UNIVERSITI PUTRA MALAYSIA

KINETIC AND CRYSTALLIZATION BEHAVIOUR OF PALM OIL, PALM KERNEL OIL AND THEIR LIPASE-CATALYZED INTERESTIFIED BLENDS USING VISCOMETRY

CHEN CHIT WAY.

FSMB 2004 4

KINETIC AND CRYSTALLIZATION BEHAVIOR OF PALM OIL, PALM KERNEL OIL AND THEIR LIPASE-CATALYZED INTERESTERIFIED BLENDS USING VISCOMETRY

By

CHEN CHIT WAY

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

January 2004

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

KINETIC AND CRYSTALLIZATION BEHAVIOR OF PALM OIL, PALM KERNEL OIL AND THEIR LIPASE-CATALYZED INTERESTERIFIED BLENDS USING VISCOMETRY

By

CHEN CHIT WAY

January 2004

Chairwoman : Associate Professor Lai Oi Ming, Ph.D.

Faculty : Food Science and Biotechnology

The oil crystallization induction time is an important kinetic parameter that provides fundamental information in elucidating their physical and chemical properties. The main criterion for the selection of technique in determining oil induction time is sensitivity in order to apply the data into the Fisher-Turnbull model for estimation of nucleation activation free energy. However, most currently available techniques possessed certain limitations and drawbacks. This study investigates viscometry's potential as a new technique for oil induction time determination. Palm oil, palm kernel oil and their mixtures in different proportions were used as model systems. The catalytic nature, the efficacy and differences in chemical composition changes of the different regiospecific lipases used on the various oil substrates were also looked into. The changes in crystallization behavior of these lipase-catalyzed interesterified oils were studied by means of viscometry in complement with other techniques.

In this study, lipase-catalyzed interesterification of palm oil and palm kernel oil using non-specific *Pseudomonas* sp. and 1,3-specific *Rhizomucor miehei* lipases demonstrated distinct chemical composition changes. Interesterification of palm oil tends to form more medium and long chain triacylglycerols while interesterification of palm kernel oil synthesized medium and short chain triacylglycerols. The two lipases showed high affinity in hydrolyzing palm oil fatty acids, resulting in high amounts of triacylglycerol losses and formation of partial glycerides. For palm kernel oil, the synthesis is enhanced. The hydrolysis preference of the two lipases toward palm oil acylglycerols were postulated to decrease in the order of triacylglycerol, diacylglycerol and monoacylglycerol while the opposite order is true for palm kernel oil.

For the three palm oil – palm kernel oil binary blends (3:1; 1:1 and 1:3; w/w), interesterification caused similar chemical composition changes especially on the synthesis of medium chain triacylglycerols. The lipase catalytic process was influenced by the major fraction in the blends. Among these blends, 1:1 ratio exhibited the highest degree of interesterification in terms of the total triacylglycerols concentration that increased in value while the other two blends indicated similar degrees of increment. The diversity and amount of triacylglycerols available are postulated to be the main reasons for the different catalytic activity in these binary blends. *Pseudomonas* sp. lipase showed higher degree and rate of interesterification compared to *R. miehei* lipase under all conditions. Also, differences in the regiospecificity of the two lipases did not cause different chemical composition changes to all interesterified products. However, the positional specificity and the fatty acids preference of the two lipases had effects on the lipases' degree of interesterification.

Viscometry was an excellent and simple technique in monitoring oil crystallization process where it was able to determine the various stages of crystallization induction time. This unique capability of viscometry has merits in oil crystallization studies: correlating the phenomenon of oil fractionation (various crystallization stages) to polymorphism, polymorphic transformation and metastability of the crystal polymorphs. However, viscometry was relatively insensitive in comparison to laser polarized light anisotropy in detection of the initial nucleation event.

Under isothermal cooling, palm oil and the binary blend at 3:1 ratio exhibited fractionation behavior at lower temperature ranges. A two-stage crystallization process was observed where the initial stearin crystallized in the α form while olein crystallized later in the β′ form. At higher isothermal temperature, no fractionation occurred and the β′ form was the only polymorph observed in a single stage crystallization process. The transition temperature for the two-stage to a single-stage crystallization process varied with different oil compositions, indicating the effect of chemical composition on crystallization behavior. Palm kernel oil, 1:1 and 1:3 blends only crystallized in a single-stage process, in the β′ polymorphic form with different microstructures. The coexistence possibility of α and β′ crystals and the metastability of α crystal in fat crystallization were also discussed.

Interesterification using the two lipases displayed opposing effect towards crystallization behavior and nucleation rate of the two native oils. Lipase-catalyzed interesterification promoted fractionation behavior at higher temperatures and rate in palm oil. This was not observed for palm kernel oil. Crystallization behavior changes of the 3:1 and 1:3 blends confirmed our hypothesis that the two lipases showed high affinity toward the major

fraction in the blend. The trend of crystallization behavior changes in the 1:1 blend indicated that palm oil triacylglycerols were synthesized first before palm kernel oil. The occurrence of sequential synthesis process signified that the lipases used had higher affinity towards palm oil than palm kernel oil fatty acids. Differential scanning calorimeter thermal profiles of the various lipase-catalyzed oils were in agreement with their chemical composition and crystallization behavior changes. These thermal profiles also provided detailed information of the minor component changes that was not revealed in their crystallization behavior.

The applicability of the Fisher-Turnbull model in fat and oil crystallization is discussed in the final part of this study. A review of published literatures indicated that numerous modifications and assumptions had been made to justify the application of this model for fats and oils nucleation. In this study, nucleation data obtained from laser polarized light anisotropy for the various fat systems were used to investigate those proposed modifications and assumptions. The study showed that a single slope constant might not fully represent the complex fat crystallization phenomenon as compared to multiple slope constants. The multiple slope constants established in fats nucleation may be correlated to the nucleation of compound nucleus with different triacylglycerol compositions. However, the present study also showed that application of multiple slope constants resulted in inconsistencies during calculation of the nucleation energies when statistically different induction data from viscometry and laser techniques were used. The results of this study thus indicate that the validity of the various modifications and assumptions made to the Fisher-Turnbull equation need further verification while the application of this model in fats and oils crystallization need further refinement.

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

KINETIK DAN PELAKUAN PEMBEKUAN MINYAK SAWIT, MINYAK ISIRONG SAWIT DAN CAMPURAN LELEMAKNYA SECARA INTERESTERIFIKASI BERMANGKINKAN LIPASE MENGGUNAKAN VISKOMETRI

Oleh

CHEN CHIT WAY

Januari 2004

Pengerusi : Professor Madya Lai Oi Ming, Ph.D.

Fakulti : Sains Makanan dan Bioteknologi

Masa induksi pembekuan minyak merupakan parameter kinetik penting yang membekalkan maklumat asas dalam penentuan ciri-ciri fizikal dan kimia minyak. Kriteria utama dalam pemilihan teknik untuk penentuan masa induksi minyak adalah ciri kepekaan teknik untuk membolehkan data diaplikasikan pada model Fisher-Turnbull dalam penganggaran tenaga pengaktifan bebas pengnuklearan. Bagaimanapun, kebanyakan teknik yang digunakan kini mempunyai had dan kelemahan tertentu. Kajian ini bertujuan menyelidik potensi penggunaan viskometri sebagai satu teknik baru dalam penentuan masa induksi pembekuan minyak. Minyak sawit, minyak isirong sawit dan campuran kedua minyak tersebut dalam pelbagai nisbah digunakan sebagai model pengajian. Sifat pemangkinan, kecekapan pemangkinan dan perubahan komposisi kimia yang disebabkan oleh sifat kespesifikan posisi lipase terhadap pelbagai substrat minyak juga dikaji. Perubahan pelakuan pembekuan untuk minyak yang telah dimangkinkan

secara interesterifikasi berenzim menggunakan viskometri berbanding dengan teknik-teknik lain juga diselidiki.

Dalam pengajian ini, tindakbalas pemangkinan berenzim terhadap minyak sawit dan minyak isirong sawit dengan lipase tidak-spesifik *Pseudomonas* sp. dan 1,3-spesifik *Rhizomucor miehei* menunjukkan perubahan komposisi kimia yang berbeza. Pemangkinan minyak sawit membentuk lebih trigliserida berantai sederhana dan panjang manakala pemangkinan minyak isirong sawit mensintesis trigliserida berantai sederhana dan pendek. Kedua-dua lipase menunjukkan kecenderungan tinggi menghidrolisis asid lemak minyak sawit, menyebabkan kehilangan kandungan trigliserida yang tinggi dan pembentukan peratusan mono- dan digliserida yang tinggi. Untuk minyak isirong sawit, proses sintesis pula menunjukkan peningkatan. Kecenderungan hidrolisis kedua-dua lipase tersebut terhadap gliserida minyak sawit dipostulasi menurun daripada tri-, di- ke monogliserida manakala keadaan sebaliknya dilihat untuk minyak isirong sawit.

Untuk ketiga-tiga campuran lelemak minyak sawit dan isirong sawit (3:1; 1:1 dan 1:3; b/b), pemangkinan berenzim menyebabkan perubahan komposisi kimia yang seakan-akan sama terutamanya pada sintesis trigliserida berantai sederhana. Tindakbalas pemangkinan dipengaruhi oleh fraksi utama campuran lelemak. Di antara campuran lelemak, nisbah 1:1 menunjukkan darjah interesterifikasi yang paling tinggi dari segi jumlah peningkatan kepekatan trigliserida manakala kedua-dua campuran lain menunjukkan darjah peningkatan yang sama. Kepelbagaian dan kandungan trigliserida dipostulasi sebagai faktor utama yang menyebabkan perbezaan kadar pemangkinan di antara campuran lelemak tersebut. Lipase *Pseudomonas* sp. menunjukkan kadar dan darjah interesterifikasi yang lebih tinggi berbanding dengan lipase *R. miehei* dalam

semua keadaan. Ciri kespesifikan lipase terhadap perubahan komposisi kimia tidak ketara. Sebaliknya, sifat kespesifikan posisi dan juga kecenderungan lipase terhadap asid lemak merupakan faktor penentuan kadar pemangkinan.

Penyelidikan ini telah membuktikan bahawa viskometri sesuai dan mudah digunakan dalam pemantauan proses pembekuan minyak di mana ia berupaya menentukan masa induksi pada pelbagai tahap pembekuan. Keupayaan ini boleh menambahkan pemahaman terhadap pelakuan pembekuan minyak: mengkaitkan hubungan fenomena fraksinasi (pelbagai tahap pembekuan) dengan bentuk polimorfik, transformasi polimorfik dan kestabilan kristal dalam pelbagai bentuk polimorfik. Walau bagaimanapun, viskometri didapati kurang peka berbanding dengan teknik anisotropi bercahaya laser terkutub dalam pengesanan pelakuan awal proses pembekuan.

Di bawah penyejukan isoterma, minyak sawit dan lelemak campuran pada nisbah 3:1 menunjukkan pelakuan fraksinasi pada julat suhu yang rendah. Proses pembekuan dua tahap diperhatikan di mana fraksi stearin membeku terlebih dahulu dalam bentuk α manakala olein membeku kemudian sebagai hablur β'. Pada suhu isoterma yang lebih tinggi, pelakuan fraksinasi tidak berlaku dan hablur β' merupakan satu-satunya bentuk hablur yang hadir dalam proses pembekuan satu tahap tersebut. Suhu peralihan daripada proses pembekuan dua tahap ke satu tahap berbeza bergantung pada komposisi minyak dalam campuran dan ini menunjukkan kesan perubahan komposisi kimia terhadap pelakuan pembekuan. Minyak isirong, lelemak campuran pada nisbah 1:1 dan 1:3 pula hanya membeku dalam satu tahap, dalam bentuk β' tetapi dalam pelbagai mikrostruktur. Kemungkinan kewujudan hablur α dan β' serta ciri ketidakstabilan hablur α dalam pembekuan minyak juga dibincangkan.

Pemangkinan dengan kedua-dua lipase menunjukkan kesan berlawanan terhadap pelakuan dan kadar pembekuan kedua-dua minyak asli. Pemangkinan berenzim menggalakkan pelakuan fraksinasi pada minyak sawit pada suhu yang lebih tinggi dan kadar yang lebih cepat tetapi kesannya tidak ketara terhadap minyak isirong. Perubahan pelakuan pembekuan pada lelemak campuran 3:1 dan 1:3 membuktikan hipotesis bahawa lipase menunjukkan affiniti yang tinggi terhadap fraksi utama dalam compuran lelemak. Tren perubahan pelakuan pembekuan pada lelemak campuran nisbah 1:1 menunjukkan bahawa trigliserida minyak sawit disintesis terlebih dahulu sebelum tindakbalas beralih kepada minyak isirong sawit. Proses sintesis berturutan ini juga menunjukkan bahawa lipase mempunyai affiniti yang lebih tinggi terhadap asid lemak minyak sawit berbanding dengan asid lemak minyak isirong. Terma profil permeteran kalori pengimbasan untuk pelbagai minyak pemangkinan berenzim adalah sejajar dengan perubahan komposisi kimia dan pelakuan pembekuan. Terma profil tersebut juga membekalkan maklumat terperinci yang penting dalam pengajian perubahan komponen surih yang tidak dapat dikesan dalam perubahan pelakuan pembekuan.

Penggunaan model Fisher-Turnbull terhadap pembekuan minyak dibincangkan di bahagian terakhir projek ini. Sorotan literatur menunjukkan bahawa pelbagai pengubahsuaian dan andaian perlu dibuat sebelum model ini boleh diaplikasikan dalam sistem nukleasi minyak. Dalam kajian ini, data nukleasi yang diperolehi daripada anisotropi bercahaya laser terkutub untuk pelbagai sistem minyak telah digunakan untuk mengkaji kesesuaian pengubahsuaian dan andaian tersebut. Ia menunjukkan bahawa pemalar kecerunan tunggal mungkin tidak dapat memaparkan keadaan sebenar sifat pembekuan minyak yang kompleks jika dibandingkan dengan pemalar kecerunan gandaan. Pemalar kecerunan gandaan yang diterbitkan daripada nukleasi minyak

mungkin berkaitan dengan nukleus sebatian yang terdiri daripada komposisi trigliserida yang berlainan. Bagaimanapun, keputusan kajian ini juga menunjukkan bahawa penggunaan pemalar kecerunan gandaan boleh menyebabkan perbezaan dalam pengiraan tenaga pengaktifan nukleasi bebas daripada data induksi yang diperolehi daripada viskometri dan teknik laser. Keputusan penyelidikan ini menunjukkan bahawa penggunaan pelbagai pengubahsuaian dan andaian ke atas model Fisher-Turnbull perlu diimbas kembali dan penggunaan model ini dalam pembekuan minyak memerlukan kajian lanjutan.

ACKNOWLEDGEMENTS

I wish to express my heartfelt appreciation and gratitude to my supervisor, Dr Lai Oi Ming for taking me as one of her very first postgraduate student and introduced me to this project. I hope I did not disappoint her. Thanks for her constant guidance, invaluable advice, encouragement and supports whenever sought throughout the course of my studies. My deepest appreciation also extended to Dr Chong Chiew Let of MPOB, a member of my supervisory committee, whom I have learnt a great deal, especially the many aspects of knowledge and technology in the field of fat and oil crystallization. I am indebted to him for giving me the opportunity to work out this project, his guidance and the many hours of stimulating discussions. Special thanks to another member of my committee, Prof. Dr Hasanah Mohd. Ghazali for her advice and comments on the contents of my thesis.

To Dr Tan Chin Ping, sincere appreciation for his technical assistance in statistical analysis. Thanks to Mr Lo Seong Koon for his advice and comments for the lipase works in my thesis. My gratitude also extended to Dr Siew Wai Lin (MPOB) and Mr Lim Wen Huei (AOTC, MPOB) for the use of their polarized microscope. Thanks to Andy for his assistance in translation of the abstract. Thank you also to the entire Physic Lab member who at one time and another, assisted in XRD and DSC analysis in this project. I am also grateful to MPOB that offered me a Graduate Research Assistantship, which funded this project and financially supporting my studies.

In the three years of my studies, I am fortunate to be surrounded by a group of wonderful friends whom I share most of my days with, whom willing to share their care, joys,

frustrations, excitement, wisdom and knowledge, which make this journey more bearable and enjoyable. A million thanks to Ms Chua Hooi Ling, Ms Koh Soo Peng, Ms Thang Yin Mee, Ms Chin Wai Hun and Ms Lee Yim Leng for being around as friends and guiding light all these while.

Last but not least, I wish to express my greatest appreciation and gratitude to my family for their understanding, encouragement and support. To my loving girlfriend, Jessie, thank you for her patience, support, encouragement and unconditional love. Thanks for the assurance that you are always there for me.

TABLE OF CONTENTS

LIST OF FIGURES