



UNIVERSITI PUTRA MALAYSIA

**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**

MARGARET LIEW YOON BING

FSMB 2001 9

**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**

MARGARET LIEW YOON BING

**DOCTOR OF PHILOSOPHY
UNIVERSITI PUTRA MALAYSIA
2001**



**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**

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

By

MARGARET LIEW YOON BING

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 PKO₀:AMF (70:30, w/w), which are amongst the desired properties of ice cream emulsion. This further support that TE of PKO₀: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 transesterifikasi 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 lemak 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 diinginkan dalam emulsi ais krim. Ini bermakna tindakbalas transesterifikasi dapat menghasilkan lemak yang sesuai digunakan dalam penghasilan emulsi ais krim.



ACKNOWLEDGEMENTS

I wish to express my sincere gratitude to my chief supervisor, Prof. Dr. Hasanah Mohd. Ghazali of the Department of Biotechnology, Faculty of Food Science and Biotechnology for her invaluable guidance, support and constructive suggestions throughout the course of my graduate study and for her editorial assistance during the preparation of the dissertation.

My grateful thanks also goes to my co-supervisors, Dr. Lai Oi Ming, Dr. Mohd. Yazid and Dr. Kamariah Long for their support, advise and comments throughout this study. A special thanks is also due to Dr. Lai Oi Ming for her editorial assistance at various stages of the preparation of my dissertation. Many thanks are due also to my co-supervisors from MPOB, Dr. Chow Mee Chin and Dr. Mohd. Suria Affandi Yusoff for their guidance and technical assistance in product processing and rheology work.

I also wish to thank staffs and research assistants of the Department of Biotechnology and Department of Food Science for their kind cooperation and technical support during the course of my study. A special thanks goes to Mr. Khalid G. Fandi from the Enzyme Lab for his assistance and support. Appreciation and acknowledgement also due to Prof. Yaakob Che Man and Tan Chin Ping from the Fats and Oils Lab and the technical staffs from the Physics, Ice Cream Processing and Effluent Labs in MPOB for their assistance and support in performing some of the analysis for my research projects.



Acknowledgement is also due to the Government of Malaysia for the PASCA scheme scholarship granted to me to pursue my PhD Degree.

Heartfelt appreciation also due to my dearest husband, Benedict Quek, my children, Bennilyn, Zachary and Mabel Quek, my parents, brothers and sisters for their enormous amount of love, moral and financial support, encouragement, patience and sacrifice, which have kept me motivated and made possible the completion of my research and dissertation.



I certify that an Examination Committee met on 19th June 2001 to conduct the final examination of Margaret Liew Yoon Bing on her Doctor of Philosophy thesis entitled “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” in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulation 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follow:

Yaakob Che Man, Ph.D.
Professor
Deputy Dean
Faculty of Food Science and Biotechnology
Universiti Putra Malaysia
(Chairman)

Hasanah Mohd. Ghazali, Ph.D.
Faculty of Food Science and Biotechnology
Universiti Putra Malaysia
(Member)

Mohd. Yazid Abdul Manap, Ph.D.
Faculty of Food Science and Biotechnology
Universiti Putra Malaysia
(Member)


Lai Oi Ming, Ph.D.
Faculty of Food Science and Biotechnology
Universiti Putra Malaysia
(Member)

Kamariah Long, Ph.D.
Malaysian Agricultural Research and Development Institute
(Member)

Chow Mee Chin, Ph.D.
Malaysian Palm Oil Board
(Member)

Mohd Suria Affandi Yusoff, Ph.D.
Malaysian Palm Oil Board
(Member)

Fereidon Shahidi, Ph.D.
Memorial University of Newfoundland
Department of Biochemistry
Canada
(Independent Examiner)



MOHD. GHAZALI MOHAYIDIN, Ph.D.,
Professor/Deputy Dean of Graduate School,
Universiti Putra Malaysia
Date: 22 JUN 2001

This thesis submitted to the Senate of Universiti Putra Malaysia has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy.



AINI IDERIS, Ph.D,
Professor,
Dean of Graduate School,
Universiti Putra Malaysia

Date:

DECLARATION

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



Name: Margaret Liew Yoon Bing
Date: 22nd June 2001

TABLE OF CONTENTS

		Page
	DEDICATION.....	ii
	ABSTRACT.....	iii
	ABSTRAK.....	v
	ACKNOWLEDGEMENTS.....	ix
	APPROVAL SHEETS	xi
	DECLARATION	xiii
	LIST OF TABLES.....	xvii
	LIST OF FIGURES.....	xix
	LIST OF ABBREVIATIONS	xxv
CHAPTER		
1	INTRODUCTION.....	1
2	LITERATURE REVIEW.....	6
	Lipases.....	6
	Definition.....	6
	Lipase Specificity.....	7
	Properties of Lipases.....	10
	Microbial Lipases.....	12
	Mycelium-Bound Lipases.....	14
	Production of Microbial Lipases.....	16
	Lipase from <i>Rhizomucor miehei</i>	19
	Fat Modification Process.....	23
	Interesterification.....	24
	Enzymatic Interesterification.....	25
	Properties of Interesterified Fats and Oils.....	27
	Ice Cream and Its Properties.....	33
	Ice Cream.....	33
	Milk Fat.....	36
	Interesterification of Milk Fat.....	41
	Rheology of Oil-in-Water Emulsion.....	43
	Rheological Measurement.....	46
3	PRODUCTION AND TRANSESTERIFICATION ACTIVITY OF MYCELIUM-BOUND LIPASE FROM <i>RHIZOMUCOR</i> <i>MIEHEI</i>	51
	Introduction.....	51
	Materials and Methods.....	53
	Materials.....	53
	Preparation of Spore Suspension.....	53
	Preparation of Mycelium-Bound <i>R. miehei</i> Lipase.....	54



	Mycelium-Bound Lipase (MBL) and Extracellular Lipase Activity.....	55
	Transesterification Reaction.....	56
	Results and Discussion.....	58
	Effect of Incubation Period.....	58
	Effect of Glucose and Oil Concentration.....	60
	Transesterification Activity of MBL.....	64
4	FACTORS AFFECTING THE CATALYTIC ACTIVITY OF MYCELIIUM-BOUND LIPASE FROM <i>RHIZOMUCOR MIEHEI</i> .	72
	Introduction.....	72
	Materials and Methods.....	73
	Materials.....	73
	Preparation of Mycelium-Bound <i>R. miehei</i> Lipase.....	74
	Transesterification Reaction.....	74
	Determination of Hydrolytic Activity.....	75
	Results and Discussion.....	76
	Effect of Reaction Time on Transesterification by MBL.....	76
	Effect of Repeated Usage of MBL on Transesterification....	78
	Effect of Substrates on Transesterification by MBL.....	81
5	PHYSICAL PROPERTIES OF PALM KERNEL OLEIN-ANHYDROUS MILK FAT MIXTURES TRANSESTERIFIED USING MYCELIIUM-BOUND LIPASE FROM <i>RHIZOMUCOR MIEHEI</i> .	91
	Introduction.....	91
	Materials and Methods.....	93
	Materials.....	93
	Preparation of Mycelium-Bound <i>R. miehei</i> Lipase.....	93
	Transesterification	93
	Hydrolytic Activity.....	94
	Slip Melting Point (SMP).....	94
	Solid Fat Content (SFC).....	95
	Thermal Properties by Differential Scanning Calorimetry (DSC) Analysis.....	95
	Results and Discussion.....	96
	Effect of Transesterification on Triglyceride Profiles.....	96
	Effect of Transesterification on Slip Melting Point and Solid Fat Content.....	103
	Thermal Analysis of Transesterified PKO:AMF (70:30).....	108
	Effect of Transesterification on Polymorphic Form.....	115



6	RHEOLOGICAL PROPERTIES OF ICE CREAM EMULSION PREPARED FROM LIPASE-CATALYSED TRANSESTERI- FIED PALM KERNEL OLEIN:ANHYDROUS MILK FAT MIXTURE.....	117
	Introduction.....	117
	Materials and Methods.....	121
	Materials	121
	Production of Oil Blends.....	121
	Ice Cream Formulation.....	122
	Ice Cream Processing.....	123
	Rheological Measurement of Ice Cream Emulsion.....	124
	Results and Discussion	125
	Linear Viscoelastic Region of Samples.....	125
	Effect of Fat Blends on Viscosity and Complex Viscosity...	127
	Effect of Fat Blends on Viscoelasticity and Frequency Dependence.....	135
	Effect of Fat Blends on Emulsion Strength.....	137
	Effect of Aging on Viscoelastic Properties.....	141
	Effect of Aging on Creep Compliance.....	149
7	SUMMARY, CONCLUSIONS AND RECOMMENDATIONS.....	153
	Summary.....	153
	Conclusions and Recommendations.....	156
	BIBLIOGRAPHY.....	157
	APPENDIX A.....	184
	BIOGRAPHICAL SKETCH.....	185



LIST OF TABLES

Table		Page
1	Composition of the Dairy Ice Cream, Percent Weight Basis.....	33
2	Main Lipid Classes and Their Percentages in Milk Fat.....	37
3	The International Dairy Federation (IDF) Specification for Anhydrous Milk Fat.....	41
4	%TGR and Degree of Transesterification (%TE) of Transesterified Palm Stearin-Palm Kernel Olein (1:1) Mixtures Using MBL Produced from Different Culture Conditions.....	65
5	Degree of Transesterification (%TE), % TG Remaining and Amount of FFA Liberated (μmol) after TE Reaction Catalysed with Mycelium-Bound Lipase (MBL) from <i>R. miehei</i> and Lipozyme IM 60 Using Different Oil Substrates.....	82
6	Amount of FFA (in μmol) Liberated and Degree of TE (%) of Transesterified Palm Kernel Olein:Anhydrous Milk Fat (PKO _o :AMF) Blends Using Mycelium-Bound Lipase (MBL) from <i>R. miehei</i> and Lipozyme IM60.....	97
7	Slip Melting Points of Palm Kernel Olein:Anhydrous Milk Fat (PKO _o :AMF) Blends Before (control) and After TE with Mycelium-Bound Lipase (MBL) from <i>R. miehei</i> and Lipozyme IM60.....	107
8	Polymorphic Forms of PKO _o :AMF Blends Before (control) and After Transesterification with Mycelium-Bound Lipase from <i>R. miehei</i> (MBL) and Lipozyme IM60.....	116
9	Ice Cream Formulation.....	123



10	Solid Fat Content (SFC) of Fat Mixtures.....	134
11	Tan Delta ($\tan \delta$) Value for the Respective Ice Cream Mix Obtained from Frequency Sweep.....	149



LIST OF FIGURES

Figure		Page
1	Various Types of Creep-Recovery Behaviours in Response to the Applied Shear Stress (σ). (a) Response of a Perfectly Elastic Solid, (b) Response of a Perfectly Viscous Liquid, (c) Response of a Viscoelastic Solid, and (d) Response of a Viscoelastic Liquid.....	48
2	Effect of Incubation Period on MBL Lipolytic Activity, Relative Amount of Extracellular Lipase and Dry Mycelium Weight of the MBL Produced in Growth Medium Containing 0.1% Glucose and 1% Oil Concentrations.....	59
3	Effect of Glucose Concentration on the Lipolytic Activity of the MBL Produced with Liquid Culture Medium Containing 1% Added Oil.....	62
4	Effect of Oil Concentration on the Lipolytic Activity of the MBL Produced when Glucose Concentration of 0.1% was Used.....	63
5	TG Profiles of Palm Stearin-Palm Kernel Olein (1:1) Mixture Before (a) and After TE Using MBL from 2-Day Incubation (b), 3-Day Incubation (c), 4-Day Incubation (d) and 5-Day Incubation (e). TGs Represented by Arrows Indicate Increases in TG Concentrations as Compared to the Respective Peaks in the Unreacted Mixture. TE Reaction was Carried Out at 60°C at 200 rpm for 24 h Using MBL Produced in Medium Containing 0.1% Glucose and 1% Oil Concentrations.....	67
6	TG Profiles of Palm Stearin-Palm Kernel Olein (1:1) Mixture Before (a) and After TE Using MBL Cultured in Medium Containing 0.5% Oil (b), 1% Oil (c), 2% Oil (d) and 5% Oil (e). TGs Represented by Arrows Indicate Increases in TG Concentrations as Compared to the Respective Peaks in the Unreacted Mixture. TE Reaction was Carried Out at 60°C at 200 rpm for 24 h.....	68



7	TG Profiles of Palm Stearin-Palm Kernel Olein (1:1) Mixture Before (a) and After TE Using MBL Cultured in Medium Containing 1% Oil with Addition of 0.1% Glucose (b), 0.25% Glucose (c), 0.5% Glucose (d), 0.75% Glucose (e) and 1% Glucose (f). TGs Represented by Arrows Indicate Increases in TG Concentrations as Compared to the Respective Peaks in the Unreacted Mixture. TE Reaction was Carried Out at 60°C at 200 rpm for 24 h.....	69
8	Effect of Reaction Time on the %TE and %TGR after TE of PKO _o :PO _s (1:1) Using Mycelium-Bound Lipase (MBL) from <i>R. miehei</i>	77
9	Effect of Reaction Time on the %TE and %TGR After TE of PO _o Using Mycelium-Bound Lipase (MBL) from <i>R. miehei</i>	77
10	Effect of Repeated Usage of MBL from <i>R. miehei</i> on %TE and %TGR After TE of PKO _o :PO _s (1:1).....	79
11	Catalytic Stability of MBL from <i>R. miehei</i> in Repeated Batch TE of a Mixture Containing PKO _o :PO _s (1:1). The TG Profile of the Unreacted Mixture (a), TG Profile of Mixture Transesterified Using Fresh MBL (b), Second Run of MBL (c), Third Run of MBL (d), Forth Run of MBL (e), and Fifth Run of MBL (f).....	80
12a	TG Profiles of Palm Kernel Olein:Sunflower Oil (PKO _o :SFO) Mixture at 1:1 Ratio Before (i) and After Transesterified with MBL (ii) and Lipozyme (iii). TGs Represented by Arrows Indicates Increase in TG concentrations.....	84
12b	TG Profiles of Palm Kernel Olein:Canola Oil (PKO _o :CNO) Mixture at 1:1 Ratio Before (i) and After Transesterified with MBL (ii) and Lipozyme (iii). TGs Represented by Arrows Indicate Increases in TG concentrations.....	85
12c	TG Profiles of Palm Kernel Olein:Groundnut Oil (PKO _o :GNO) Mixture at 1:1 Ratio Before (i) and After Transesterified with MBL (ii) and Lipozyme (iii). TGs Represented by Arrows Indicate Increases in TG Concentrations.....	86



12d	TG Profiles of Palm Kernel Olein:Anhydrous Milk Fat (PKO _o :AMF) Mixture at 1:1 Ratio Before (i) and After Transesterified with MBL (ii) and Lipozyme (iii). TGs Represented by Arrows Indicate Increases in TG Concentrations.....	87
12e	TG Profiles of Palm Kernel Olein:Palm Stearin (PKO _o :PO _s) Mixture at 1:1 Ratio Before (i) and After Transesterified with MBL (ii) and Lipozyme (iii). TGs Represented by Arrows Indicate Increases in TG Concentrations.....	88
12f	TG Profiles of Palm Kernel Olein:Corn Oil (PKO _o :CO) Mixture at 1:1 Ratio Before (i) and After Transesterified with MBL (ii) and Lipozyme (iii). TGs Represented by Arrows Indicate Increases in TG Concentrations.....	89
13a	TG Profiles of Palm Kernel Olein:Anhydrous Milk Fat (PKO _o :AMF) Mixture at 100:0 Ratio Before (i) and After TE with MBL (ii) and Lipozyme IM60 (iii). TGs Represented by arrows Indicate Increases in TG Concentrations.....	98
13b	TG Profiles of Palm Kernel Olein:Anhydrous Milk Fat (PKO _o :AMF) Mixture at 70:30 Ratio Before (i) and After TE with MBL (ii) and Lipozyme IM60 (iii). TGs Represented by arrows Indicate Increases in TG Concentrations.....	99
13c	TG Profiles of Palm Kernel Olein:Anhydrous Milk Fat (PKO _o :AMF) Mixture at 60:40 Ratio Before (i) and After TE with MBL (ii) and Lipozyme IM60 (iii). TGs Represented by arrows Indicate Increases in TG Concentrations.....	100
13d	TG Profiles of Palm Kernel Olein:Anhydrous Milk Fat (PKO _o :AMF) Mixture at 50:50 Ratio Before (i) and After TE with MBL (ii) and Lipozyme IM60 (iii). TGs Represented by arrows Indicate Increases in TG Concentrations.....	101
13e	TG Profiles of Palm Kernel Olein:Anhydrous Milk Fat (PKO _o :AMF) Mixture at 0:100 Ratio Before (i) and After TE with MBL (ii) and Lipozyme IM60 (iii). TGs Represented by arrows Indicate Increases in TG Concentrations.....	102



14a	Solid Fat Content (SFC) of Palm Kernel Olein:Anhydrous Milk Fat (PKO _o :AMF) Mixture at 100:0 Ratio Before (Control) and After TE with MBL and Lipozyme IM60.....	104
14b	Solid Fat Content (SFC) of Palm Kernel Olein:Anhydrous Milk Fat (PKO _o :AMF) Mixture at 70:30 Ratio Before (Control) and After TE with MBL and Lipozyme IM60.....	105
14c	Solid Fat Content (SFC) of Palm Kernel Olein:Anhydrous Milk Fat (PKO _o :AMF) Mixture at 60:40 Ratio Before (Control) and After TE with MBL and Lipozyme IM60.....	105
14d	Solid Fat Content (SFC) of Palm Kernel Olein:Anhydrous Milk Fat (PKO _o :AMF) Mixture at 50:50 Ratio Before (Control) and After TE with MBL and Lipozyme IM60.....	106
14e	Solid Fat Content (SFC) of Palm Kernel Olein:Anhydrous Milk Fat (PKO _o :AMF) Mixture at 0:100 Ratio Before (Control) and After TE with MBL and Lipozyme IM60.....	106
15a	Differential Scanning Calorimetry (DSC) Heating Thermograms of Palm Kernel Olein Before (i) and After TE with MBL (ii) and Lipozyme IM60 (iii). Pretreatment: Cooled from 70°C to -30°C at 10°C/min. Samples were Heated from -30°C to 70°C at 5°C/min.....	109
15b	Differential Scanning Calorimetry (DSC) Heating Thermograms of Palm Kernel Olein:Anhydrous Milk Fat (PKO _o :AMF) Mixture at 70:30 Before (i) and After TE with MBL (ii) and Lipozyme IM60 (iii). Pretreatment: Cooled from 70°C to -30°C at 10°C/min. Samples were Heated from -30°C to 70°C at 5°C/min.....	110
15c	Differential Scanning Calorimetry (DSC) Heating Thermograms of Palm Kernel Olein:Anhydrous Milk Fat (PKO _o :AMF) Mixture at 60:40 Before (i) and After TE with MBL (ii) and Lipozyme IM60 (iii). Pretreatment: Cooled from 70°C to -30°C at 10°C/min. Samples were Heated from -30°C to 70°C at 5°C/min.....	111



15d	Differential Scanning Calorimetry (DSC) Heating Thermograms of Palm Kernel Olein:Anhydrous Milk Fat (PKO _o :AMF) Mixture at 50:50 Before (i) and After TE with MBL (ii) and Lipozyme IM60 (iii). Pretreatment: Cooled from 70°C to -30°C at 10°C/min. Samples were Heated from -30°C to 70°C at 5°C/min.....	112
15e	Differential Scanning Calorimetry (DSC) Heating Thermograms of Anhydrous Milk Fat Before (i) and After TE with MBL (ii) and Lipozyme IM60 (iii). Pretreatment: Cooled from 70°C to -30°C at 10°C/min. Samples were Heated from -30°C to 70°C at 5°C/min.....	113
16	A Typical Stress Sweep Profile Indicating the Linear VE Region of Ice Cream Emulsion.....	126
17	Log Viscosity (η) versus Log Shear Rate of Ice Cream Emulsions Prepared with the Respective Fat Sources.....	128
18	Complex Viscosity (η^*) versus Frequency of Ice Cream Emulsion Prepared with the Respective Fat Sources.....	130
19	Loss Modulus (G'') and Storage Modulus (G') versus Frequency of Ice Cream Emulsion Prepared with the Respective Fat Sources..	131
20	Creep Compliance of Ice Cream Emulsion Prepared with the Respective Fat Sources.....	138
21	Three Deformation Mechanisms Identified on the Creep Side of the Creep and Recovery Analysis. J_0 = Instantaneous Compliance, J_e = Retarded Elastic Mechanism and t/η = Newtonian Flow (Gladwell <i>et al.</i> , 1985a).....	139
22a	Storage Modulus (G') and Loss Modulus (G'') versus Frequency of Ice Cream Emulsion Prepared with Anhydrous Milk Fat (AMF) Aged for 2 h, 4 h and 6 h, Respectively.....	143

