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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DOCTOR OF PHILOSOPHY
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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
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≤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 (POs) and palm kernel olein (PKOo) (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 PKOo: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 PKOo: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 PKOo:AMF mixture (70:30, w/w) (control mixture) and transesterified PKOo:AMF (70:30, w/w) were used in the preparation of ice cream emulsions. Results obtained show that emulsion prepared using TE-PKOo:AMF (70:30,
w/w) blend exhibited higher viscosity and greater strength than that of either AMF or unmodified PKO_{6}:AMF (70:30, w/w), which are amongst the desired properties of ice cream emulsion. This further support that TE of PKO_{6}: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 (POs) dan lemak isirong olein kelapa sawit (PKOs) (1:1, b/b) pada 60°C.

MBL didapati mencapai aktiviti transesterifikasi yang optimum selepas 24 jam tindakbalas dan kestabilan aktiviti pemangkinannya dapat ditunjukkan berikut 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, sepetimana 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 PKOs 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 polimorifik 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 PKOs: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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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.

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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:

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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

DEDICATION........................................................................................................ ii
ABSTRACT.......................................................................................................... iii
ABSTRAK.......................................................................................................... iii
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 Rhizomucor miehei...................................................... 19
      Fat Modification Process................................................................... 23
         Interesterification........................................................................... 24
         Enzymatic Interestereification...................................................... 25
         Properties of Interestereified Fats and Oils................................ 27
      Ice Cream and Its Properties.......................................................... 33
         Ice Cream...................................................................................... 33
         Milk Fat............................................................................................ 36
         Interestereification of Milk Fat..................................................... 41
         Rheology of Oil-in-Water Emulsion............................................. 43
         Rheological Measurement............................................................. 46

3 PRODUCTION AND TRANSESTERIFICATION ACTIVITY
   OF MYCELIUM-BOUND LIPASE FROM RHIZOMUCOR MIEHEI............. 51
   Introduction.............................................................................................. 51
      Materials and Methods..................................................................... 53
      Materials............................................................................................. 53
      Preparation of Spore Suspension.................................................... 53
      Preparation of Mycelium-Bound R. miehei Lipase.......................... 54

xiv
### 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

---

#### FACTORS AFFECTING THE CATALYTIC ACTIVITY OF MYCELIUM-BOUND LIPASE FROM RHIZOMUCOR MIEHEI

Introduction

- 72

Materials and Methods

- 73

#### Materials

- 73

#### Preparation of Mycelium-Bound R. miehei 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

---

#### PHYSICAL PROPERTIES OF PALM KERNEL OLEIN-ANHYDROUS MILK FAT MIXTURES TRANSESTERIFIED USING MYCELIUM-BOUND LIPASE FROM RHIZOMUCOR MIEHEI

Introduction

- 91

Materials and Methods

- 93

#### Materials

- 93

#### Preparation of Mycelium-Bound R. miehei 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

---

xv
THEORETICAL PROPERTIES OF ICE CREAM EMULSION PREPARED FROM LIPASE-CATALYSED TRANSESTERIFIED PALM KERNEL OLEIN: ANHYDROUS MILK FAT MIXTURE

Introduction
Materials and Methods
Materials
Production of Oil Blends
Ice Cream Formulation
Ice Cream Processing
Rheological Measurement of Ice Cream Emulsion
Results and Discussion
Linear Viscoelastic Region of Samples
Effect of Fat Blends on Viscosity and Complex Viscosity
Effect of Fat Blends on Viscoelasticity and Frequency Dependence
Effect of Fat Blends on Emulsion Strength
Effect of Aging on Viscoelastic Properties
Effect of Aging on Creep Compliance

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS
Summary
Conclusions and Recommendations

BIBLIOGRAPHY
APPENDIX A
BIOGRAPHICAL SKETCH
<table>
<thead>
<tr>
<th>Table</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Composition of the Dairy Ice Cream, Percent Weight Basis</td>
</tr>
<tr>
<td>2</td>
<td>Main Lipid Classes and Their Percentages in Milk Fat</td>
</tr>
<tr>
<td>3</td>
<td>The International Dairy Federation (IDF) Specification for Anhydrous Milk Fat</td>
</tr>
<tr>
<td>4</td>
<td>%TGR and Degree of Transesterification (%TE) of Transesterified Palm Stearin-Palm Kernel Olein (1:1) Mixtures Using MBL Produced from Different Culture Conditions</td>
</tr>
<tr>
<td>5</td>
<td>Degree of Transesterification (%TE), % TG Remaining and Amount of FFA Liberated (μmol) after TE Reaction Catalysed with Mycelium-Bound Lipase (MBL) from R. miehei and Lipozyme IM 60 Using Different Oil Substrates</td>
</tr>
<tr>
<td>6</td>
<td>Amount of FFA (in μmol) Liberated and Degree of TE (%) of Transesterified Palm Kernel Olein:Anhydrous Milk Fat (PKO₆: AMF) Blends Using Mycelium-Bound Lipase (MBL) from R. miehei and Lipozyme IM60</td>
</tr>
<tr>
<td>7</td>
<td>Slip Melting Points of Palm Kernel Olein:Anhydrous Milk Fat (PKO₆:AMF) Blends Before (control) and After TE with Mycelium-Bound Lipase (MBL) from R. miehei and Lipozyme IM60</td>
</tr>
<tr>
<td>8</td>
<td>Polymorphic Forms of PKO₆:AMF Blends Before (control) and After Transesterification with Mycelium-Bound Lipase from R. miehei (MBL) and Lipozyme IM60</td>
</tr>
<tr>
<td>9</td>
<td>Ice Cream Formulation</td>
</tr>
</tbody>
</table>
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Figure Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Various Types of Creep-Recovery Behaviours in Response to the Applied Shear Stress ($\sigma$). (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.</td>
<td>48</td>
</tr>
<tr>
<td>2</td>
<td>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.</td>
<td>59</td>
</tr>
<tr>
<td>3</td>
<td>Effect of Glucose Concentration on the Lipolytic Activity of the MBL Produced with Liquid Culture Medium Containing 1% Added Oil.</td>
<td>62</td>
</tr>
<tr>
<td>4</td>
<td>Effect of Oil Concentration on the Lipolytic Activity of the MBL Produced when Glucose Concentration of 0.1% was Used.</td>
<td>63</td>
</tr>
<tr>
<td>5</td>
<td>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.</td>
<td>67</td>
</tr>
<tr>
<td>6</td>
<td>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.</td>
<td>68</td>
</tr>
</tbody>
</table>
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.

Effect of Reaction Time on the %TE and %TGR after TE of PKO₆:PO₆ (1:1) Using Mycelium-Bound Lipase (MBL) from R. miehei.

Effect of Reaction Time on the %TE and %TGR After TE of PO₆ Using Mycelium-Bound Lipase (MBL) from R. miehei.

Effect of Repeated Usage of MBL from R. miehei on %TE and %TGR After TE of PKO₆:PO₆ (1:1).

Catalytic Stability of MBL from R. miehei in Repeated Batch TE of a Mixture Containing PKO₆:PO₆ (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).

TG Profiles of Palm Kernel Olein: Sunflower Oil (PKO₆: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.

TG Profiles of Palm Kernel Olein: Canola Oil (PKO₆: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.

TG Profiles of Palm Kernel Olein: Groundnut Oil (PKO₆: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.
12d TG Profiles of Palm Kernel Olein: Anhydrous Milk Fat (PKO<sub>a</sub>: 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<sub>a</sub>:PSt) 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<sub>a</sub>: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<sub>a</sub>: AMF) Mixture at 100:0 Ratio Before (i) and After Transesterified 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<sub>a</sub>: AMF) Mixture at 70:30 Ratio Before (i) and After Transesterified 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<sub>a</sub>: AMF) Mixture at 60:40 Ratio Before (i) and After Transesterified 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<sub>a</sub>: AMF) Mixture at 50:50 Ratio Before (i) and After Transesterified 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<sub>a</sub>: AMF) Mixture at 0:100 Ratio Before (i) and After Transesterified 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<sub>6</sub>: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<sub>6</sub>: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<sub>6</sub>: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<sub>6</sub>: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<sub>6</sub>: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<sub>6</sub>: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<sub>6</sub>: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
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.

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.

A Typical Stress Sweep Profile Indicating the Linear VE Region of Ice Cream Emulsion.

Log Viscosity (η) versus Log Shear Rate of Ice Cream Emulsions Prepared with the Respective Fat Sources.

Complex Viscosity (η*) versus Frequency of Ice Cream Emulsion Prepared with the Respective Fat Sources.

Loss Modulus (G'') and Storage Modulus (G') versus Frequency of Ice Cream Emulsion Prepared with the Respective Fat Sources.

Creep Compliance of Ice Cream Emulsion Prepared with the Respective Fat Sources.


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.