quantify the compounds. Chromstar 6.3 Software was used to calculate the peak areas. The percentage of the peak area was calculated to be the percentage of conversion of the corresponding compounds.

The composition of the jatropha oil based wax esters was determined using the Trace GC ultra Gas Chromatography coupled to an ISQ Mass Spectroscopy detector (Thermo Scientific, Massachusetts, USA). A TR-5MS capillary column (30 m length, 0.25 mm internal diameter, 0.25 μm film thickness) was used for the GC system. The oven temperature was set at an initial temperature of 150 °C with a hold time for 1 min. Then, the temperature was ramped to 225 °C at 20 °C/min with a hold time for 1 min, increased to 300 °C at 5 °C/min with a hold time for 40 min. The final temperature was to 340 °C at 10 °C/min with a final hold time of 10 min. The mass spectrometer was set to 290 °C for the transfer line heater temperature and operated in the scan mode from 35 amu to 500 amu. Electron impact ionization was employed with an ion source temperature of 250 °C.

2.4. Comparison of wax ester synthesis by immobilized lipase EQ3 and Lipozyme RM IM

The wax ester synthesis was carried out by alcoholysis of jatropha oil and oleyl alcohol. The reaction mixture contained 1:3 mol/mol of jatropha oil (150 μmol, 180 mg): oleyl alcohol (450 μmol, 120 mg), hexane (1 mL) and immobilized lipase (immobilized lipase EQ3: 0.14 U/mg carrier, 12.65 U/mg protein and a_w = 0.672; or Lipozyme RM IM: 0.60 U/mg carrier, 10.85 U/mg protein and a_w = 0.802). The reaction mixture was placed in a screw-capped tube and incubated at 37 °C by shaking at 150 rpm for 72 h. All experiments were done in triplicate. The samples were diluted in chloroform (1:2 v/v). The percentage of wax ester synthesis for each reaction was determined using the TLC-FID analysis.

2.4.1. Effect of enzyme concentration

The influence of enzyme concentration on wax ester synthesis was determined using different amounts of immobilized enzyme (2, 5 and 10 U). The reaction mixture was incubated using the above mentioned conditions.

2.4.2. Effect of reaction temperature

To determine the effect of temperature on the transesterification, the reaction mixtures were incubated by shaking at 150 rpm at various temperatures (30, 37 and 45 °C).

2.4.3. Effect of the molar ratio of substrate

The reaction mixture was incubated with different molar ratios of jatropha oil and oleyl alcohol at 1:1, 1:2, 1:3 and 1:4 (mol/mol).