UNIVERSITI PUTRA MALAYSIA

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By

MARGARET LIEW YOON BING

Thesis Submitted in Fulfilment of the Requirement for the Degree of Doctor of Philosophy in the Faculty of Food Science and Biotechnology
Universiti Putra Malaysia

June 2001

DEDICATION

Specially Dedicated to.....

My Beloved

Husband and Children

Benedict, Bennilyn, Zachary and Mabel Quek

Parents

Mr. Liew Chee Kan and Mdm. Anna Lai

Brothers and Sisters

Agatha, Catherine, Christina, Johnny and Peter Liew

Together We Soar for Success

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

PRODUCTION OF MYCELIUM-BOUND LIPASE FROM *RHIZOMUCOR MIEHEI* AND ITS APPLICATION IN THE MODIFICATION OF PALM KERNEL OLEIN: ANHYDROUS MILK FAT FOR ICE CREAM EMULSION

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June 2001

Chairman: **Professor Dr. Hasanah Mohd. Ghazali**

Faculty: **Food Science and Biotechnology**

The production conditions and characteristics of mycelium-bound lipase (MBL) from *Rhizomucor miehei*, its catalytic performance in fat transesterification (TE) and the feasibility of using the enzyme to produce a transesterified fat suitable for use in ice cream formulation were investigated. The production of MBL was studied over a 5-day incubation period. Maximum dry mycelium weight and highest lipolytic activity were obtained after 3 days incubation in a medium containing 0.1% (w/v) glucose and 1.0% (w/v) palm olein. Production of MBL was demonstrated to be inducible where the addition of 0.5 – 5.0% (w/v) of palm oil resulted in a significantly ($p \geq 0.05$) higher lipolytic activity than culture without any added oil. High levels (>0.5%) of glucose concentrations were found to inhibit the synthesis of MBL and insufficient lipase was

produced to catalyse the TE reaction of palm stearin (PO_s) and palm kernel olein (PKO_o) (1:1, w/w) mixture at 60°C.

MBL was found to achieve optimum TE activity after 24 h reaction time and its catalytic stability was demonstrated following five repeated usage in TE reaction. Results obtained also illustrated the capability of MBL in transesterifying different oil substrates in a manner similar to that of Lipozyme IM 60 (Novo Nordisk Industries, Copenhagen, Denmark), which is a commercial preparation of immobilised *R. miehei* lipase.

Mixtures of PKO_o:anhydrous milk fat (AMF) at different ratios (100:0, 70:30, 60:40, 50:50 and 0:100, w/w) were transesterified using MBL in a solvent-free system. The triglyceride (TG) profile, slip melting point (SMP), solid fat content (SFC), melting thermogram and the polymorphic forms of the unreacted and transesterified mixtures were evaluated. Results indicated that TE by MBL produced oil mixtures with new TG profiles, relatively lower slip melting points and solid fat contents. The melting thermograms from differential scanning calorimetric analysis indicated an overall shift to lower melting TG. This study also demonstrated that the transesterified PKO_o:AMF mixture at 70:30 (w/w) ratio completely melted at 25°C which meets the melting criteria for fat used in ice cream formulation.

AMF, unmodified PKO_o:AMF mixture (70:30, w/w) (control mixture) and transesterified PKO_o:AMF (70:30, w/w) were used in the preparation of ice cream emulsions. Results obtained show that emulsion prepared using TE-PKO_o:AMF (70:30,

w/w) blend exhibited higher viscosity and greater strength than that of either AMF or unmodified PKO_o:AMF (70:30, w/w), which are amongst the desired properties of ice cream emulsion. This further support that TE of PKO_o:AMF (70:30, w/w) is capable of producing fat that is suitable for use in the preparation of ice cream emulsion.

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

PENGHASILAN LIPASE TERIKAT MISELIA DARI *RHIZOMUCOR MIEHEI* DAN APLIKASINYA DALAM TRANSESTERIFIKASI CAMPURAN LEMAK ISIRONG OLEIN KELAPA SAWIT: LEMAK SUSU UNTUK EMULSI AIS KRIM

Oleh

MARGARET LIEW YOON BING

Jun 2001

Pengerusi: **Profesor Dr. Hasanah Mohd. Ghazali**

Faculti: **Sains Makanan and Bioteknologi**

Keadaan penghasilan dan sifat lipase terikat miselia (MBL) dari *Rhizomucor miehei*, aktiviti transesterifikasi MBL dan keupayaan enzim ini menghasilkan campuran lemak yang dapat digunakan dalam formulasi ais krim adalah dikaji. Penghasilan MBL dikaji selama lima hari pengeraman. Berat maksimum miselia kering dan aktiviti lipolitik maksimum telah dicapai selepas tiga hari pengeraman dalam media yang mempunyai kepekatan glukosa dan minyak kelapa sawit sebanyak 0.1% (b/i) dan 1% (b/i), masing-masing. Penambahan 0.5 – 5.0 % (b/i) minyak menyebabkan aktiviti lipolitik yang lebih tinggi berbanding dengan kultur tanpa-minyak dan ini menunjukkan bahawa penghasilan MBL adalah sintesis-teraruh. Kepekatan glukosa > 0.5% didapati menyebabkan pengurangan aktiviti lipolitik yang signifikan dimana MBL yang dihasilkan tidak dapat

memangkin tindakbalas transesterifikasi diantara campuran lemak stearin kelapa sawit (PO_o) dan lemak isirong olein kelapa sawit (PKO_o) (1:1, b/b) pada 60°C.

MBL didapati mencapai aktiviti transesterifikasi yang optimum selepas 24 jam tindakbalas dan kestabilan aktiviti pemangkinannya dapat ditunjukkan berikutan dengan kemampuan MBL digunakan sebanyak lima kali berulang dalam tindakbalas transesterifikasi. Keputusan juga menunjukkan bahawa MBL boleh memangkin tindakbalas transesterifikasi keatas pelbagai jenis campuran minyak, sepertimana yang dapat dilakukan juga oleh Lipozyme IM 60 (Novo Nordisk Industries, Copenhagen, Denmark), sejenis lipase tersekat–gerak dari *R. miehei* yang diperolehi secara komersil.

Campuran PKO_o dan lemak susu (AMF) pada nisbah 100:0, 70:30, 60:40, 50:50 dan 0:100 (b/b) telah ditransesterifikasi dalam media tanpa-pelarut menggunakan MBL. Profil trigliserida, titik lebur gelincir, kandungan lemak pejal, temogram pencairan dan bentuk polimorfik campuran minyak transesterikasi telah dikaji. Keputusan menunjukkan bahawa tindakbalas transesterifikasi boleh menghasilkan campuran minyak yang mempunyai profil trigliserida yang baru, dan titik lebur gelincir dan kandungan lemak pejal yang lebih rendah. Termogram pencairan dari analisis "differential scanning calorimetry" menunjukkan perubahan menyeluruh trigliserida ke tahap lebur yang lebih rendah. Kajian juga menunjukkan bahawa transesterifikasi campuran PKO_o:AMF (70:30, b/b) dapat menghasilkan campuran lelemak yang cair sepenuhnya pada 25°C, dan ini memenuhi kriteria lemak yang sesuai digunakan dalam formulasi ais krim.

AMF, campuran PKO_o:AMF (70:30, b/b) tanpa-tindakbalas (campuran kawalan) dan campuran PKO_o:AMF (70:30, b/b) yang telah ditransesterifikasi (TE-PKO_o:AMF) digunakan dalam penyediaan emulsi ais krim. Keputusan kajian menunjukkan bahawa emulsi TE-PKO_o:AMF mempamerkan kelikatan dan kekuatan emulsi yang lebih tinggi daripada emulsi lain. Ciri-ciri ini adalah ciri-ciri fizikal yang diingini dalam emulsi ais krim. Ini bermakna tindakbalas transesterifikasi dapat menghasilkan lemak yang sesuai digunakan dalam penghasilan emulsi ais krim.

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