

## ABSTRACT

The main objective of present study is to establish the relationship between positional fatty acids in triacylglycerols and obesity. The inferences drawn from the *in vivo* studies have been used in the design of prospective structured lipids to reduce obesity risk. In the effort to evaluate the effect of positional fatty acids on obesity, an accurate and precise analytical method to analyse positional distribution of fatty acids within the glycerol moiety is an important prerequisite. An elegant, user-friendly and accurate analytical method using quantitative  $^{13}\text{C}$  NMR spectroscopy (qCNMR) has been fully established in the present study. A regiospecific analysis data using qCNMR was attainable within 44 minutes, much shorter relative to the conventional methods which use a combination of chromatographic techniques. Besides, the current method provides quantitative results with high accuracy by virtue of direct measurement and negligible sample preparation prior to the analysis. Occurrence of acyl migration during the course of sample pre-treatment is omitted. The applicability as well as the versatility of the present method had been accessed in various oils and fats, reaction intermediates of chemical interesterification and extracted lipids from biological samples with systematic errors were less than 2.0 mol%. As a summary, a cookbook approach for qCNMR in the regiospecific analysis of oils and fats from diverse sources has been established.

Correlation between the positional fatty acids within glycerol moiety and the accretion of fat was investigated in two *in vivo* studies using C57BL/6 mouse model. The positional distribution of saturated fatty acid (SFA) was found to exert a more pronounced effect on body fat deposition than the total SFA content. The extent of fat deposition will be lessened in the event of long chain SFA (C16:0 and above) occur predominantly at the *sn*-1,3 positions of triacylglycerols. Among the different chain

length of SFA, stearic acid (C18:0) was found to be more efficient in reducing fat deposition than palmitic acid (C16:0) at the similar positions. It is postulated that after the action of 1,3-specific pancreatic lipase, non-esterified SFA will suffer delayed absorption and being excreted in the faeces due to the formation of insoluble calcium soaps. Consequently, the resynthesis of triacylglycerols in chylomicron and eventually their deposition in adipose tissues will be reduced.

In accordance with the findings from both *in vivo* studies, structured lipid which aims to alleviate the risk of obesity has been designed. An even longer SFA, namely behenic acid (C22:0), was incorporated into the *sn*-1,3 positions of triacylglycerols in palm olein iodine value (IV) 56 and high-oleic sunflower oil. To achieve sustainable chemistry, immobilised lipases from the strain of *Rhizomucor miehei* (Lipozyme RM IM, Novozymes<sup>TM</sup>) and *Thermomyces lanuginosa* (Lipozyme TL IM, Novozymes<sup>TM</sup>) were employed in the current synthesis work. The synthesized structured lipids which contain high amount of BOO and BOB molecular species may serve potential applications in functional dietary fats, for instance, bakery shortening, *trans*-fat-free margarine, vanaspati and cocoa butter equivalent, with the additional health benefit in terms of giving lower risk towards the body fat deposition.

## ABSTRAK

Objektif utama kajian ini adalah untuk menentukan hubungan antara kedudukan asid lemak dalam triasilgliserol dengan obesiti. Kesimpulan yang didapati daripada kajian *in vivo* telah digunakan dalam reka bentuk dan sintesis lipid berstruktur yang bertujuan untuk mengurangkan risiko obesiti. Dalam usaha mengkaji kesan kedudukan asid lemak dalam obesiti, satu kaedah analisa yang tepat adalah penting untuk menganalisa komposisi asid lemak pada kedudukan yang berbeza dalam triasilgliserol. Satu kaedah analisa yang elegan, mudah dan tepat telah dioptimumkan sepenuhnya dengan menggunakan kuantitatif spektroskopi  $^{13}\text{C}$  NMR (qCNMR). Data analisa regiospesifik dapat diperoleh dalam 44 minit dengan menggunakan qCNMR. Ini memerlukan masa yang lebih pendek daripada kaedah konvensional yang menggabungkan teknik-teknik kromatografi. Selain itu, kaedah ini memberi keputusan kuantitatif yang lebih tepat kerana penyediaan sampel tidak diperlukan sebelum analisa. Fenomena pemindahan asid semasa penyediaan sampel dapat dielakkan. Kesesuaian serta keserbabolehan kaedah ini telah diuji dalam pelbagai jenis minyak dan lemak, antaranya produk interesterifikasi kimia dan ekstrak lipid daripada sampel biologi dengan ralat sistematik yang kurang daripada 2.0 mol%. Kesimpulannya, protokol qCNMR dalam analisa regiospesifik minyak dan lemak telah dioptimumkan.

Hubungan antara kedudukan asid lemak dalam triasilgliserol dan pendedapan lemak badan telah dikaji dalam dua kajian *in vivo* yang menggunakan model tikus C57BL/6. Kedudukan asid lemak tepu (SFA) didapati memberi kesan yang lebih ketara atas penyerapan lemak badan jika dibandingkan dengan kandungan SFA keseluruhan. Penyerapan lemak badan dapat dikurangkan sekiranya kebanyakan SFA berantai panjang (C16:0 dan ke atas) berada di kedudukan *sn*-1,3 dalam triasilgliserol. Antara

panjang rantai SFA yang berbeza, asid stearik (C18:0) didapati lebih berkesan dalam mengurangkan penyerapan lemak daripada asid palmitik (C16:0) pada kedudukan yang sama. Ini adalah kerana selepas tindakan pankreas *lipase* yang bertindak khusus pada kedudukan *sn*-1,3, SFA yang bebas akan mengalami penangguhan dalam penyerapan dan akhirnya akan dikeluarkan dalam najis sebab pembentukan sabun kalsium tidak terlarut. Oleh itu, resynthesis triasilgliserol di *chylomicron* dan akhirnya penyerapan lemak dalam tisu-tisu adipos akan dikurangkan.

Selaras dengan penemuan dari kedua-dua kajian *in vivo*, lipid berstruktur yang bertujuan untuk mengurangkan risiko obesiti telah direka dan disintesis. Rantai SFA yang lagi panjang, iaitu asid behenik (C22:0), telah digabungkan pada kedudukan *sn*-1,3 dalam triasilgliserol yang diperolehi daripada olein sawit bernilai iodin (IV) 56 dan minyak bunga matahari yang beroleik tinggi. Untuk mencapai kemampunan, *lipases* semulajadi *Rhizomucor miehei* (Lipozyme RM IM, Novozymes<sup>TM</sup>) dan *Thermomyces lanuginosa* (Lipozyme TL IM, Novozymes<sup>TM</sup>) telah digunakan dalam kerja-kerja sintesis. Lipid berstruktur yang disintesis mengandungi komposisi BOO dan BOB spesies yang tinggi. Ini membolehkan aplikasi-aplikasi dalam pemakanan lemak berfungsi, sebagai contoh, roti lemak sayuran, marjerin yang bebas dari *trans*-asid lemak, vanaspati dan kesamaan lemak koko, dengan manfaat kesihatan dari segi memberi risiko yang lebih rendah terhadap penyerapan lemak badan.

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## TABLE OF CONTENTS

	Page
Abstract	iii
<i>Abstrak</i>	v
Acknowledgements	vii
Table of Contents	viii
List of Figures	xiv
List of Tables	xix
List of Abbreviations	xxii

### CHAPTER ONE: INTRODUCTION

1.1	GENERAL REVIEW ON OILS AND FATS	
1.1.1	Fatty Acid	1
1.1.2	Triacylglycerol	4
1.1.3	Fatty Acid Profile of Triacylglycerol	5
1.2	ANALYSIS OF POSITIONAL DISTRIBUTION OF FATTY ACIDS IN TRIACYLGLYCEROLS	
1.2.1	Historical Development of Stereospecific and Regiospecific Analysis Method	9
1.2.2	Shortcomings in Conventional Regiospecific Analysis	14
1.2.3	<sup>13</sup> C NMR Regiospecific Analysis	16
1.3	POSITIONAL FATTY ACIDS AND THEIR HEALTH IMPACT	
1.3.1	Lipid Digestion and Absorption	18
1.3.2	Serum Lipid Profile and Blood Cholesterol Level	19
1.3.3	Hypothesis on Obesity	21
1.4	STRUCTURED LIPIDS	
1.4.1	Introduction	22
1.4.2	Synthesis of Structured Lipids	23
	1.4.2.1 Direct Esterification	24
	1.4.2.2 Transesterification/Alcoholysis	25
	1.4.2.3 Acidolysis	27
	1.4.2.4 Interesterification	29

1.4.3	Applications of Structured Lipids	34
1.5	OBJECTIVES OF PRESENT STUDY	36

## **CHAPTER TWO: REGIOSPECIFIC ANALYSIS OF TRIACYLGLYCEROLS USING <sup>13</sup>C NMR**

2.1	BACKGROUND	
2.2	OPTIMISATION OF NMR DATA ACQUISITION PARAMETERS	
2.2.1	Materials	45
2.2.2	Sample Preparation	45
2.2.3	Statistical Analysis	46
2.2.4	Instrumentation	46
2.2.4.1	Pulse Sequence	46
2.2.4.2	Repetition Time	47
2.2.4.3	Spectral Width	47
2.2.4.4	Temperature	47
2.2.4.5	Other Acquisition Parameters	47
2.2.5	Results and Discussion	48
2.2.5.1	The Effect of Pulse Sequence Selection	48
2.2.5.2	The Effect of Repetition Time	51
2.2.5.3	The Effect of Pulse Angle Selection	54
2.2.5.4	The Effect of Spectral Width	57
2.2.5.5	The Effect of Data Points	59
2.2.5.6	The Effect of Acquisition Time	62
2.2.5.7	The Effect of Experimental Temperature	64
2.2.5.8	The Effect of Number of Scans	67
2.3	OPTIMISATION OF NMR DATA PROCESSING PARAMETERS	
2.3.1	Sample	69
2.3.2	Instrumentation	69
2.3.2.1	Zero Filling	70
2.3.2.2	Apodisation	70
2.3.2.3	Smoothing Algorithm	70
2.3.2.4	Integration Method	70
2.3.3	Results and Discussion	71
2.3.3.1	The Effect of Zero Filling	71

2.3.3.2	The Effect of Broadening Factor in Exponential Apodisation	72
2.3.3.3	The Effect of Smoothing Algorithm	73
2.3.3.4	The Effect of Waveform Function in Deconvolution	75
2.4	APPLICATION ON NATURALLY OCCURRING OILS AND FATS	
2.4.1	Experimental	77
2.4.1.1	Materials	77
2.4.1.2	Sample Preparation	78
2.4.1.3	<sup>13</sup> C NMR Spectroscopy	78
2.4.1.4	Gas Chromatography	79
2.4.2	Results and Discussion	79
2.4.2.1	Application on Oils with Distinctive Fatty Acid Profile	79
2.4.2.2	Regiospecific Analysis of World's Four Major Vegetable Oils	84
2.4.2.3	Regiospecific Analysis of Other Naturally Occurring Oils and Fats	86
2.4.2.4	Regiospecific Analysis of Fish Oil	90
2.5	NEW REGIOSPECIFIC DATA ON PALM OIL AND KERNEL OIL FRACTION	
2.5.1	Experimental	92
2.5.1.1	Materials	92
2.5.1.2	Methodology	92
2.5.2	Results and Discussion	93
2.6	CONCLUSION	

### **CHAPTER THREE: INVESTIGATION OF THE EFFECT OF POSITIONAL FATTY ACIDS IN TRIACYLGLYCEROLS ON OBESITY**

3.1	BACKGROUND	
3.2	<i>IN VIVO</i> STUDY 1: DIFFERENT SATURATION LEVELS AT THE SN-1,3 POSITIONS OF TRIACYLGLYCEROLS ON FAT DEPOSITION	
3.2.1	Experimental	105



3.2.1.1	Diets	105
3.2.1.2	Animal Experiment	108
3.2.1.3	Lipid Extraction	108
3.2.1.4	Lipid Analysis	108
3.2.1.5	Statistical Analysis	111
3.2.2	Results	112
3.2.2.1	Food Consumption	112
3.2.2.2	Body Mass Gain	113
3.2.2.3	Fat Deposition	115
3.2.2.4	Faecal Fatty Acids	118
3.2.2.5	Adipose Tissue	121
3.2.3	Discussion	124
3.3	<i>IN VIVO</i> STUDY 2: DIFFERENT CHAIN LENGTH OF LONG CHAIN SATURATED FATTY ACIDS AT THE <i>SN</i> -1,3 POSITIONS OF TRIACYLGLYCEROLS ON FAT DEPOSITION	
3.3.1	Experimental	130
3.3.1.1	Diets	130
3.3.1.2	Animal Experiment	132
3.3.1.3	Lipid Extraction	133
3.3.1.4	Lipid Analysis	133
3.3.1.5	Statistical Analysis	133
3.3.2	Results	134
3.3.2.1	Food Consumption	134
3.3.2.2	Body Mass Gain	134
3.3.2.3	Fat Deposition	135
3.3.2.4	Faecal Fatty Acids	138
3.3.2.5	Adipose Tissue	140
3.3.3	Discussion	144
3.4	CONCLUSION	

## **CHAPTER FOUR: SYNTHESIS OF PROSPECTIVE STRUCTURED LIPIDS FOR OBESITY ALLEVIATION**

4.1	BACKGROUND	
4.1.1	Literature Review	149
4.1.2	The Approach of Present Study	153
4.2	ENZYMATIC INTERESTERIFICATION BETWEEN PALM OLEIN AND ETHYL BEHENATE	
4.2.1	Experimental	154
4.2.1.1	Materials	154
4.2.1.2	Preparation of Ethyl Behenate	155
4.2.1.3	Enzymatic Interesterification	155
4.2.1.4	<sup>13</sup> C NMR Regiospecific Analysis	156
4.2.1.5	High Performance Liquid Chromatography (HPLC)	156
4.2.2	Results and Discussion	157
4.2.2.1	Positional Distribution of Fatty Acids in Palm Olein and Structured Lipids Synthesised	157
4.2.2.2	Identification of Triacylglycerol Molecular Species	163
4.2.2.3	Triacylglycerol Composition	173
4.3	ENZYMATIC INTERESTERIFICATION BETWEEN HIGH-OLEIC SUNFLOWER OIL AND ETHYL BEHENATE	
4.3.1	Experimental	181
4.3.1.1	Materials	181
4.3.1.2	Preparation of Ethyl Behenate	181
4.3.1.3	Enzymatic Interesterification	181
4.3.1.4	<sup>13</sup> C NMR Regiospecific Analysis	181
4.3.1.5	High Performance Liquid Chromatography (HPLC)	181
4.3.2	Results and Discussion	182
4.3.2.1	Positional Distribution of Fatty Acids in High-Oleic Sunflower Oil and Structured Lipids Synthesised	182
4.3.2.2	Triacylglycerol Composition	187
4.4	PURIFICATION AND CHARACTERISATION OF STRUCTURED LIPIDS FOR OBESITY ALLEVIATION	
4.4.1	Experimental	196
4.4.1.1	Purification and Isolation of Structured Triacylglycerols	196

4.4.1.2 Determination of Acidity and Iodine Values	196
4.4.1.3 Gas Chromatography (GC)	197
4.4.1.4 Differential Scanning Calorimetry (DSC)	197
4.4.2 Results and Discussion	197
4.4.2.1 Selection of Ideal Structured Lipids	197
4.4.2.2 Acidity and Iodine Values	201
4.4.2.3 Total Fatty Acid Composition	202
4.4.2.4 Thermal Properties	204
4.4.2.5 Prospective Applications	215
4.5 CONCLUSION	
References	217
List of Publications and Papers Presented	238

## LIST OF FIGURES

Figure 1.1	Fischer projection of a triacyl- <i>sn</i> -glycerol	5
Figure 1.2	Examples of major triacylglycerol molecular species in cocoa butter equivalent	8
Figure 1.3	<i>sn</i> -1,3 specific lipase from <i>Rhizopus delemar</i> hydrolysis of trioleoylglycerol to yield intermediate partial oleoylglycerols	13
Figure 1.4	Non-specific Grignard hydrolysis of trioleoylglycerol to yield wide range of partial oleoylglycerols	14
Figure 1.5	Problem of acyl migration in intermediate monoacylglycerol during sample preparation	15
Figure 1.6	<sup>13</sup> C NMR spectrum of acyl chain carbonyl resonances of synthetic mixture of triacylglycerols	17
Figure 1.7	Pathway of free long chain SFA from <i>sn</i> -1,3 positions after hydrolysis of pancreatic lipase	22
Figure 1.8	General scheme for common reactions used in synthesis of structured lipids	24
Figure 1.9	Mechanism of base-catalysed transesterification of triacylglycerols (Eckey, 1956)	26
Figure 1.10	Mechanism of acid-catalysed transesterification of triacylglycerols (Eckey, 1956)	26
Figure 1.11	Formation of enolate anion and its mesomeric structures (Weiss <i>et al.</i> , 1961)	30
Figure 1.12	Intramolecular rearrangement of the acyl group <i>via</i> Claisen condensation (Liu, 2004)	31
Figure 1.13	Intermolecular rearrangement of the acyl group <i>via</i> Claisen condensation (Liu, 2004)	32
Figure 2.1	Three dimensional illustration of carbonyl carbons in tripalmitoylglycerol (PPP)	49
Figure 2.2	Pulse sequence of Single Pulse with Inverse Gated Heteronuclear Decoupling. <sup>1</sup> H decoupler was turned on during acquisition time only to suppress NOE	51
Figure 2.3	Relationship between targeted accuracy and the minimum required repetition time, <i>t</i>	53

Figure 2.4	$^{13}\text{C}$ NMR spectrum of carbonyl carbon resonances of triacylglycerols in canola (Pulse angle = $90^\circ$ )	55
Figure 2.5	$^{13}\text{C}$ NMR spectrum of carbonyl carbon resonances of triacylglycerols in canola (Pulse angle = $30^\circ$ )	56
Figure 2.6	$^{13}\text{C}$ NMR spectrum of carbonyl carbon resonances of triacylglycerols in crude palm oil (Spectral width = 18000 Hz)	58
Figure 2.7	$^{13}\text{C}$ NMR spectrum of carbonyl carbon resonances of triacylglycerols in crude palm oil (Spectral width = 1500 Hz)	59
Figure 2.8	Time-domain signals of $^{13}\text{C}$ NMR regiospecific analysis of soybean oil. The number of data points as varied as follows (a) data points = 4096, (b) data points = 16384 and (c) data points = 8192	60
Figure 2.9	$^{13}\text{C}$ NMR spectrum of carbonyl carbon resonances of triacylglycerols in soybean oil (Number of data points = 4096)	60
Figure 2.10	$^{13}\text{C}$ NMR spectrum of carbonyl carbon resonances of triacylglycerols in soybean oil (Number of data points = 16384)	61
Figure 2.11	$^{13}\text{C}$ NMR spectrum of carbonyl carbon resonances of triacylglycerols in soybean oil acquired under optimum number of data points (8192)	62
Figure 2.12	$^{13}\text{C}$ NMR spectrum of carbonyl carbon resonances of triacylglycerols in soybean oil (Acquisition time = 10.8 s)	63
Figure 2.13	$^{13}\text{C}$ NMR spectrum of carbonyl carbon resonances of triacylglycerols in soybean oil (Acquisition time = 5.4 s)	64
Figure 2.14	Effect of experimental temperature ( $T = 20^\circ\text{C}$ , $30^\circ\text{C}$ , $40^\circ\text{C}$ and $50^\circ\text{C}$ ) on peak width and S/N ratio of <i>cis</i> -9 monoene acyl chain carbonyl resonances of triacylglycerols in rapeseed oil (canola)	65
Figure 2.15	Effect of shimming quality on peak width and S/N ratio of <i>cis</i> -9 monoene acyl chain carbonyl resonances of triacylglycerols in rapeseed oil (canola) at $40^\circ\text{C}$	66
Figure 2.16	$^{13}\text{C}$ NMR spectrum of carbonyl carbon resonances of triacylglycerols in canola (Number of scans = 64)	67
Figure 2.17	$^{13}\text{C}$ NMR spectrum of carbonyl carbon resonances of triacylglycerols in canola (Number of scans = 128)	68
Figure 2.18	$^{13}\text{C}$ NMR spectrum of carbonyl carbon resonances of triacylglycerols in canola (Number of scans = 256)	69
Figure 2.19	$^{13}\text{C}$ NMR spectrum of carbonyl carbon resonances of triacylglycerols in rapeseed oil (canola) (No smoothing)	74

Figure 2.20	$^{13}\text{C}$ NMR spectrum of carbonyl carbon resonances of triacylglycerols in rapeseed oil (canola) (after 9-point weighted Savitzky-Golay signal smoothing)	74
Figure 2.21	$^{13}\text{C}$ NMR spectrum of acyl chain carbonyl resonances of synthetic triacylglycerol mixture. Curve fitting was used as the integration method	75
Figure 2.22	Waveforms produced by using different curve fitting function in deconvolution of carbonyl carbon resonances of synthetic triacylglycerol mixture	76
Figure 2.23	$^{13}\text{C}$ NMR spectrum of acyl carbonyl resonances of triacylglycerols in canola (Fatty acid profile UUU)	81
Figure 2.24	$^{13}\text{C}$ NMR spectrum of acyl carbonyl resonances of triacylglycerols in mango fat (Fatty acid profile SUS)	82
Figure 2.25	$^{13}\text{C}$ NMR spectrum of acyl carbonyl resonances of triacylglycerols in coconut oil (Fatty acid profile SSS)	83
Figure 2.26	Probable acyl migration of oleoyl acyl chain ( <i>cis</i> -9-monoene) from <i>sn</i> -2 to <i>sn</i> -1 or <i>sn</i> -3 position in intermediate diacylglycerols	86
Figure 2.27	400 MHz $^{13}\text{C}$ NMR spectrum of acyl chain carbonyl resonances (spectral width = 1500 Hz) of triacylglycerols in menhaden fish oil	91
Figure 2.28	Relationship between total fatty acid composition and iodine values of palm oil fraction	96
Figure 2.29	Correlation between fatty acid composition at <i>sn</i> -1,3 positions of triacylglycerols and iodine values of palm oil fraction	97
Figure 2.30	Correlation between fatty acid composition at <i>sn</i> -2 position of triacylglycerols and iodine values of palm oil fraction	97
Figure 3.1	Enzymatic hydrolysis and fat absorption in intestine as illustrated by 1,3-dipalmitoyl-2-oleoylglycerol (POP)	103
Figure 3.2	Calibration graph for palmitic, stearic and oleic acids in the determination of the absolute amount of faecal fatty acids	111
Figure 3.3	Effect of total saturation level at the <i>sn</i> -1,3 positions of triacylglycerols on fat deposition (mass of fat deposited/total feed consumed)	116
Figure 3.4	Effect of different extent of unsaturation at the <i>sn</i> -1,3 positions of triacylglycerols on fat deposition (mass of fat deposited/total feed consumed)	117

Figure 3.5	Effect of fatty acid composition at the <i>sn</i> -1,3 positions of triacylglycerols on fat deposition (mass of fat deposited/total feed consumed)	137
Figure 4.1	Kinetics of the enzymatic interesterification between palm olein IV 56 and ethyl behenate catalysed by (a) Lipozyme RM IM (b) Lipozyme TL IM, measured by the positional distribution of saturated fatty acids (SFA) in triacylglycerol	160
Figure 4.2	Kinetics of the enzymatic interesterification between palm olein IV 56 and ethyl behenate catalysed by (a) Lipozyme RM IM (b) Lipozyme TL IM, measured by the positional distribution of mono- (MUFA) and polyunsaturated fatty acids (PUFA) in triacylglycerol	162
Figure 4.3	Separation of triacylglycerol molecular species with identical PN values in chromatogram of palm olein IV 56	166
Figure 4.4	Graph of the logarithm of the retention volumes of saturated triacylglycerols, relative to trioleoylglycerol, against the total number of carbon atoms in the fatty acid acyl chains	167
Figure 4.5	Graph of the logarithm of the retention volumes of triacylglycerols, relative to trioleoylglycerol, against the total number of double bonds in the fatty acid acyl chains	169
Figure 4.6	Peak identification of various triacylglycerol molecular species in the chromatogram of palm olein IV 56	171
Figure 4.7	Peak identification of various triacylglycerol molecular species in the chromatogram of high-oleic sunflower oil	172
Figure 4.8	Peak identification of various triacylglycerol molecular species in the chromatogram of structured lipids	172
Figure 4.9	Enzymatic interesterification reaction scheme between ethyl behenate and 1-palmitoyl-2,3-dioleoylglycerol (POO) in palm olein IV 56	175
Figure 4.10	Intesterification reaction between palm olein IV 56 and ethyl behenate catalysed by Lipozyme RM IM	177
Figure 4.11	Intesterification reaction between palm olein IV 56 and ethyl behenate catalysed by Lipozyme TL IM	180
Figure 4.12	Kinetics of the enzymatic interesterification between high-oleic sunflower oil and ethyl behenate catalysed by (a) Lipozyme RM IM (b) Lipozyme TL IM, measured by the positional distribution of saturated fatty acids (SFA) in triacylglycerol	185

Figure 4.13	Kinetics of the enzymatic interesterification between high-oleic sunflower oil and ethyl behenate catalysed by (a) Lipozyme RM IM (b) Lipozyme TL IM, measured by the positional distribution of mono- (MUFA) and polyunsaturated fatty acids (PUFA) in triacylglycerol	186
Figure 4.14	Intesterification reaction between high-oleic sunflower oil and ethyl behenate catalysed by Lipozyme RM IM	191
Figure 4.15	Enzymatic interesterification reaction scheme between ethyl behenate and trioleoylglycerol (OOO) in high-oleic sunflower oil	193
Figure 4.16	Intesterification reaction between high-oleic sunflower oil and ethyl behenate catalysed by Lipozyme TL IM	194
Figure 4.17	Differential scanning calorimetry melting curves of palm olein IV 56 and its selected interesterified products	207
Figure 4.18	Differential scanning calorimetry melting curves of high-oleic sunflower oil and its selected interesterified products	208
Figure 4.19	Differential scanning calorimetry crystallisation curves of palm olein IV 56 and its selected interesterified products	209
Figure 4.20	Differential scanning calorimetry crystallisation curves of high-oleic sunflower oil and its selected interesterified products	210
Figure 4.21	Differential scanning calorimetry melting curves of the selected structured lipids produced from the enzymatic interesterification between oil and ethyl behenate	212
Figure 4.22	Differential scanning calorimetry crystallisation curves of the selected structured lipids produced from the enzymatic interesterification between oil and ethyl behenate	213



## LIST OF TABLES

Table 1.1	Systematic, trivial, shorthanded nomenclatures and structure of natural occurring fatty acids	2
Table 1.2	Total fatty acid composition (FAC) of World's major edible oils	6
Table 2.1	NOE (1+ $\eta$ ) factor for carbonyl carbons of triacylglycerols in selected oils	50
Table 2.2	T <sub>1</sub> relaxation times for carbonyl carbons of triacylglycerol in different type of oils and fats	52
Table 2.3	Study of the effect of zero filling on the accuracy and precision of qCNMR regiospecific analysis of synthetic triacylglycerol mixture	71
Table 2.4	Study of the line broadening effect on the accuracy and precision of qCNMR regiospecific analysis of synthetic triacylglycerol mixture	73
Table 2.5	Comparison of different curve fitting function in processing of regiospecific data of synthetic triacylglycerol mixture	77
Table 2.6	Regiospecific analyses of four major vegetable oils by experimental (by <sup>13</sup> C NMR) and literature values (by conventional method)	85
Table 2.7	Regiospecific analyses data of selected oils and fats by <sup>13</sup> C NMR	87
Table 2.8	Regiospecific analyses data of menhaden fish oil by <sup>13</sup> C NMR	92
Table 2.9	New regiospecific analyses data of palm fraction by <sup>13</sup> C NMR	94
Table 3.1	The composition of the autoclavable standard pellet feeds for mice	106
Table 3.2	Total fatty acid composition (FAC) of dietary oils and fats for <i>in vivo</i> study 1	107
Table 3.3	Positional fatty acid composition of dietary oils for <i>in vivo</i> study 1	108
Table 3.4	Total food consumption of C57BL/6 mice over 15 weeks ( <i>In vivo</i> study 1)	112
Table 3.5	Weekly food consumption of C57BL/6 mice from different dietary groups ( <i>In vivo</i> study 1)	112
Table 3.6	Body mass of C57BL/6 mice over 15 weeks ( <i>In vivo</i> study 1)	114

Table 3.7	Weekly body mass gain of C57BL/6 mice from different dietary groups ( <i>In vivo</i> study 1)	114
Table 3.8	Fat deposition in C57BL/6 mice over 15 weeks ( <i>In vivo</i> study 1)	118
Table 3.9	Composition of free fatty acids in the faeces of mice from each test group ( <i>In vivo</i> study 1)	119
Table 3.10	The absolute amount of free fatty acids in the faeces of mice from different dietary groups ( <i>In vivo</i> study 1)	120
Table 3.11	The excretion indices for each fatty acid in the faeces of mice from different dietary groups ( <i>In vivo</i> study 1)	121
Table 3.12	Fatty acid composition of the extracted lipids from subcutaneous adipose tissue of mice ( <i>In vivo</i> study 1)	122
Table 3.13	Fatty acid composition of the extracted lipids from visceral adipose tissue of mice ( <i>In vivo</i> study 1)	123
Table 3.14	Positional fatty acid composition of the adipose tissues of mice ( <i>In vivo</i> study 1)	124
Table 3.15	Total fatty acid composition of dietary oils and fats for <i>in vivo</i> study 2	131
Table 3.16	Positional fatty acid composition of dietary oils for <i>in vivo</i> study 2	131
Table 3.17	Total food consumption of C57BL/6 mice after 15 weeks ( <i>In vivo</i> study 2)	134
Table 3.18	Body mass of C57BL/6 mice over 15 weeks ( <i>In vivo</i> study 2)	135
Table 3.19	Fat deposition in C57BL/6 mice after 15 weeks ( <i>In vivo</i> study 2)	138
Table 3.20	Composition of free fatty acid in the faeces of mice from each test group ( <i>In vivo</i> study 2)	139
Table 3.21	The absolute amount of free fatty acids in the faeces of mice from different dietary groups ( <i>In vivo</i> study 2)	139
Table 3.22	The excretion index for each free fatty acid in the faeces of mice from different dietary groups ( <i>In vivo</i> study 2)	140
Table 3.23	Fatty acid composition of the extracted lipids from subcutaneous adipose tissue of mice ( <i>In vivo</i> study 2)	141
Table 3.24	Fatty acid composition of the extracted lipids from visceral adipose tissue of mice ( <i>In vivo</i> study 2)	142
Table 3.25	Positional fatty acid composition of the adipose tissues of mice ( <i>In vivo</i> study 2)	143

Table 4.1	Regiospecific data of the starting material (palm olein IV 56) and the interesterification reaction intermediates catalysed by Lipozyme RM IM with ethyl behenate	158
Table 4.2	Regiospecific data of the starting material (palm olein IV 56) and the interesterification reaction intermediates catalysed by Lipozyme TL IM with ethyl behenate	159
Table 4.3	Equivalent carbon numbers (ECN) for various triacylglycerol molecular species	165
Table 4.4	Triacylglycerol composition of the starting material (palm olein IV 56) and the interesterification reaction intermediates catalysed by Lipozyme RM IM with ethyl behenate	173
Table 4.5	Triacylglycerol composition of the starting material (palm olein IV 56) and the interesterification reaction intermediates catalysed by Lipozyme TL IM with ethyl behenate	176
Table 4.6	Evolution of targeted structured lipids, co-products and reactant triacylglycerols as a function of time in interesterification of palm olein IV 56 catalysed by Lipozyme RM IM	177
Table 4.7	Evolution of targeted structured lipids, co-products and reactant triacylglycerols as a function of time in interesterification of palm olein IV 56 catalysed by Lipozyme TL IM	178
Table 4.8	Regiospecific data of the starting material (high-oleic sunflower oil) and the interesterification reaction intermediates catalysed by Lipozyme RM IM with ethyl behenate	183
Table 4.9	Regiospecific data of the starting material (high-oleic sunflower oil) and the interesterification reaction intermediates catalysed by Lipozyme TL IM with ethyl behenate	184
Table 4.10	Triacylglycerol compositions of the starting material (high-oleic sunflower oil) and the interesterification reaction intermediates catalysed by Lipozyme RM IM with ethyl behenate	188
Table 4.11	Triacylglycerol compositions of the starting material (high-oleic sunflower oil) and the interesterification reaction intermediates catalysed by Lipozyme TL IM with ethyl behenate	190
Table 4.12	Evolution of targeted structured lipids, co-products and reactant triacylglycerols as a function of time in interesterification of high-oleic sunflower oil catalysed by Lipozyme RM IM	191
Table 4.13	Evolution of targeted structured lipids, co-products and reactant triacylglycerols as a function of time in interesterification of high-oleic sunflower oil catalysed by Lipozyme TL IM	194

Table 4.14	Triacylglycerol compositions of the selected structured lipids from different batches	199
Table 4.15	Regiospecific data of the selected structured lipids	200
Table 4.16	Physicochemical properties of the starting materials and the selected structured lipids	201
Table 4.17	Fatty acid composition of palm olein IV 56 and the selected structured lipids produced by Lipozymes RM IM and TL IM	203
Table 4.18	Fatty acid composition of high-oleic sunflower oil and the selected structured lipids produced by Lipozymes RM IM and TL IM	204
Table 4.19	Comparison of the transition temperatures for melting and crystallisation curves of starting materials and selected structured lipids	205
Table 4.20	Comparison of the onset, offset and range of temperatures for thermograms of the starting materials and selected structured lipids	211

## LIST OF ABBREVIATIONS

%	per cent
ACQTM	acquisition time
ACUC	Animal Care and Use Committee
ANOVA	analysis of variance
AOCS	American Oil Chemists' Society
BBB	tribehenoylglycerol
BBP	1,2-dibehenoyl-3-palmitoylglycerol
BBS	1,2-dibehenoyl-3-stearoylglycerol
Bhd.	Berhad (limited)
BOB	1,3-dibehenoyl-2-oleoylglycerol
BOO	1-behenoyl-2,3-dioleoylglycerol
BOS <sub>t</sub>	1-behenoyl-2-oleoyl-3-stearoylglycerol
BSTFA	N,N-Bis(trimethylsilyl)trifluoroacetamide
°C	degree Celsius
<sup>13</sup> C	carbon-13
Ca	calcium
CDCl <sub>3</sub>	deuterated chloroform
CLA	conjugated linoleic acid
CMC	critical micelle concentration
CN	carbon number of the three acyl chains
COB	cocoa butter
Cr(acac) <sub>3</sub>	chromium(III) acetylacetonate
DB	number of double bonds
DHA	docosahexaenoic acid
DNPU	dinitrophenyl urethane
DSC	differential scanning calorimetry
ECN	equivalent carbon number
ELSD	evaporative light scattering detector
EPA	eicosapentaenoic acid
<i>etc.</i>	et cetera
FA	fatty acid
FAC	fatty acid composition

FID	free induction decay
GC	gas chromatography
G(x)	Gaussian function
$^1\text{H}$	proton
HDL-C	high-density-lipoprotein cholesterol
HOS	high-oleic sunflower oil
HPLC	high performance liquid chromatography
HS_RM	structured lipids produced from high-oleic sunflower oil under action of Lipozyme RM IM
HS_TL	structured lipids produced from high-oleic sunflower oil under action of Lipozyme TL IM
Hz	Hertz
i.d.	internal diameter
<i>i.e.</i>	id est
IM	immobilized
IPOo	chemical interesterified palm olein
IRRPW	irradiation pulse width
IUPAC	International Union of Pure and Applied Chemistry
IV	iodine value
KOH	potassium hydroxide
L	polyunsaturated fatty acid acyl chain (in NMR spectrum)
LB	line broadening
LDL-C	low-density-lipoprotein cholesterol
LLL	trilinoleoylglycerol
LSD	Fisher's Least Significant Difference test
L(x)	Lorentzian function
Mg	magnesium
$M_0$	equilibrium magnetisation
MUFA	monounsaturated fatty acid
m/v	mass/volume ratio
M(x)	hybrid function of Lorentzian and Gaussian functions
$M_y$	transverse magnetisation
$M_z$	longitudinal magnetisation
NA	not applicable
ND	not detected

NMR	nuclear magnetic resonance
NOE	Nuclear Overhauser enhancement
NRC	National Research Council
NUFA	number of unsaturated fatty acids
O	<i>cis</i> -9 monoene fatty acid acyl chain (in NMR spectrum)
OLL	1,2-dilinoleoyl-3-oleoylglycerol
OOL	1,2-dioleoyl-3-linoleoylglycerol
OOO	trioleoylglycerol
PD	pulse delay
pH	$-\log [H^+]$
PMF	palm mid fraction
PN	partition number
PO <sub>o</sub>	palm olein
POO	1,2-dioleoyl-3-palmitoylglycerol
POP	1,3-dipalmitoyl-2-oleoylglycerol
PO_RM	structured lipids produced from palm olein under action of Lipozyme RM IM
POSt	1-palmitoyl-2-oleoyl-3-stearoylglycerol
PO_TL	structured lipids produced from palm olein under action of Lipozyme TL IM
ppm	parts per million
PPP	tripalmitoylglycerol
PUFA	polyunsaturated fatty acid
PW	observation pulse width
qCNMR	Quantitative <sup>13</sup> C NMR
RBD	refined, bleached and deodorised
RM	<i>Rhizomucor miehei</i>
S	saturated fatty acid acyl chain (in NMR spectrum)
SAL	sal stearin
SD	standard deviation
Sdn.	Sendirian (private)
SEM	standard error of the mean
SFA	saturated fatty acid
SFC	solid fat content
SL	structured lipids

<i>sn</i>	stereospecific-numbering
S/N	signal-to-noise
SOY	soybean oil
SSS	saturated-saturated-saturated fatty acid profile
StOP	1-stearoyl-2-oleoyl-3-palmitoylglycerol
StOSt	1,3-distearoyl-2-oleoylglycerol
StStSt	tristearoylglycerol
SUS	saturated-unsaturated-saturated fatty acid profile
<i>t</i>	repetition time
T <sub>o</sub>	Onset temperature
T <sub>f</sub>	Offset temperature
T <sub>1</sub>	spin-lattice relaxation time
T <sub>2</sub>	spin-spin relaxation time
TL	<i>Thermomyces lanuginose</i>
UUU	unsaturated-unsaturated-unsaturated fatty acid profile
V	<i>cis</i> -11 monoene fatty acid acyl chain (in NMR spectrum)
<i>viz.</i>	videlicet
WHO	World Health Organisation