UNIVERSITI PUTRA MALAYSIA

ENZYMATIC TRANSESTERIFICATION OF PALM OLEIN AND COD LIVER OIL BY IMMOBILIZED RHIZOMUCOR MIEHEI AND PSEUDOMONAS CEPACIA LIPASES

CHEW XUI SIM PAULINE

FSMB 2001 38

ENZYMATIC TRANSESTERIFICATION OF PALM OLEIN AND COD LIVER OIL BY IMMOBILIZED *RHIZOMUCOR MIEHEI* AND *PSEUDOMONAS CEPACIA* LIPASES

By

CHEW XUI SIM PAULINE

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

July 2001

Specially Dedicated

To……

Dad (Chew Boon Tai)

Mom (Wong Ah Sep)

Younger Sister (Chew Hui Yee)

Youngest Sister (Chew Lip Yin)

And All My Dear Friends in Christ

For their love and support……...

Abstract of the thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

ENZYMATIC TRANSESTERIFICATION OF PALM OLEIN AND COD LIVER OIL BY IMMOBILIZED *RHIZOMUCOR MIEHEI* AND *PSEUDOMONAS CEPACIA* LIPASES

By

CHEW XUI SIM PAULINE

July 2001

Chairman: Professor Hasanah Mohd. Ghazali Ph.D.

Faculty: Food Science and Biotechnology

Enzymatic transesterification of palm olein (POo) and cod liver oil (CLO) in a solvent free system was studied using immobilized enzymes, with the aim of enriching POo with essential fatty acids/polyunsaturated fatty acids (PUFA) from CLO. The effects of enzyme loading, different ratio of POo and CLO blends, and the enzyme's regiospecificity on the triglyceride (TG) profile and fatty acid composition were evaluated. Reverse-phase high performance liquid chromatography (HPLC) and alkaline titration evaluated the catalytic performance of the lipase in terms of degree of transesterification and hydrolysis, respectively. The fatty acid (FA) composition of glycerides was studied using gas chromatography (GC). The 1,3-specific and non-specific lipases used were *Rhizomucor miehei* lipase (Lipozyme IM60) and *Pseudomonas cepacia* lipase, respectively. Results obtained show that the degree of transesterification and hydrolysis increased with an increase of *R. miehei* lipase concentration. The fatty

acid composition based on relative concentrations of the mixtures before and after reaction showed no significant changes. The substrates were then fractionated into 6 fractions from HPLC runs based on retention time. Analysis of FA composition of these fractions show that PUFA of CLO were found mainly in Fractions 2, 5 and 6. Various possible TG structures found in Fraction 2 onwards were predicted based on the FA types detected by GC and equivalent carbon number (ECN). Different ratio of POo:CLO blends did not significantly change the degree of transesterification, hydrolysis and %TG remaining as well as FA composition of the blends. Incorporation of eicosanoic acid (Ei) in Fraction 5 to form Ei-containing TG was almost double its concentration in the *P. cepacia* lipase-reacted mixture. It was also found that both lipases are able to incorporate docosaenoic acid (Do) to form Do-containing TG in F6. In both cases, the palmitic acid was incorporated into TG that possesses higher ECN. Thermal studies using differential scanning calorimetry (DSC) showed that the increase of OOL, OOO and PPP were found to be correlated with the melting temperature of a newly formed exotherm C in cooling thermogram when the enzyme concentration increased. It was found that *P. cepacia* produced mixtures with higher degree of saturation.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

TRANSESTERIFIKASI CAMPURAN MINYAK OLEIN KELAPA SAWIT DAN MINYAK IKAN KOD DENGAN LIPASE TERSEKAT-GERAK DARIPADA *RHIZOMUCOR MIEHEI* DAN *PSEUDOMONAS CEPACIA*

Oleh

CHEW XUI SIM PAULINE

Julai 2001

Pengerusi : Profesor Hasanah Mohd. Ghazali, Ph.D.

Fakulti : Sains Makanan dan Bioteknologi

Transesterifikasi secara enzimatik ke atas campuran minyak olein kelapa sawit (POo) dan minyak ikan kod (CLO) di dalam sistem tanpa pelarut organik telah dijalankan dengan menggunakan lipase tersekat-gerak supaya POo dapat diperkayakan dengan asid lemak perlu (PUFA). Kesan-kesan kepekatan enzim, pecahan antara POo dan CLO, dan regiospesifisiti lipase terhadap profil trigliserida dan komposisi asid lemak (FA) telah dikaji. Keupayaan pemangkinan enzim ditentukan dengan melihat perubahan kepekatan dan komposisi trigliserida (TG) (tindakbalas transesterifikasi) dengan fasa berbalik kromatografi cecair berprestasi tinggi (HPLC) dan tindakbalas hidrolisis oleh kaedah titratan alkali. Komposisi asid lemak gliserida ditentukan dengan kromatografi gas (GC). Enzim 1,3-spesifik and bukan spesifik yang digunakan adalah lipase daripada *Rhizomucor miehei* and *Pseudomanas cepacia* masing-masing. Tindakbalas transesterifikasi and hidrolisis bertambah apabila kepekatan lipase *R. miehei*

bertambah. Komposisi FA berdasarkan kepekatan relatif dalam campuran sebelum dan selepas tindakbalas menunjukkan tiada perubahan yang signifikan. Substrak-substrak kemudiannya dibahagikan kepada 6 fraksi melalui HPLC berdasarkan masa retensi masing-masing. Analisis daripada komposisi FA menunjukkan bahawa PUFA daripada CLO terdapat di fraksi-fraksi ke-2, 5 dan 6. Pelbagai jenis TG yang mungkin dijumpai daripada fraksi ke-2 dan fraksi seterusnya telah dijangka berdasarkan jenis-jenis FA terjumpa daripada GC and nombor karbon yang bersamaan (ECN). Pecahan antara POo:CLO yang berbeza tidak menunjukkan perubahan signifikan dari segi aktiviti-aktiviti transesterifikasi dan hidrolisis, %TG tertinggal serta komposisi FA. Kepekatan asid eikosanoid dalam bahagian ke-5 hampir berganda dua kali dalam tindakbalas yang dilakukan dengan lipase *P. cepacia*. Adalah didapati bahawa kedua-dua lipase berupaya menambahkan kepekatan asid dokosaenoid (Do) untuk membentuk TG yang mengandungi Do dalam fraksi ke-6. Dalam kedua-dua kes ini, asid palmitik (P) juga dipindahkan kepada TG yang mengandungi ECN yang lebih tinggi. Kajian terma dengan menggunakan 'differential scanning calorimetry' menunjukkan bahawa peningkatan kepekatan OOL, OOO dan PPP mempunyai hubungan dengan suhu peleburan satu eksoterma C yang baru terbentuk dalam profil penyejukan apabila kepekatan enzim bertambah. Adalah didapati *P. cepacia* berupaya menghasilkan produk yang mempunyai darjah ketepuan yang lebih tinggi.

ACKNOWLEDGEMENTS

I wish to express my sincere gratitude and thanks to my supervisor, Professor Dr. Hasanah Mohd. Ghazali for her guidance, suggestions and encouragement to make this project a success.

I also would like to express my heartfelt appreciation and thanks to Dr. Lai Oi Ming, one of my committee members who has encouraged me in many ways with endless patience and advice. My gratitude also go to Prof. Dr. Yaakob B. Che Man and Dr. Kamariah Long. Their support, comments and encouragement have helped me to persevere on this project.

Special thanks and appreciation to all the laboratory staffs and technicians, especially Mr. Tan and Mr. Chan who have been helping me on HPLC and GC. Also, to all my fellow graduates and undergraduates, I thank them for their concern and moral support. Great appreciation to my housemates and church members too who have been praying for me and support me in many ways, especially Ms. Fong, Ms. Phang and Ms. Lee. Deepest appreciation and gratitude are also expressed to my family for their endless understanding, care, support and love throughout my studies in UPM.

Last but not least, I thank God for his mercy and grace to guide me through this master project with many lessons learned.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS

A	C20; Arachidic acid
CLO	Cod liver oil
CN	Carbon number
CPO	Crude palm oil
D or DHA	C22:6; Docosahexaenoic acid
DDD	Tridocosahexaenoylglycerol
DDO	Didocosahexaenoyloleoylglycerol
DG	Diglyceride
Do or U3	C22:1; docosaenoic acid
DOO	Dioleindocosahexaenoylglycerol
DPA	Docosapentaenoic acid
DSC	Differential Scanning Calorimetry
Ei or U1	C20:1; eicosaenoic acid
Ep or EPA	C20:5; Eicosapentaenoic acid
ECN	Equivalent carbon number
EEE	Trieicosapentaenoylglycerol
EEP	Dieicosapentaenoylpalmitoylglycerol
FA	Fatty acid
FAEE	Fatty acid ethyl ether
FAME	Fatty acid methyl ether
FFA	Free fatty acid
FO	Fish oil

GC	Gas chromatography
HOSO	High-oleic sunflower oil
HPLC	High performance liquid chromatography
HUFA	Highly unsaturated fatty acid
IFOMA	International Association of Fish Meal and Fish Oil Manufacturers
IV	Iodine value
L	C18:2; Linoleic acid
La	C12; Lauric acid
M	C14; Myristic acid
MG	Monoglyceride
MUFA	Monounsaturated fatty acid
O	C18:1; Oleic acid
OLL	Oleoyl-dilinolein
OOL	Linoleoyl-diolein
OOO	Triolein
P	C16; Palmitic acid
P1 or U2	C16:1; palmitoleic acid
PO	Palm oil
POo	Palm olein
PLP	Linoleoyl-dipalmitin
POL	Palmitoyl-oleoyl-linolein
POO	Palmitoyl-diolein

POP	Oleoyl-dipalmitin
PORLA	Palm Oil Registration and Licensing Authority
PORIM	Palm Oil Research Institute Malaysia
PPP	Tripalmitin
PUFA	Polyunsaturated fatty acid
RBD	Refined, bleached and deodorized
S	C18; Stearic acid
SAT	Saturated
SFA	Saturated fatty acid
SOO	Stearoyl-diolein
SOS	Oleoyl-distearin
TLC	Thin layer chromatography
TG	Triglyceride
UFA	Unsaturated fatty acid
ω	Omega

CHAPTER I

INTRODUCTION

Refined, bleached and derodorized (RBD) palm olein (POo) is the largest traded oil in the world market (PORLA, 2000a). It possesses high resistance to oxidation (due to low unsaturation and presence of tocopherol, a natural antioxidant) and gumming, has low level of free fatty acid (FFA) and smoking point, low rate of foaming, darkening and melting point, has no *trans* or iso-acid, and no unpleasant room odor due to the absence of linolenic acid (Pantzaris, 1987). In view of its superior physical characteristics and stability, POo is very popular in commercial and industrial establishments where deep-frying is a norm.

POo contains 46% saturated FA (myristic, palmitic and stearic acids), 43% monounsaturated FA (oleic, *n-9*) and 11% polyunsaturated fatty acid (PUFA) (linoleic, *n-6*) (Gunstone, 1986; Siew *et al.*, 1992, 1993). Similar to other vegetable oils, POo lacks in *n-3* PUFA that is present in marine oils. Marine oils, such as fish oil (FO) and fish liver oil are rich sources of *n-3* PUFA (Best, 1987). This makes them unique dietary fats since most common animal and vegetable fats are virtually devoid of *n-3* PUFA especially the eicosapentaenoic acid (EPA) and docasahexaenoic acid (DHA). Even though EPA and DHA can be synthesized by elongation and desaturation of linolenic acid, ingestion of the pre-formed molecules usually is more effective, especially