



UNIVERSITI PUTRA MALAYSIA

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

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

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November 2008

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In this study, olein and stearin of rambutan kernel fat was produced by fractionation at 15°C. 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 °C-19.5°C to 37 °C-38.5°C. Interesterified blend had lower SFC at 0 °C to 20°C but higher SFC from 25°C to 37.5°C when compared to non-EIE products. All interesterified blends were in solid form at room temperature and had ~0 % SFC at 37.5 °C. All non-EIE blends were in liquid form at room temperature and gave 0% SFC at 35°C. 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 enthalpy of melting and crystallization of the blends. An optimization study for enzymatic interesterification (EIE) of rambutan kernel stearin (RKF), 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 (16°C-83°C) were important determining factors affecting EIE. Based on SFC profile, ratio that has been chosen for optimization study was 30% of RKF, 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 47.82°C 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 10°C and 19.40% at 20°C. 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°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 47.5°C to 36.5°C while sample D was decreased from 50°C to 34°C. 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 10.92°C and 36.20°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 50°C for 4 h with 4.39% of Lipozyme TL IM.



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

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Dalam kajian ini, pemisahan pemeringkatan pada suhu 15°C dilakukan ke atas lemak rambutan mentah untuk mendapatkan fraksi olein dan stearin. Seterusnya proses pengubahsuaian 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 bebas (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 pengubahsuaian lemak melalui interesterifikasi berenzim. Secara perbandingan, campuran selepas interesterifikasi telah meningkatkan nilai SMP dari 17°C-19.5°C ke 37°C-38.5°C. Adunan EIE memberikan nilai SFC yang rendah pada suhu 0°C ke 20°C tetapi mempunyai nilai SFC yang lebih tinggi pada suhu 25°C ke 37.5°C 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 37.5°C. Berdasarkan profil SFC, nisbah 30% RKF, 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 tindakbalas yang optimum bagi menghasilkan ciri-ciri marjerin yang baik adalah pada suhu 47.82°C dengan menggunakan 6.30% lipase selama 12 jam. Marjerin yang terhasil mempunyai nilai SFC 21.80% dan 19.40% pada suhu 10°C dan 20°C. Ciri-ciri marjerin yang dihasilkan mempunyai kebolehsbaran 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 37.5°C 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°C manakala SMP bagi adunan D menurun dari 50°C ke 34°C. 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 10.92°C dan puncak peleburan yang tajam pada suhu 36.20°C 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°C selama 4 jam dengan menggunakan 4.39% enzim Lipozyme TL IM.

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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.

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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.

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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 acknowledgment. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

NAZARUDDIN RAMLI

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