



UNIVERSITI PUTRA MALAYSIA

**ENZYMATIC SYNTHESIS OF OLEYL OLEATE, A LIQUID WAX
ESTER, IN A STIRRED TANK REACTOR**

SALINA MAT RADZI.

FS 2006 36

**ENZYMATIC SYNTHESIS OF OLEYL OLEATE, A LIQUID WAX
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By

SALINA BINTI MAT RADZI

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

June 2006



Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

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June 2006

Chairman : Professor Hjh. Mahiran Basri, PhD

Faculty : Science

High performance enzymatic synthesis of oleyl oleate, a liquid wax ester was successfully synthesized via enzymatic esterification reaction of oleic acid and oley alcohol. Immobilised *Candida antarctica* lipase B (Novozym 435) was used as biocatalyst. The study was divided into four parts which are the optimisation of reaction synthesis at different scales, the reactor study in term of mixing efficiency, the stability of immobilised enzyme and the analysis and characterization of the product of the reaction.

Preliminary synthesis of oleyl oleate was carried out in a small scale reaction with a total volume of 3.5 mL using screw-capped vials. Optimisation reaction study via conventional method of varying one parameter at-a-time approach was carried out. A high percentage



conversion yield of >90% was achieved at optimum reaction time of 5 min, reaction temperature of 40-60°C, molar ratio of substrates (oleyl alcohol/oleic acid) of 2:1, amount of enzyme of 0.4 g and organic solvents of Log P \geq 3.5 at fixed agitation speed of 150 rpm.

Investigation in larger scale production of oleyl oleate was performed using 2 L stirred tank reactor (STR). The reaction was scaled-up to 300X with a total volume of 1.05 L. A high percentage conversion of oleyl oleate was achieved of >95% by conventional experiment method at reaction time of 30 min, agitation speed of 400 rpm, reaction temperature of 45-50°C, molar ratio of substrate (oleyl alcohol/oleic acid) of 2:1 and amount of enzyme of 90 g.

The reaction synthesis was further optimised by response surface method (RSM) based on five-level, three-variable central composite rotatable design (CCRD) to evaluate the interactive effects of important parameters in larger scale processing. Generally, simultaneously increasing amount of enzyme, agitation speed and reaction temperature would improved the yields. A high percentage conversion of 97.4% was achieved under the optimum condition, which compared well with the maximum predicted value of 97.7%.

In order to improve the production and productivity of oleyl oleate to the highest amount that can be produced in a 2 L STR, the reaction was



synthesized in a solvent-free system. Maximum scaling-up of substrate concentration that can be achieved in the reactor vessel was 900X as compared to 300X previously. The production and productivity of oleyl oleate were successfully improved from 295.39 g/L/h to 705.76 g/L/h and 310.16 g/h to 952.78 g/h, respectively.

Reactor study on the performance of 2 L STR as a mixing device was evaluated to improve the mixing efficiency. The rheological property of the reaction mixture exhibited Newtonian behaviour. Rushton turbine impeller showed better performance in degree of mixing, as compared to AL Hydrofoil impeller whereby a high Reynolds number of $>10^4$ was achieved at 400 rpm, which exhibit a turbulent flow pattern. There was significant effect to the mixing improvement on the enzyme particles distribution by using a 2 impellers system with spacing of 30 mm.

The enzyme showed high stability against heat as shown by the high percentage conversion of wax ester. Novozym 435 retained its synthetic activity up to 9 uses and 4 uses in screw-capped vials and STR, respectively. The effect of shear forces due to the mechanical agitation speed on the enzyme morphology was determined by scanning electron microscope (SEM). Although small rupture on the surface of enzyme was observed when increasing the agitation speed, the enzyme activity was very high even at high agitation speed.

Analysis of product was evaluated by spectroscopy method of Fourier transform-infrared spectroscopy (FT-IR) and gas chromatography-mass spectroscopy (GC-MS) to identify the product obtained. Characteristics of oleyl oleate were also examined, which include iodine value, saponification value, acid value and ester value. Solubility of oleyl oleate in methanol and ethanol was comparatively lower as compared to the solubility at higher chain length of alcohols. This compound seems compatible in most of oils and stable even after heating up to 90°C and overnight storage at room temperature.

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

**SINTESIS ENZIM BAGI OLEIL OLEAT, CECAIR ESTER LILIN, DI
DALAM TANGKI REAKTOR BERGERAK**

Oleh

SALINA BINTI MAT RADZI

Jun 2006

Pengerusi : Profesor Hjh. Mahiran Basri, PhD

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Sintesis enzim berprestasi tinggi bagi oleil oleat, cecair ester lilin telah disintesis dengan jayanya melalui tindakbalas esterifikasi enzim oleh asid oleik dan alkohol oleil. Lipase B *Candida antartica* tersekatgerak (Novozim 435) telah digunakan sebagai biopemangkin dalam tindakbalas ini. Kajian ini telah dibahagikan kepada empat bahagian iaitu pengoptimuman sintesis tindakbalas pada skala berbeza, kajian reaktor berhubung dengan keefisienan percampuran, kestabilan enzim tersekatgerak dan analisis dan pencirian produk tindakbalas.

Permulaan sintesis tindakbalas bagi oleil oleat telah dijalankan pada skala kecil dengan jumlah isipadu adalah 3.5 mL menggunakan botol kecil bertutup-skru. Kajian pengoptimuman tindakbalas melalui kaedah

konvensional iaitu memvariasikan satu parameter pada-satu-masa telah dijalankan. Peratusan penukaran hasil yang tinggi iaitu >90% telah dicapai pada masa tindakbalas optimum iaitu 5 min, suhu tindakbalas pada 40-60°C, nisbah molar bahan tindakbalas (alkohol oleil/ asid oleik) pada 2:1, amaun enzim pada 0.4 g dan pelarut organik pada $\log P \geq 3.5$ dengan kelajuan putaran yang ditetapkan pada 150 rpm.

Penyelidikan pada pengeluaran skala lebih besar bagi oleil oleat telah dihasilkan dengan menggunakan 2 L tangki reaktor bergerak (STR). Skala tindakbalas telah ditingkatkan kepada 300X dengan jumlah isipadu pada 1.05 L. Peratusan penukaran oleil oleat yang tinggi telah dicapai pada >95% dari kaedah eksperimen secara konvensional pada masa tindakbalas pada 30 min, kelajuan putaran pada 400 rpm, suhu tindakbalas pada 45-50°C, nisbah molar bahan tindakbalas (alkohol oleil/asid oleik) pada 2:1 dan amaun enzim sebanyak 90 g.

Sintesis tindakbalas telah dioptimumkan selanjutnya dengan kaedah permukaan respon (RSM) berdasarkan lima-peringkat, tiga-pembolehubah rekaan pusat komposit berputar (CCRD) untuk menilai kesan interaktif bagi parameter-parameter yang penting di dalam pemprosesan pada skala lebih besar. Secara amnya, dengan peningkatan secara serentak bagi amaun enzim, kelajuan putaran dan suhu tindakbalas akan meningkatkan hasil. Peratusan penukaran yang

tinggi iaitu 97.4% diperolehi di bawah keadaan optimum, di mana ia telah dibandingkan dengan baik dengan nilai anggaran maksimum iaitu 97.7%. Bagi meningkatkan pengeluaran dan produktiviti oleil oleat kepada amaun yang paling tinggi yang dapat dihasilkan dalam 2 L STR, tindakbalas telah disintesis di dalam sistem tanpa pelarut. Peningkatan skala bahan tindakbalas yang maksimum dapat dihasilkan di dalam bekas reaktor adalah 900X berbanding 300X sebelumnya. Pengeluaran dan produktiviti oleil oleat telah ditingkatkan masing-masing dengan jayanya daripada 295.39 g/L/j kepada 705.76 g/L/j dan 310.16 g/j kepada 952.78 g/j.

Kajian reaktor ke atas prestasi 2 L STR sebagai peralatan percampuran telah dilakukan untuk meningkatkan keefisienan percampuran. Sifat riologi campuran tindakbalas telah menunjukkan sifat Newtonian. Penggerak turbin Rushton telah menunjukkan prestasi terbaik di dalam percampuran, berbanding dengan penggerak AL Hidrofoil, di mana nombor Reynolds yang tinggi $>10^4$ telah dicapai pada 400 rpm, di mana ia menunjukkan gerakan aliran bergelora. Terdapat kesan yang baik pada keefisienan percampuran dengan menggunakan sistem 2 penggerak dengan jarak 30 mm.

Enzim menunjukkan kestabilan yang tinggi terhadap haba dengan peratusan penukaran ester lilin yang tinggi. Aktiviti sintetik Novozim 435 kekal masing-masing, sehingga 9 kali penggunaan dan 4 kali penggunaan

di dalam botol kecil bertutup-skru dan STR. Kesan tekanan daripada kelajuan putaran mekanikal terhadap morfologi enzim telah ditentukan dengan pengimbas mikroskop electron (SEM). Walaupun rekahan kecil pada permukaan enzim telah didapati semasa kelajuan putaran ditingkatkan, aktiviti enzim adalah sangat tinggi walaupun pada kelajuan putaran yang tinggi.

Analisis produk telah dilakukan dengan kaedah spektroskopi iaitu spektroskopi inframerah (FT-IR) dan spektroskopi jisim-kromatografi gas (GC-MS) untuk mengenali produk yang telah di perolehi. Ciri-ciri oleil oleat juga diperiksa, termasuk nilai iodin, nilai saponifikasi, nilai asid dan nilai ester. Kelarutan oleil oleat dalam metanol dan etanol adalah lebih rendah berbanding dengan kelarutan dalam alkohol berantai panjang. Kompaun ini di dapati sesuai di dalam kebanyakan minyak dan stabil walaupun selepas dipanaskan sehingga 90°C dan disimpan sepanjang malam pada suhu bilik.

ACKNOWLEDGEMENTS

Alhamdulillah, all praises to Allah S.W.T., for giving me the strength to endure all challenges and complete this study.

My sincere appreciation goes out to Prof. Dr. Hjh. Mahiran Basri, Prof. Dr. Abu Bakar Salleh, Prof. Dr. Arbakariya Ariff, Dr. Rosfarizan Mohamad and other members in Enzyme and Microbial Technology Research Group for their great concern, advice, patience, persistent encouragement and invaluable assistance from the beginning till the end of this study.

A special thank to Mr. Rizal Kapri, Mr. Sobri Mohd. Akhir and members of the Fermentation and Enzyme Technology Laboratory, Institute of Bioscience, whose help and cooperation were invaluable during the course of the study.

I am grateful to staff of Microscopy and Microanalysis Unit, Institute of Bioscience especially to Assoc. Prof. Dr. Fauziah Othman and Mr. Safari for their help in the progress of this study.

Love and thanks to my family for their understanding, constant supports and simply being here, loving me with all their hearts.

A special thank also goes to Kak Erin, Salhah, Pei Sin, Ita, Yamin, Yati and other members of Lab 401, without whom life would be a bore and uneventful. Thank you for the bond of friendship and for making my stay in UPM a bearable one with many sweet memories and experiences. Thank you for being friends in need.

Finally, thank you to the Ministry of Science, Technology and Environment and Universiti Putra Malaysia for their financial support and facilities.



I certify that an Examination Committee has met on 8th June 2006 to conduct the final examination of Salina Binti Mat Radzi on her Doctor of Philosophy thesis entitled "Enzymatic Synthesis of Oleyl Oleate, a Liquid Wax Ester, in a Stirred Tank Reactor" 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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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.



SALINA BINTI MAT RADZI

Date: 21 / 6 / 2006

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