



UNIVERSITI PUTRA MALAYSIA

**FACTORS AFFECTING THE OPTIMIZATION OF LIPASE-
CATALYSED PALM-BASED ESTERS SYNTHESIS**

ERIN RYANTIN GUNAWAN.

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CATALYSED PALM-BASED ESTERS SYNTHESIS**

By

ERIN RYANTIN GUNAWAN

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

2005



DEDICATION

I dedicate this thesis to all my family and Ibu Pertiwi.....Indonesia



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

**FACTORS AFFECTING THE OPTIMIZATION OF LIPASE-
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July 2005

Chairman: Professor Mahiran Basri, PhD

Faculty: Science

The lipase synthesis of esters using palm oil fractions and long chain alcohol as substrates was carried out. Various lipases were tested for their suitability for the reaction. Among the enzymes tested, Lipozyme IM exhibited the highest percentage yield of palm oil ester (more than 75%) compared with the other lipases. Two methods, classical method (one-variable at-a-time) and response surface methodology (RSM), were employed for optimization of the reaction.

By the classical method, five parameters such as reaction time, temperature, amount of enzyme, molar ratio of substrates and various organic solvents of the reaction system were investigated.



The optimum yield was achieved at the reaction temperature of 40 - 50°C for palm oil (PO) and 40°C for palm kernel oil (PKO) alcoholysis, a reaction time of 5 – 7 h for PO and 7 – 10 h for PKO, 0.15 g of enzyme for both PO and PKO alcoholysis, molar ratio at 3: 1 (alcohol: PO or PKO), and the best solvent for the reactions was hexane. Percentage yields of esters obtained at these optimum reaction conditions were 83, 80 and 81% for refined, bleached and deodorized (RBD) palm oil, RBD palm stearin (PS) and RBD palm olein (PL), respectively and 87, 90 and 86% for RBD palm kernel oil, RBD palm kernel stearin (PKS) and RBD palm kernel olein (PKL), respectively.

The classical method of optimization involves varying one parameter at a time and keeping the other parameters constant. However, this method is inefficient as it fails to understand relationships between the variables (reaction time, temperature, molar ratio and amount of enzyme) and the response (percentage yield). RSM is an effective statistical technique for the investigation of complex processes. RSM comprising of a five-level, four-factor central composite rotatable design (CCRD) was used to evaluate the interactive effects of synthesis reaction time (2.5 – 10 h of PO, 5 – 15 h of PKO), temperature (30 - 70°C of PO, 30 - 50°C of PKO), amount of enzyme (0.1 - 0.2 g of PO or PKO) and substrates molar ratio (1: 1 - 5: 1 alcohol to PO or PKO) on the percentage yield of



esters and to obtain the optimum conditions for enzyme-catalyzed alcoholysis of palm-based ester.

The optimum conditions derived by RSM of PO and PKO were: reaction time at 7.38 and 10 h, temperature of 53.9°C and 44.2°C, amount of enzyme of 0.149 and 0.157g, and substrates molar ratio 3.41: 1 and 3.78: 1 (alcohol: PO or PKO), respectively. The actual experimental yield of PO and PKO were 84.6% and 90.8% under these optimum conditions, which compared well with the maximum predicted value of 85.4% and 91.8% for PO and PKO, respectively.

The composition of esters synthesized from PO at optimum reaction condition are 0.8% of oleyl laurate, 3.8% of oleyl myristate, 35.5% of oleyl palmitate, 4.5% of oleyl stearate, 33.3% oleyl oleate and 6.2% of oleyl linoleate. Meanwhile, The composition of esters synthesized from PKO are 0.7% of oleyl caproate, 5.7% of oleyl caprylate, 3.7% of oleyl caprate, 36.7% of laurate, 13.10% of oleyl myristate, 8.8% of oleyl palmitate, 2.5% oleyl stearate, 14.1% of oleyl oleate and 2.4% of oleyl linoleate. These optimum conditions were also used in alcoholysis of PS, PL, PKS and PKL, which gave the average percentage yield of more than 80%.

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

**FAKTOR-FAKTOR MEMPENGARUHI PENGOPTIMUMAN
SINTESIS ESTER BERASASKAN KELAPA SAWIT
DIMANGKINKAN OLEH LIPASE**

Oleh

ERIN RYANTIN GUNAWAN

Julai 2005

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Sintesis ester lilin menggunakan pecahan minyak kelapa sawit and alkohol rantai panjang sebagai substrat yang dimungkinkan oleh lipase telah dijalankan. Dua kaedah iaitu kaedah klasik (satu- variasi pada satu-masa) dan kaedah permukaan respon, telah digunakan untuk mencari keadaan optima. Pelbagai jenis lipase telah diuji kesesuaiannya untuk tindak balas. Di antara enzim yang telah diuji, Lipozyme IM memberi hasil peratusan ester lilin kelapa sawit paling tinggi (lebih dari 75 %) berbanding lipase lain yang diuji.



Dalam kaedah klasik, lima parameter iaitu masa tindak-balas, suhu, jumlah enzim, nisbah molar substrat dan kepelbagaian pelarut organik telah di kajiselidik.

Hasil optima telah dicapai pada suhu tindak balas alkoholisis 40-50°C bagi minyak kelapa sawit (PO) dan 40°C bagi minyak isirung kelapa sawit (PKO), masa tindak balas pada 5 – 7 jam bagi PO dan pada 7 – 10 jam bagi PKO, jumlah enzim bagi 0.15 g untuk alkoholisis PO dan PKO, nisbah molar substrat pada 3: 1 (alcohol: PO atau PKO) dan pelarut terbaik bagi tindak balas adalah heksana. Hasil peratusan ester lilin yang diperolehi menggunakan keadaan optima ini adalah 83, 80 dan 81% bagi minyak kelapa sawit yang dibersih, diluntur dan dinyahbau (RBD), RBD kelapa sawit sterin (PS) dan RDB kelapa sawit olin, masing-masing 87, 90 dan 86% bagi RBD minyak kelapa sawit (PKL), RBD kelapa sawit isirung sterin (PKS) dan RBD kelapa sawit isirung olin (PKL).

Kaedah klasik bagi pengoptimuman termasuk memvariasikan satu parameter pada satu masa dan mengekalkan selainnya boleh digunakan. Walaubagaimanapun, kaedah ini tidak mencukupi kerana ia gagal untuk memahami hubungkait di antara variable (masa tindak balas, suhu, nisbah molar dan jumlah enzim) dan respon (peratusan hasil). RSM adalah teknik statistik untuk



menyelidik proses yang kompleks. RSM mengandungi lima-peringkat, empat-faktor jenis pusat komposit putaran (CCRD) telah digunakan untuk mengkaji kesan interaktif dalam sintesis bagi masa tindak balas (2.5 – 10h bagi PO, 5 – 15 jam bagi PKO), suhu (30 - 70°C bagi PO, 30 - 50°C bagi PKO), jumlah enzim (0.1 – 0.2 g bagi PO atau PKO) dan nisbah molar susbstrat (1: 1 –5: 1 alkohol bagi PO atau PKO) peratusan hasil ester lilin dan untuk mendapatkan keadaan optima untuk alkoholisis bermangkinkan enzim yang menghasilkan ester lilin berasaskan kelapa sawit.

Keadaan optima diperolehi melalui RSM bagi PO dan PKO adalah; masa tindak balas pada 7.38 dan 10 jam, suhu pada 53.9 dan 44.2°C, jumlah enzim pada 0.179 dan 0.157 g dan nisbah molar substrat 3.41: 1 dan 3.78: 1 (alkohol: PO atau PKO), masing-masing. Hasil sebenar dari eksperimen bagi PO dan PKO adalah 84.6% dan 90.8% di bawah keadaan optima, di mana ia sesuai dengan nilai yang dianggar iaitu 85.4% dan 91.8% bagi PO dan PKO. Keadaan optimum ini juga digunakan pada alkoholisis PS, PL, PKS dan PKL, di mana ia telah memberikan purata hasil peratusan lebih daripada 80%.



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In The Name of ALLAH, The Most Merciful and Most Beneficent

All praises do to Allah, Lord of the universe. Only by His grace and mercy this thesis can be completed.

This work was carried out with a hope to contribute towards the expansion of our currently limited knowledge on Oleochemistry. The completion of this thesis would have been impossible if not for the assistance and direct involvement of so many kindhearted individuals. Thus, I am very much indebted to my previous mentors and I have no way of repaying such a debt except to express my sincerest gratitude.

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I certify that an Examination Committee met on 18th July 2005 to conduct the final examination of Erin Ryantin Gunawan on her Doctor of Philosophy thesis entitled "Factors Affecting the Optimization of Lipase-Catalysed Palm-Based Esters Synthesis" 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.



ERIN RYANTIN GUNAWAN

DATE: 20/8/2005

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