UNIVERSITI PUTRA MALAYSIA

ENZYMATIC INTERESTERIFICATION OF RAMBUTAN KERNEL OLEIN AND STEARIN FOR SPECIALTY FATS PRODUCTION

NAZARUDDIN BIN RAMLI

IB 2008 12

ENZYMATIC INTERESTERIFICATION OF RAMBUTAN KERNEL OLEIN AND STEARIN FOR SPECIALTY FATS PRODUCTION

NAZARUDDIN BIN RAMLI

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

November 2008

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

ENZYMATIC INTERESTERIFICATION OF RAMBUTAN KERNEL OLEIN AND STEARIN FOR SPECIALTY FATS PRODUCTION

By:
Nazaruddin Ramli

November 2008

Chairman : Professor Dr Arbakariya Ariff, Ph.D.

Faculty : Institute of Bioscience

In this study, olein and stearin of rambutan kernel fat was produced by fractionation at 15ºC. Purification of crude rambutan fat was also carried out. Fat modification was further developed to generate specialty fat through both fractions of rambutan fat. Besides that, palm mid (PMF), palm olein (POo) and palm stearin IV=34 (POs) were also used in the blending studies. Enzymatic interesterification (EIE) was used in fat modification using lipase TL IM with specific position at 1,3. Among the physico-chemical analyses carried out were solid fat content solid (SFC), melting and crystallization behaviour, free fatty acid (FFA), fatty acid methyl ester composition (FAME), slip melting point (SMP), triacylglyceride (TAG), iodine value (IV) and peroxide value (PV). These characteristics were compared with those of noninteresterification mixture.

The interesterified products showed an increase in SMP values ranging from 17 ºC-19.5ºC to 37 ºC-38.5ºC. Interesterified blend had lower SFC at 0 ºC to 20ºC but higher SFC from 25ºC to 37.5ºC when compared to non-EIE products. All interesterified blends were in solid form at room temperature and had ~0 % SFC at 37.5 ºC. All non-EIE blends were in liquid form at room temperature and gave 0% SFC at 35ºC. EIE blends produced significantly higher FFA (17-27 %) than non-EIE blends with only 2-5% FFA. High value of FFA showed that EIE of RKS: PMF:POo with *Thermomyces lanuginosa* lipase caused a high degree of TAG loss due to increased in rate of hydrolysis. Statistical analysis showed that there was no significant difference ($p>0.05$) for peroxide value and iodine value between non-EIE blends and EIE blends. Thermal analysis showed that enzymatic interesterification of RKS:PMF:POo had decreased the entalpy of melting and crisytallization of the blends. An optimization study for enzymatic interesterification (EIE) of rambutan kernel stearin (RKFs), palm mid fraction (PMF, IV=34), and palm olein (POo) blends was carried out by Response Surface Method (RSM) to obtain desired blended products. Enzyme load (1.95%-12.05%), reaction time (1.20-29.80 h), and temperature (16ºC-83ºC) were important determining factors affecting EIE. Based on SFC profile, ratio that has been chosen for optimization study was 30% of RKFs, 32.5% of PMF and 37.5% of POo. The optimal conditions of variables which produced the desired properties for use as margarine was at 47.82ºC with 6.30% an enzyme dosage for 12 h of reaction. Fat blends that were produced at optimal parameters had been used to produce margarine. Margarine that was produced had SFC value of 21.80 % at 10ºC and 19.40% at 20ºC. These profiles indicated that the product has good spreadability effect at refrigerating temperatures and

resistance to oil exudation at room temperature. Beside that, margarine that was produced in this study had a good microstructure similar to commercial margarine.

Cocoa butter substitute (CBS) was produced through enzymatic interesterification (EIE) of palm stearin (POs), palm mid fraction (PMF) and rambutan kernel olein (RKOo) by using Lipozyme TL IM (*Thermomyces lanuginous*). Generally, the percentage of SFC and SMP was reduced after EIE process. The percentage of SFC at 37.5^{o}C for sample B (20%POs: 60% PMF: 20%RKOo) and D (20% POs: 50% PMF: 30% RKOo) after EIE process were the lowest SFC (4.51% and 0.51%, respectively). The SMP for sample B was decreased from 47.5ºC to 36.5ºC while sample D was decreased from 50ºC to 34ºC. Free fatty acid and peroxide value for sample B and D were increased after EIE while the fatty acid composition and iodine value did not show any difference after EIE. The DSC thermogram of sample D showed small peak and sharp peak at 10.92ºC and 36.20^{o}C which were similar to cocoa butter profile. Therefore, sample D was chosen to undergo optimization process using Response Surface Method (RSM). Finally, the optimize reaction parameters to produce good CBS was at 50ºC for 4 h with 4.39% of Lipozyme TL IM.

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

INTERESTERIFIKASI BERENZIM KE ATAS OLEIN DAN STEARIN LEMAK BIJI RAMBUTAN DI DALAM PENGHASILAN LEMAK ALTERNATIF

Oleh:
Nazaruddin Ramli

November 2008

Pengerusi : Professor Arbakariya Ariff, Ph.D.

Fakulti : Institut Biosains

Dalam kajian ini, pemisahan pemeringkatan pada suhu 15ºC dilakukan ke atas lemak rambutan mentah untuk mendapatkan fraksi olein dan stearin. Seterusnya proses pengubahsuian struktur lemak telah dijalankan untuk menghasilkan lemak istimewa melalui kedua-dua fraksi tersebut. Proses ini melibatkan interesterifikasi berenzim (EIE) dengan menggunakan lipase khusus posisi 1,3. Penulinan terhadap lemak rambutan yang diekstrak turut dilakukan. Antara analisis fizikokimia yang telah dilakukan adalah seperti kandungan lemak pepejal (SFC), kelakuan peleburan dan penghabluran, asid lemak bebes (FFA), komposisi asid lemak (FA), takat lebur gelincir (SMP), triasilgliserida (TAG), nilai iodin (IV) dan nilai peroksida (PV). Pembangunan lemak istimewa akan dilakukan ke atas setiap fraksi bergantung kepada profil kandungan lemak pepejal dan takat lebur gelincir. Daripada profil kedua-dua parameter ini, maka dua jenis produk telah dikenalpasti untuk dihasilkan iaitu margerin dan lemak koko gantian. Di samping lemak stearin dan olein rambutan, lemak pecahan pertengahan

sawit (PMF), olein sawit (POo) dan stearin sawit IV=34 (POs) telah turut digunakan dalam kajian adunan minyak ini. Penghasilan lemak istimewa ini telah dilakukan dengan kaedah pengubahusiuan lemak melalui interesterifikasi berenzim Secara perbandingan, campuran selepas interesterifikasi telah meningkatkan nilai SMP dari 17ºC-19.5ºC ke 37ºC-38.5ºC. Adunan EIE memberikan nilai SFC yang rendah pada suhu 0ºC ke 20ºC tetapi mempunyai nilai SFC yang lebih tinggi pada suhu 25ºC ke 37.5ºC berbanding dengan adunan ringkas. Jadi, adunan EIE yang terhasil berada dalam keadaan pepejal pada suhu bilik dan semua sampel adunan IE mempunyai ~0% SFC pada suhu 37.5ºC. Berdasarkan profil SFC, nisbah 30% RKFs, 32.5% PMF dan 37.5% POo telah dipilih untuk kajian pengoptimuman.

Kajian tentang kesan parameter tindakbalas semasa interesterifikasi seperti masa tindakbalas, peratus enzim dan suhu tindakbalas telah dilakukan. Proses pengoptimuman turut dilakukan ke atas proses EIE di mana rekabentuk kajian yang digunakan ialah D-Optimal (adunan tigaan) dan kaedah respon permukaan (KRP) dengan menggunakan program Design Expert Versi 6.0. Lipase *Thermomyces lanuginose* (Lipozyme® TL IM) telah digunakan dalam kajian ini. Parameter tindak balas yang optimum bagi menghasilkan ciri-ciri marjerin yang baik adalah pada suhu 47.82ºC dengan menggunakan 6.30% lipase selama 12 jam. Marjerin yang terhasil mempunyai nilai SFC 21.80% dan 19.40% pada suhu 10**ºC** dan 20ºC. Ciri-ciri marjerin yang dihasilkan mempunyai kebolehsebaran baik pada suhu sejuk dan stabil terhadap ekstrusi minyak pada suhu bilik. Marjerin yang dihasilkan juga mempunyai mikrostruktur yang baik seperti marjerin komersial.

Lemak koko gantian (CBS) dihasilkan melalui interesterifikasi berenzim (EIE) adunan tigaan minyak stearin sawit (POs), minyak pertengahan fraksi (PMF) dan minyak kernel olein rambutan (RKOo) dengan menggunakan Lipozyme TL IM (*Thermomyces lanuginosus*). Secara umumnya, peratus SFC dan suhu SMP berkurangan selepas proses EIE. Peratus SFC pada suhu 37.5ºC bagi adunan B (20 %POs: 60 % PMF: 20 %RKOo) dan D (20 % POs: 50 % PMF: 30% RKOo) ialah 4.51% dan 0.51%. Suhu SMP bagi adunan B menurun dari 47.5 ke 36^{o}C manakala SMP bagi adunan D menurun dari 50**ºC** ke 34ºC. Bagi nilai asid lemak bebas dan nilai peroksida, kedua-dua adunan B dan D meningkat selepas proses EIE. Manakala komposisi asid lemak dan nilai iodin bagi adunan B dan D tidak banyak berbeza selepas proses EIE. Selain itu, adunan D juga menunjukkan puncak peleburan yang kecil pada suhu 10.92ºC dan puncak peleburan yang tajam pada suhu 36.20ºC yang menyerupai termogram peleburan lemak koko. Berdasarkan profil SFC dan termogram peleburan ini, adunan D telah dipilih untuk kajian pengoptimuman dengan menggunakan kaedah respon permukaan (KRP). Secara rumusan, tindakbalas optimum untuk menghasilkan gantian lemak koko yang baik daripada adunan D boleh dilakukan pada suhu 50^{o}C selama 4 jam dengan menggunakan 4.39% enzim Lipozyme TL IM.

ACKNOWLEDMENTS

I wish to express my foremost appreciation to Professor Dr Arbakariya Ariff, Professor Dr Abu Bakar Salleh and Dr Ling Tau Chuan for patiently guiding me through the course of this thesis to its eventual end, enlightening me scientifically and resolving my many technical crises.

The author wish to express their gratitude to the Intercontinental Specialty Fats, Klang, Alami Tech Sdn Bhd, Teluk Panglima Garang, Banting, Selangor, Malaysia, and Delima Oil Sdn Bhd, Klang, Malaysia for providing the samples in this research. This work was supported by the Ministry of Science and Technology grant (01-02-02-0016 EA 175).

Further thanks are extended to my colleagues for their cheerful assistance on occasions too numerous to mention. Gratitude it also extended to the staff of Institute of Biosciene for so generously sharing his technical expertise.

Last but not least, I am eternally grateful to my family for their present love and support, without which I would never have succeeded in my academic endeavours.

I certify that a Thesis Examination Committee has met on 25th November 2008 to conduct the final examination of Nazaruddin Bin Ramli on his thesis entitled "Enzymatic Interesterification of Rambutan Kernel Olein and Stearin For Specialty Fats Production" 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:

Maheran Basri, Ph.D
Professor,
Faculty of Science
Universiti Putra Malaysia
(Chairman)

Lai Oi Ming, PhD
Associate Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Internal Examiner)

Suraini Abd. Aziz, PhD
Associate Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Internal Examiner)

Ramachandran K.B, PhD
Professor
Department of Biotechnology
India Institute Technology
India
(External Examiner)

BUJANG KIM HUAT, PhD
Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 19 February 2009

Saya mengesahkan bahawa satu Jawatankuasa Peperiksaan Tesis telah berjumpa pada 25 November 2008 untuk menjalankan peperiksaan akhir bagi Nazaruddin Bin Ramli bagi menilai tesis beliau yang bertajuk "Interesterifikasi Berenzim Ke Atas Olein dan Stearin Lemak Biji Rambutan di dalam Penghasilan Lemak Alternatif" mengikut Akta Universiti dan Kolej Universiti 1971 dan Perlembagaan Universiti Putra Malaysia [P.U.(A) 106] 15 Mac 1998. Jawatankuasa tersebut telah memperakukan bahawa calon ini layak dianugerahi Ijazah Doktor Falsafah.

Ahli Jawatankuasa Peperiksaan Tesis adalah seperti berikut:

Maheran Basri, PhD
Profesor
Fakulti Sains
Universiti Putra Malaysia
(Pengerusi)

Lai Oi Ming, PhD
Profesor Madya
Fakulti Bioteknologi & Sains Biomolekul
Universiti Putra Malaysia
(Pemeriksa Dalam)

Suraini Abd. Aziz, PhD
Profesor Madya
Fakulti Bioteknologi & Sains Biomolekul
Universiti Putra Malaysia
(Pemeriksa Dalam)

Ramachandran K.B, PhD
Profesor
Jabatan Bioteknologi
Institut Teknologi India
India
(Pemeriksa Luar)

BUJANG KIM HUAT, PhD
Profesor dan Timbalan Dekan
Sekolah Pengajian Siswazah
Universiti Putra Malaysia

Tarikh: 19 Februari 2009

DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledgment. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

_____

NAZARUDDIN RAMLI

Date:

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES