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Solvent-free lipase-catalyzed synthesis of a novel hydroxyl-fatty acid derivative of kojic acid

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ABSTRACT

The aim of this work was the synthesis of a novel hydroxyl-fatty acid derivative of kojic acid rich in kojic acid monoricinoleate (KMR) which can be widely used in the cosmetic and food industry. The synthesis of KMR was carried out by lipase-catalysed esterification of ricinoleic and kojic acids in solvent-free system. Three immobilized lipases were tested and the best KMR yields were attained with Lipozyme TL IM and Novozym 435. Since Lipozyme TL IM is the cheapest, it was selected to optimize the reaction conditions. The optimal reaction conditions were 80 °C for the temperature, 1:1 for the alcohol/acid molar ratio, 600 rpm for stirring speed and 7.8% for the catalyst concentration. Under these conditions, the reaction was scaled up in a 5×10^{-3} m^3 stirred tank reactor. ^1H–^{13}C HMBC-NMR showed that the primary hydroxyl group of kojic acid was regioselectively esterified. The KMR has more lipophilicity than kojic acid and showed antioxidant activity that improves the oxidation stability of biodiesel.

1. Introduction

Kojic acid (5-hydroxy-2-(hydroxymethyl)-1,4-pyrone) is a cheap fungal metabolite produced by many species of *Aspergillus* and *Penicillium*, made from carbohydrates particularly glucose and starch [1]. Kojic acid is widely used as a food additive to prevent the browning reaction or in cosmetics as a skin whitening agent [2–4]. However, kojic acid is water-soluble and unstable at high temperature for long term storage, prohibiting it to be directly incorporated in oil base cosmetic products. To improve the kojic acid properties, such as storage stability, compatibility and oil-solubility, many kojic acid derivatives have been synthesized, usually by modifying the C-5 hydroxyl group to form hydroxyphenyl ethers or esters or by using this group to form glycosides or peptide derivatives [5–8]. The main reason is that the kojic acid derivatives were approximately 15 times more stable than kojic acid itself [6].

The esterification protocol of kojic acid with long chain fatty acids in the presence of acid or alkaline catalysts usually results in a complex mixture and makes easy the formation of esters at C-5, the secondary hydroxyl group of kojic acid. Kojic acid possesses two different hydroxyl groups: the secondary hydroxyl group at C-5 position and the primary hydroxyl group at C-7. The hydroxyl group at the C-5 position of kojic acid is essential to the radical scavenging activity and tyrosinase interference activity, respectively [9]. The use of immobilized lipases eliminates the inherent problems associated with the use of chemical catalysts. Liu and Shaw [10] improved the lipophilic property of kojic acid by lipase-catalysed acylation with lauric and oleic acids in presence of acetonitrile as solvent. In this case, the acylation was also carried out at C-5 hydroxyl group (secondary hydroxyl group of kojic acid). Subsequently, Khamaruddin et al. [11] tried to improve Liu yields by esterification of kojic acid and oleic acid using lipase from *Candida rugosa* and *Aspergillus Niger*, in organic media. The maximum yield was not exceeded 45%. Optimized enzymatic synthesis of kojic acid monooleate has been reported by Ashari et al. [12] but with an unsatisfactory yield (40% after 48 h reaction time). In both cases, the kojic acid was esterified at C-5 hydroxyl group.

The most common fatty acids used in kojic acid esterification were: oleic, palmitic and lauric acids, and the reactions were carried out in presence of organic solvent. However, to the best of our knowledge, there are not studies to date on the enzymatic esterification of kojic acid with hydroxyl-fatty acids in solvent free systems. The best known example of hydroxyl fatty acid is the ricinoleic acid (cis-12-hydroxy-9-octadecenoic acid), which constitutes between 80 and 90% in castor oil [13]. This acid is characterized by the presence of the hydroxyl group which imparts

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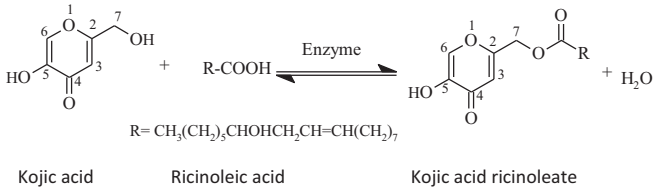

Scheme 1. Kojic acid monoricinoleate synthesis.

several unique chemical and physical properties. The use of this hydroxyl fatty acid for the lipase-regioselective esterification of diols was reported previously by our research group [14,15].

It is studied here the lipase-catalysed esterification of the kojic acid with ricinoleic acid in a solvent-free system. Three different commercially available lipases were evaluated for their catalytic activity in the reaction. For the selected lipase, the optimization of the reaction was performed with respect to the temperature, the catalyst concentration and stirring speed as independent variables and the yield of KMR as a response variable. Furthermore, the synthesis of KMR (Scheme 1) at the optimum conditions was tested, using a 5×10^{-3} m^3 batch reactor in order to investigate the possibility of large-scale production. Antioxidant activities and biodiesel solubility of the purified KMR were measured.

2. Experimental

2.1. Equipment

Reactions were performed in a solvent-free system using a batch stirred reactor of 500 cm^3 volume, under fixed conditions of pressure and temperature. Pressure, stirring speed and temperature controllers were provided. The propeller used was marine-type and the speed was set at 600 rpm. The desired working pressure (60 mmHg) was maintained by a vacuum pump. This permitted ready elimination of water from the system in range of temperature studied, without significant variations of viscosity of the liquid phase or reaction volume. The reaction temperature was achieved by immersing the reactor into a thermostatic bath with an electrical device connected to a PID controller which allows a temperature control of ±0.1 °C.

2.2. Materials

Kojic acid (purity, 98%) was kindly donated by Institute of Bioscience (Malaysia) and ricinoleic acid (purity, 95%) was supplied by Fluka (Spain). Immobilized thermostable lipase from *Candida antarctica* (Novozym 435), from *Rhizomucor miehei* (Lipozyme RM IM) and from *Thermomyces lanuginose* (Lipozyme TL IM) were kindly provided by Novozymes A/S (Bagsvaerd, Denmark).

2.3. Analytical methods

2.3.1. Gas chromatography (GC)

Kojic acid monoricinoleate (KMR) was monitored by capillary column GC, using a Hewlett-Packard 5890 series II equipped with a flame ionization detector (FID). The injection system was split-splitless. The carrier gas was helium at a flow rate of 1 mL/min. The analytical procedures and the operating conditions have been already described in a previous work [16]. The quantification was based on external calibration using standard solutions of octyl octanoate, over the range of 0.02–0.06 mg/mL and reaction aliquots were always prepared in CS_2 to a final concentration of 3 mg/mL.

2.3.2. Nuclear magnetic resonance (NMR)

^{1}H, ^{13}C, Dept and ^{1}H–^{13}C HMBC spectra were measured on a Brucker Avance-DPX-300 spectrometer at 300 MHz, in $CDCl_3$ solution. The chemical shifts (δ) were expressed in parts per million (ppm) relative to tetramethylsilane (TMS), and the coupling constants J were given in hertz (Hz).

2.3.3. Infra-red (IR)

IR spectra were recorded as a thin film using NaCl plate on a PERKIN-ELMER 781 spectrophotometer (ν_{max} in cm^{-1}).

2.3.4. Oxidative stability

The oxidation stability of rapeseed biodiesel was analyzed according to Rancimat method using Metrohm 743 Rancimat equipment (Herisau, Switzerland). Three grams of sample were placed in a heating block at 110 °C; the air flow rate was 10 L/h and volatile products were discharge in a flask containing 50 mL of distilled water where conductivity changes were measured. The time corresponding to the inflection point in the oxidation curve is the Induction Period (IP). Each sample was run in duplicate.

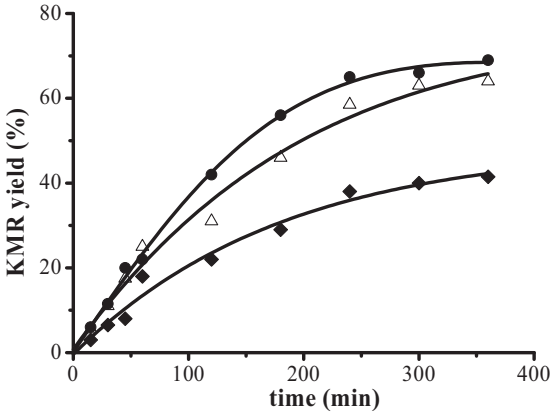

Fig. 1. Effect of different enzymes on KMR yield: Novozym 435 (●), Lipozyme TL IM (△) and Lipozyme RM IM (♦). This experiment was performed at a molar ratio of 1:1 (kojic acid to ricinoleic acid), temperature of 75 °C and catalyst concentration of 3% of the total weight of the substrate.

2.4. Lipase-catalyzed synthesis of kojic acid monoricinoleate

Ricinoleic and kojic acids were added to the reactor and the stirring was started. When the desired temperature was reached, the catalyst was added and the vacuum pump was turned on in order to displace the equilibrium towards the KMR synthesis. The reactants were stirred during 360 min. The samples were taken at regular intervals and analyzed by GC. The product was purified by a silica gel 60 (with a mesh size 40–63 mm) column chromatography using hexane/ethyl acetate (75:25, v/v) as eluents. The purified KMR is viscous oil. IR (neat): 3421, 2932, 2855, 1737 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.79 (1H, s), 6.43 (1H, s), 5.47–5.31 (2H, dt, J = 13.7, J = 2.3), 4.68 (2H, s), 3.57–3.53 (1H, m), 2.32 (2H, t, J = 2.5), 2.16–2.11 (2H, m), 2.00–1.93 (2H, m), 1.60–1.53 (2H, m), 1.40–1.33 (2H, m), 1.23–1.20 (18H, m), 0.88–0.63 (3H, m); ^{13}C NMR (300 MHz, $CDCl_3$): δ = 174.2, 172.6, 162.8, 146.1, 138.6, 133.0, 125.3, 111.5, 71.5, 61.1, 36.8, 35.3, 33.8, 31.8, 29.7, 29.5, 29.4, 29.0, 28.9, 27.3, 25.7, 24.7, 22.6, 14.1.

3. Results and discussion

3.1. Enzyme screening

The first step in this work is to find the most suitable lipase to carry out the optimization of the process esterification. The effects of the three lipases (Novozym 435, Lipozyme TL IM and Lipozyme RM IM) from three different sources were investigated for their ability to produce KMR ester by esterification of kojic acid with ricinoleic acid and the results were represented in Fig. 1. For these trials, the temperature, the catalyst concentration, the reaction time and the substrate molar ratio were held constant at 75 °C, 3% of the total weight of the substrate, 360 min and 1:1 (kojic acid to ricinoleic acid). The initial reaction rates were similar for Novozym 435 and Lipozyme TL IM. The KMR yield increased sharply at the first 180 min of reaction time. The highest KMR yield was obtained with Novozym 435 (68%) followed by Lipozyme TL IM (65%). However, by using Lipozyme RM IM from *Rhizomucor miehei*, the reaction rate was much lower, and the maximum KMR yield achieved with this lipase was around 40%, after 360 min of reaction time. Although the best KMR yield was attained with Novozym 435 from *Candida antarctica*, Lipozyme TL IM from *Thermomyces lanuginose* was selected to optimize the esterification of kojic and ricinoleic acids because it is the cheapest.

3.2. Effect of stirring speed

A preliminary study has been carried out to study the effect of stirring speed on the process. As can be seen in Fig. 2, the