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OPTIMIZATION AND KINETIC STUDY OF LIPASE-CATALYZED SYNTHESIS OF PALM-BASED KOJIC ACID ESTER

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By

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

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Chairman : Assoc. Prof. Rosfarizan Binti Mohamad, PhD

Faculty : Biotechnology and Biomolecular Sciences

Palm-based kojic acid ester was produced through lipase-catalyzed esterification of kojic acid with oleic acid using lipase as a catalyst. The chemical and physical characterization of pure palm-based kojic acid ester was analyzed in order to be effectively applied in cosmetic application. The chemical characterization was determined by Fourier-Transform Infrared spectroscopy, Gas Chromatography- Flame Ionization Detector and Nuclear Magnetic Resonance in order to verify and elucidate the structure of product. It was found that kojic acid was esterified at C-5 position to produce palm-based kojic acid ester and the chemical's name was (E)-6-(hydroxymethyl)-4-oxo-4*H*-pyran-3-yloctadec-9-enoate. The important physicochemical

property of the ester such as skin irritancy test was found to be non-irritating with Human Irritancy Equivalent (HIE) score between 0.55-0.83.

Response Surface Methodology and 5-level-4-factor central composite rotatable design were employed in optimizing the synthesis of palm-based kojic acid ester catalyzed by immobilized lipase from *Rhizomucor meihei* (Lipozyme *RM* IM) in acetonitrile. Four parameters such as temperature (°C), amount of enzyme (g), substrate molar ratio (kojic acid: oleic acid) and reaction time (h) were studied and their interaction effects were investigated. The optimized reaction conditions obtained after analysis with backward elimination are 0.17 g of enzyme and molar ratio of substrates (OA:KA) corresponding to 1:4 at 52.50°C for 42 h of reaction. Under this condition, the percentage yield of palm-based kojic acid ester produced was 37.2%.

The improvement of the synthesis process was carried out in a 2 L stirred-tank reactor (STR) equipped with a multi-impeller design. The types of impeller used were Rushton Turbine (RT), High Efficiency (HE) and Half-pitched Helical Ribbon (HR). The configuration of HE-RT combination showed the highest conversion yield with 77.20% as compared to RT-HE (72.45%) and HR (63.68%). The high Reynolds Number, N_{RE} (3463.89) was achieved at 250 rev/min using HR, which exhibits a transition flow pattern. The production of palm-based kojic acid ester in a 2 L stirred-tank reactor follows Newton's Law due to the linear relationship in between the shear stress and

shear rate. A heterogenous enzyme particles suspension was obtained at 250 rev/min and 30 mm of distance in between the two impellers (RT-HE).

A kinetic study, using the experimental data obtained from the batch-mode of 2 L stirred-tank reactor was evaluated for the synthesis of palm-based kojic acid ester. This study was aimed to develop a kinetic model by focusing on the substrates concentration. Different acid and alcohol concentrations were tested systematically and the results were used to identify the best reaction scheme to describe the results obtained over an extended range of conditions. As a result, the kinetic of the reaction can be described by Ping-Pong Bi-Bi mechanism with acid inhibition (palm-based oleic acid). The values of the apparent kinetic parameters were estimated by non-linear regression analysis and computed as: υ_{max}: 4.58 x 10 mmol/L.h.g; $K_{m(KA)}$: 1.87 x 10^{-7} mmol/L.h.g; $K_{m(OA)}$: 8.31 x 10^{2} mmol/L.h.g ; $K_{i(OA)}$: 5.32 x 10^{6} mmol/L.h.g. A good agreement between the calculated and the observed value was found.

Lipase-catalyzed esterification in a solvent-free system using Novozym 435 lipase was also investigated as a method for kinetic modeling of palm-based kojic acid ester. The structure of palm-based kojic acid ester was also elucidated. It was found that kojic acid was easily esterified at C-7 position to produce palm-based kojic acid ester and the chemical's name was found to be (E)-(5-hydroxy-4-oxo-4H-pyran-2-yl)methyl octadec-9-enoate. The rate expression of this ester was developed using irreversible second-order power model as a first approach. The activation energy (E_a) shows a value of 7.90 x 10^{4}

J/mol and the kinetic reaction constant (k_o) of 5.30 x 10^8 $L.mol^{-1}.min^{-1}.g_{CAT}^{-1}$. The adsorption value ($K_{KAE}$) of 1.90 x 10^{-17} $g_{CAT}.g_{KAE}^{-1}$ between catalyst and ester was correlated with the Langmuir-Hinshelwood-Hougen-Watson model as a second approach. The Gibbs energy, enthalpy and entropy changes for reaction at different temperature were calculated from the measured equilibrium constant using the integrated Van't Hoff's equation. The calculated values for the thermodynamic quantities of Gibbs energy, enthalpy and entropy changes are 2.77 x 10^4 J/mol, 9.85 x 10^5 J/mol and 4.77 x 10^2 J/mol.K, respectively.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

PENGOPTIMUMAN DAN KINETIK SINTESIS ESTER ASID KOJIK BERASASKAN KELAPA SAWIT YANG DIMANGKINKAN OLEH LIPASE

Oleh

SITI EFLIZA ASHARI

Januari 2013

Pengerusi : Profesor Madya Rosfarizan Mohamad, PhD

Fakulti : Bioteknologi dan Sains Biomolekul

Ester asid kojik berasaskan kelapa sawit telah dihasilkan melalui pengesteran lipase oleh asid kojik dan asid oleic dalam pelarut organic menggunakan lipase sebagai pemangkin. Pencirian kimia dan fizikal bagi ketulenan ester asid kojik berasaskan kelapa sawit ini telah dianalisa untuk digunakan sebagai bahan kosmetik. Pencirian kimia telah ditentukan dengan menggunakan spektroskopi inframerah (FT-IR), spektroskopi kromatografi gas- pengesan nyala pengionan (GC-FID) dan resonans magnet nukleus (NMR) untuk mengenalpasti produk yang telah diperoleh. Struktur ester asid kojik telah dikenal pasti pada kedudukan C-5 asid kojik dengan nama spesifiknya, (E)-6-(hydroxymethyl)-4-oxo-4*H*-pyran-3-yloctadec-9-enoate. Pencirian fizikal seperti ujian

keradangan kulit telah dianalisa dan didapati bahawa ester asid kojik berasaskan kelapa sawit ini bebas dari keradangan dengan skor dari 0.55-0.83.

Kaedah tindakbalas permukaan dan lima-peringkat-empat-faktor rekaan pusat komposit berputar telah digunakan dalam pengoptimuman sintesis ester asid kojik yang dimangkinkan oleh lipase dari *Rhizomucor meihei* (Lipozym *RM* IM) dalam asetonitril. Empat parameter iaitu suhu (°C), jumlah enzim (g), nisbah molar substrat (asid kojik: asid oleik) dan masa tindakbalas telah dikaji dan kesan interaksi telah diselidik. Keadaan tindakbalas optima selepas dianalisa menggunakan penghapusan terkebelakang adalah dengan menggunakan 0.17 g enzim dan nisbah molar substrat (OA:KA), 1:4 pada 52.50°C untuk 42 j tindakbalas. Dalam keadaan ini, peratus ester asid kojik berasaskan kelapa sawit ini yang diperoleh adalah sebanyak 37.2%.

Peningkatan proses sintesis telah dijalankan dalam tangki reaktor berpengaduk 2 L dengan menggunakan pelbagai reka bentuk pengaduk. Jenis-jenis pengaduk yang digunakan adalah turbin Rushton (RT), kecekapan tinggi (HE) dan pengaduk setengah nada reben helik (HR). Peratusan ester yang tinggi telah dicapai oleh kombinasi HE-RT dengan 77.20% berbanding dengan kombinasi RT-HE (72.45%) dan HR (63.68%). Angka Reynolds (3463.89) yang tinggi telah dicapai pada kelajuan 250 pusingan/min menggunakan HR, yang mempamerkan corak peralihan campuran. Penghasilan ester asid kojik berasaskan sawit melalui tangki reaktor berpengaduk adalah berdasarkan hukum Newton kerana terdapat hubungan malar di antara tegasan ricih dan kadar ricih.

Penggantungan partikel enzim heterogen telah diperoleh pada 250 pusingan/min dengan jarak antara 30 mm antara dua pengaduk, RT dan HE.

Kajian kinetik menggunakan tangki reaktor berpengaduk telah dibuat bertujuan untuk membentuk model kinetik dengan memberi tumpuan kepada faktor kepekatan substrat. Perbezaan kepekatan asid dan alkohol telah diuji secara sistematik. Secara amnya, ciri-ciri utama kinetik yang berlaku dalam tindakbalas didapati mengikut mekanisma hukum Ping-Pong Bi-Bi yang mana perencatan oleh lebihan asid oleik berasaskan kelapa sawit telah dikenal pasti. Nilai-nilai pemboleubah kinetik telah dianggarkan oleh analisis regressi tidak malar seperti berikut: υ_{max}: 4.58 x 10^2 mmol/L.j.g; $K_{m(KA)}$:1.87 x 10^{-7} mmol/L.j.g; $K_{m(OA)}$: 8.31 x 10^2 mmol/L.j.g ; $K_{i(OA)}$: 5.32 x 10^6 mmol/L.j.g.

Pengesteran-bermangkin lipase dalam sistem pelarut bebas dengan menggunakan Novozym 435 telah dianalisa sebagai satu kaedah untuk permodelan kinetik ester asid kojik berasaskan kelapa sawit. Struktur ester telah dikenal pasti pada kedudukan C-7 asid kojik dengan nama spesifiknya, (E)-(5-hydroxy-4-oxo-4H-pyran-2-yl) methyl octadec-9-enoate. Pengkadaran ester ini telah dikaji dengan mengunakan model kuasa peringkat kedua tidak berbalik sebagai pendekatan pertama. Tenaga pengaktifan (E_a) menunjukkan nilai 7.90 x 10^4 J/mol dan kinetik tindakbalas malar (k_o) adalah 5.30 x 10^8 $L.mol^{-1}.min^{-1}.g_{CAT}^{-1}$. Nilai penjerapan antara pemangkin dan ester (K_{KAE}) iaitu sebanyak 1.90 x 10^{-17} $g_{CAT}.g_{KAE}^{-1}$ telah dikaitkan dengan model Langmuir-Hinshelwood-Hougen-Watson sebagai pendekatan kedua. Tenaga Gibbs, perubahan entalpi dan entropi bagi

tindakbalas pada suhu yang berbeza telah dikira dengan menggunakan persamaan Van't Hoff. Nilai-nilai yang dikira untuk data termodinamik ini adalah masing-masing sebanyak 2.77 x 10^4 J/mol, 9.85 x 10^5 J/mol dan 4.77 x 10^2 J/mol.K.

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I certify that a Thesis Examination Committee has met on 4th January 2013 to conduct the final examination of Siti Efliza Ashari on her thesis entitled "Optimization and Kinetic Study of Lipase-Catalyzed Synthesis of Palm-based Kojic Acid Ester" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U. (A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

Members of the Thesis Examination Committee were as follows:

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declared that it has not been previously or currently submitted for any other degree at Universiti Putra Malaysia or other institutions.

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SITI EFLIZA ASHARI

Date: 4 January 2013

TABLE OF CONTENTS