UNIVERSITI PUTRA MALAYSIA

OPTIMIZATION OF LIPASE-CATALYSED SYNTHESIS AND CHARACTERIZATION OF KOJIC ACID ESTERS

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By

NOOR HAYATI BINTI KHAMARUDDIN

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Master of Science

OPTIMIZATION OF LIPASE-CATALYSED SYNTHESIS AND CHARACTERIZATION OF KOJIC ACID ESTERS

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NOOR HAYATI BINTI KHAMARUDDIN

March 2007

Chairman : Professor Mahiran Basri, PhD

Faculty : Science

Kojic acid ester was successfully synthesized by esterification using lipase from *Pseudomanas cepacia* (Amano PS) as a biocatalyst in an organic media. The reaction mixture consisted of 90 mM of oleic acid, 360 mM of kojic acid and 0.15 g Amano PS in the presence of 2 mL of acetonitrile. The mixture was incubated at temperature of 50°C for 24 hour at shaking speed of 150 rpm. Lipozyme IM, Novozym 435, *Pseudomonas cepacia* lipase, *Aspergillus niger*, *Candida rugosa* and Eupergit C-Lipase were tested for their suitability as the enzyme in the reaction.

Among the enzymes tested, lipase from *Pseudomonas cepacia* gave the highest enzyme activity (0.015 yield/µg protein content) and specific activity (3.738×10^{-6} mmol ester/min/mg protein content) in the synthesis. Oleic acid

was found to be the best substrate to produce the ester but must be in the presence of acetonitrile as an organic solvent. The maximum percentage yield using *Pseudomonas cepacia* lipase at optimal condition was 44% without removal of water from the reaction mixture. From the optimization studies, kojic acid derivatives can be synthesized from palm oil and kojic acid, by *Pseudomonas cepacia* lipase (Amano PS).

TLC analysis showed that kojic acid ester gave an R_f value of 0.28 in the TLC plate and a retention time of 31.752 min in the gas chromatogram. The product formation and the reactant disappearance were monitored by IR spectroscopy. A strong C=O stretching of the ester at 1746 cm^{-1} and weak absorption peak of O-H in the unreacted kojic acid indicated completion of the esterification reaction. The gas chromatography-mass spectrometry (GC-MS) analysis gave a molecular ion peak at m/z 478 which was due to the kojic acid ester. ^{1}H-NMR and ^{13}C-NMR spectral data confirmed the molecular structure of the kojic acid ester.

Abstrak tesis dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

PENGOPTIMUMAN SINTESIS BERMANGKINKAN LIPASE DAN PENCIRIAN BAGI ESTER ASID KOJIK

Oleh

NOOR HAYATI BINTI KHAMARUDDIN

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Pegerusi : Profesor Mahiran Basri, PhD

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Ester bagi asid kojik dapat disediakan melalui proses pengesteran dengan menggunakan lipase berasal daripada *Pseudomonas cepacia* (Amano PS) sebagai mangkin dalam pelarut organik. Tindak balas ini mengandungi 90 mM asid oleik, 360 mM asid kojik dan 0.15 g Amano PS dengan kehadiran 2 mL asetonitril. Campuran ini ditindak balas pada suhu 50°C selama 24 jam dan digoncang pada kelajuan 150 rpm. Lipozyme IM, Novozym 435, *Pseudomonas cepacia* lipase, *Aspergillus niger*, *Candida rugosa* and Eupergit C-Lipase diuji untuk kesesuian sebagai enzim di dalam tindak balas ini.

Di antara enzim yang diuji, lipase daripada *Pseudomonas cepacia* menunjukkan aktiviti enzim yang tertinggi (0.015 hasil/µg kandungan

protein) dan aktiviti spesifik (3.7379 x 10^{-6} mmol ester/min/mg kandungan protein) di dalam sintesis. Asid oleik adalah substrat yang terbaik untuk menghasilkan produk ester tetapi dengan kehadiran asetonitril sebagai pelarut organik. Peratusan hasil maksimum untuk lipase *Pseudomonas cepacia* pada keadaan optimum adalah 44% tanpa meyingkirkan air daripada tindak balas. Hasil kajian tinda kbalas optimum, terbitan asid kojik boleh dihasilkan daripada minyak kelapa sawit dan asid kojik, dengan menggunakan lipase *Pseudomonas cepacia* (Amano PS).

Analisis TLC menunjukkan ester asid kojik memberikan nilai R_f adalah 0.28 di dalam kepingan TLC dan 31.752 minit di dalam kromatogram gas. Pembentukan produk dan reaktan dianalisis oleh spektroskopi IR. Peregangan C=O ester yang kuat pada 1746 cm^{-1} dan penyerapan puncak lemah pada O-H dalam asid kojik yang tidak bertindak balas membuktikan tindak balas pengesteran telah selesai. Analisis kromatografi gas-spektrometri jisim (GC-MS) memberikan puncak ion molekul pada *m/z* 478 adalah ester asid kojik. Data spektrum ^{1}H-NMR dan ^{13}C-NMR membuktikan struktur molekul ester asid kojik.

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I certify that an Examination Committee met on 8 March 2007 to conduct the final examination of Noor Hayati Binti Khamaruddin on her Master of Science thesis entitled "Optimization of Lipase-Catalysed Synthesis and Characterization of Kojic Acid Esters" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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This thesis submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Master of Science. The members of the Supervisory Committee are 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 declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

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NOOR HAYATI BINTI KHAMARUDDIN

Date: APRIL

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LIST OF ABBREVIATIONS

KAMO	Kojic acid monooleate
KAML	Kojic acid monolaurate
TLC	Thin Layer Chromatography
FT-IR	Fourier Transform-Infra Red
GC	Gas Chromatography
GC-MS	Gas Chromatography-Mass Spectrometry
NMR	Nuclear Magnetic Resonance
TMS	Tetramethylsilane
DMF	Dimethylformamide
γ	gamma
a_w	initial water activity
°C	degree celcius
Å	Angstrom
rpm	rotation per minute
sp.	species
mM	milimolar
cm	centimeter
mm	millimeter
m	meter
g	gram
mL	milliliter