UNIVERSITI PUTRA MALAYSIA

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KENG PEI SIN

SCALE-UP PRODUCTION OF PALM-BASED WAX ESTERS USING LIPOZYME RM IM AND CHARACTERISATION OF THE ESTERS

By

KENG PEI SIN

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

January 2008

DEDICATED TO:

My family, S.T. Ong and K.L. Tan,
for all that you are and what you mean to me.

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Chairman: Professor Mahiran Basri, PhD

Faculty: Science

The investigation of palm-based wax esters synthesis was started in a 1-L scale stirred-tank reactor (STR) with 0.5-L working volume using Rushton turbine impeller. The optimum alcoholysis conditions derived from the Response surface methodology (RSM) were: temperature 50.4°C, amount of enzyme 16 wt% of palm oil, amount of palm oil 200 mmol, amount of oleyl alcohol 600 mmol, palm oil-to-oleyl alcohol ratio 3:1 and impeller speed 242 rpm. The corresponding predicted value of percentage yield and productivity were 91.5% and 106.4 mmol/h respectively as compared to the actual experimental value of 92.3% and 110.8 mmol/h.

The optimum reaction condition derived from RSM was employed in the 1.5-L STR. Rushton turbine (RT), AL-hydrofoil (AL-H) and 2-bladed elephant ear (EE) impeller were used to investigate the mixing performance in the reactor. Of these impellers, RT was found to give the highest reaction yield of 95.8% and productivity of 344.9

mmol/h at lower agitation speed (250 rpm) as compared to the other two impellers. Homogeneous solid suspension was achieved by RT impeller at 250 rpm with almost 99% solid suspension height. The shortest mixing time was obtained by the agitation system with 2-bladed EE impeller due to the larger D_i/D_t ratio of the impeller. For all the impellers tested, the Reynolds number obtained were in the range of $10^2 < Re < 10^4$, indicated that the fluid flow pattern fell in the transition region for the agitation range tested. Although ruptures and cracks of immobilised enzymes were observed in the agitation system, the high stability of Lipozyme RM IM was proven by its reusability of more than fifteen times under agitation system of RT impeller, to give reaction percentage yield of 78.9%, which was only 16.9% reduced from the first cycle.

Subsequently, scale-up production of palm oil esters (POE) to 50-L was successfully carried out in the 75-L STR based on constant impeller tip speed method. The esters yield and productivity were increased to 97.2% and 11.67 mol/h, respectively. Higher reaction rate was observed in the 50-L scale production with the reaction equilibrium achieved earlier at 4 h of reaction time as compared to the 5 h optimum reaction time observed in bench-scale study. The esters yield and productivity were not only maintained during the scaling-up study, but increased gradually from reaction of 0.5-L, 1.5-L to 50-L.

Analyses of palm-based wax esters were carried out to establish compliance to product specifications and standards. Important physicochemical properties were analysed followed a standard Test Methods modified from American Oil Chemists' Society

(AOCS) as well as Malaysian Palm Oil Board (MPOB) standard. Simultaneous differential scanning calorimetry-thermal gravimetry analysis (DSC-TGA) showed high thermal stability profile of palm-based wax esters. The dermal irritation assay of POE showed the non-irritancy of the esters with Human Irritancy Equivalent (HIE) score below 0.9; whilst an increase of skin hydration of 40.7% after 90 min application was achieved in the acute moisturising test.

Downstream processing of POE was carried out using liquid-liquid extraction and crystallisation process to remove the remaining reactants including oleyl alcohol and lipids impurities in the esters. Ethanol was used as the solvent to remove the remaining unreacted oleyl alcohol in the POE mixture. The extraction was carried out in separatory funnel with optimum ethanol to esters ratio 4:1 at room temperature. A removal of 97% of the unreacted oleyl alcohol was achieved after 3 stages of extraction with 30 min contact time for each stage at 150 rpm agitation speed of the water bath shaker. Meanwhile, crystallisation of POE mixture at 23˚C for 24 h incubation time was shown to increase the purity of the esters from 92.0% to 96.1%.

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

PENGHASILAN SKALA BESAR ESTER LILIN BERDASARKAN KELAPA SAWIT MENGGUNAKAN LIPOZYME RM IM DAN PENCIRIANNYA

Oleh

KENG PEI SIN

Januari 2008

Pengerusi: Profesor Mahiran Basri, PhD

Fakulti: Sains

Penyelidikan sintesis ester lilin berasaskan kelapa sawit bermula dengan menggunakan tangki reactor berpengaduk (STR) skala 1-L dengan isipadu berfungsi 0.5 L dan turbin Rushton. Keadaan optimum alkoholisis diperolehi melalui Permukaan respon (RSM) adalah: suhu 50.4°C, 16 wt% amaun enzim daripada berat minyak kelapa sawit, 200 mmol amaun minyak kelapa sawit, 600 mmol amaun alkohol oleil, 3:1 nisbah minyak kelapa sawit terhadap alkohol oleil dan 242 rpm kelajuaan penggerak turbin. Peratus hasil dan produktiviti jangkaan adalah masing-masing 91.5% dan 106.4 mmol/j berbanding dengan nilai sebenar eksperimen iaitu 92.3% and 110.8 mmol/h.

Keadaan tindakbalas optimum yang diperoleh daripada RSM telah diaplikasikan dalam 2-L STR. Turbin Rushton (RT), AL-hydrofoil (AL-H) dan turbin 2-plat telinga gajah (EE) digunakan untuk mengkaji kesan pencampuran dalam STR. RT didapati

memberi hasil tertinggi iaitu 95.8% dan produktiviti 344.9 mmol/j dengan kadar pengacauan yang rendah (250 rpm) berbanding turbin-turbin lain. Pengapungan pepejal yang homogen diperolehi dengan menggunakan turbin RT pada kelajuan 250 rpm dengan ketinggian pengapungan pepejal yang hampir dengan 99%. Masa pencampuran yang tersingkat diperolehi dengan menggunakan sistem pengacaun turbin 2-plat EE kerana nisbah D_i/D_t yang lebih besar. Bagi kesemua turbin yang dikaji, nombor Reynolds yang didapati adalah dalam lingkungan 10^2 <Re< 10^4 menunjukkan corak aliran cecair berada dalam tahap translasi. Sungguhpun pemecahan dan retakan enzim tersekat-geark telah dikesan dalam sistem berpengaduk, tetapi kestabilan Lipozyme RM IM telah dibuktikan dengan kebolehannya untuk digunakan semula melebihi 15 kali dalam sistem pengacauan turbin RT dengan peratus hasilannya 78.9% iaitu berkurangan sebanyak 16.9% daripada kitar pertama.

Penghasilan skala besar POE ke 50-L telah berjaya dilaksanakan dengan menggunakan STR berisipadu 75-L berasaskan kaedah kelajuan penggerak hujung yang konstan. Peratus penghasilan dan produktiviti POE telah meningkat ke 97.2% dan 11.67 mol/j. Kadar tindakbalas yang lebih tinggi telah diperolehi pada skala penghasilan 50-L dengan keseimbangan tindakbalas tercapai lebih awal iaitu 4 j masa tindakbalas berbanding dengan 5 j yang diperlukan dalam 1.5-L STR. Peratus penghasilan dan produktiviti ester bukan sahaja dapat dikekalkan dalam kajian skala besar ini, malahan telah meningkat daripada tindakbalas 0.5-L ke 1.5-L dan seterusnya 50-L.

Analisis ester lilin berdasarkan kelapa sawit telah dijalankan berdasarkan Kaedah Ujian Piawaian Kelab Ahli Kimia Amerika (AOCS) dan Badan Kelapa Sawit Malaysia (MPOB) untuk memastikannya mematuhi piawaian dan spesifikasi produk. Analisis serentak kalorimetri imbas perbezaan-gravimetri suhu (DSC-TGA) menunjukkan ester-ester ini mempunyai kestabilan suhu yang tinggi. Ujian kegatalan/keradangan pada POE menunjukkan produk ini tidak membawa kegatalan/keradangan pada kulit dengan Kesamaan Kegatalan/Keradangan Manusia (HIE) di bawah 0.9; manakala ujian kelembapan memperlihatkan peningkatan kelembapan kulit sebanyak 40.7% selepas 90 minit pengaplikasian.

Proses penulenan POE dijalankan dengan menggunakan kaedah pengekstrakan cecair-cecair dan proses kristalisasi untuk menyingkirkan saki-baki bahan tindakbalas termasuk alcohol oleil dan sisa minyak yang terdapat dalam campuran ester. Etanol digunakan sebagai pelarut untuk menyingkirkan saki-baki alcohol oleil dalam campuran POE. Pengekstrakan dijalankan dalam corong pemisah dengan nisbah optima etanol kepada ester adalah 4:1 pada suhu bilik. Sebanyak 97% saki-baki alcohol oleil telah disingkirkan selepas pengekstrakan sebanyak 3 kali dengan 30 minit masa pengoncangan dengan kelajuan putaran pengoncang air 150 rpm. Sementara itu, kristalisasi campuran POE pada 23˚C selama 24 j telah meningkatkan ketulenan ester daripada 92.0% ke 96.1%.

ACKNOWLEDGEMENTS

My deepest gratitude and sincere appreciation is owed to Prof. Dr. Mahiran Basri for her invaluable guidance, support and patience during the course of my laboratory work and throughout the completion of this thesis. My appreciation also goes to Prof. Dr. Abu Bakar Salleh and Prof. Dr. Arbakariya Ariff for their invaluable time and great concern.

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Members of the Examination Committee are as follows:

Asmah Hj. Yahaya, PhD
Associate Professor
Faculty of Science
Universiti Putra Malaysia
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Faujan Hj. Ahmad, PhD
Associate Professor
Faculty of Science
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(Member)

Suraini Abd. Aziz, PhD
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Faculty of Biotechnology and Biomolecular Sciences
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(Member)

Masitah Hasan, PhD
Professor
Faculty of Engineering
Universiti Malaya
(Independent Examiner)

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HASANAH MOHD GHAZALI, PhD
Professor and Deputy Dean
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Universiti Putra Malaysia

Date: 21 February 2008

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Professor
Faculty of Science
Universiti Putra Malaysia
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Abu Bakar Salleh, PhD
Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
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Arbakariya Ariff, PhD
Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Member)

AINI IDERIS, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 21 February 2008

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.

_____

KENG PEI SIN

Date: 31 January 2008

DEDICATED TO:

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Faculty of Engineering
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