Contents

			Page
Abstract			(3)
Acknowledgments			(7)
Contents			(8)
List of T	ables		(15)
List of Figures			(16)
List of Abbreviations			(21)
Chapter 1.			1
Introc	luctio	n	1
Literature Review			3
1.	Paln	n Oil	3
2.	2. Lipases		
	2.1	Sources of lipases	4
	2.2	Function of lipases	5
	2.3	Specificity of lipases	7
	2.4	Application of lipase	10
3.	Imm	nobilized lipases	10
4.	Kinetics of reaction catalyzed by immobilized lipases		
5.	MA	G production and application	16
	5.1	Hydrolysis or alcoholysis of triglycerides to produce	17
		2-monoglycerides	
	5.2	Glycerolysis of triglycerides with glycerol	19
	5.3	Esterification of glycerols with fatty acid or fatty acid	20
		esters	
6.	The	influence of reaction condition on glycerolysis	21
	6.1	Sources of lipases	21
	6.2	Initial water content	22

	6.3	Glycerol/triacylglycerols molar ratio	22
	6.4	Temperature	23
	6.5	Organic solvents	23
7.	Bior	reactors for MAG production	24
	7.1	Batch stirred-tank reactors (BSTR)	27
	7.2	Continuous stirred-tank reactors (CSTR)	28
	7.3	Packed-bed reactors (PBR)	28
	7.4	Fluidized-bed reactors (FBR)	29
	7.5	Membrane reactors (MR)	30
8.	Scal	e up of MAG production	31
9.	Reco	overy of MAG	32
Objective			34
Scope of	resea	arch works	34
Chapter	2.		35
Mater	ials		35
1.	Lipa	ases	35
2.	Sup	ports	35
3.	Raw	y materials	35
4.	Che	micals	36
5.	Instr	ruments	37
Metho	ods		38
A.An	alytic	cal methods	38
1.	Acio	d value	38
2.	Iodi	ne value	38
3.	Solu	ıble protein	38
4.	Spor	nification value	38
5.	Hyd	rolytic activity of lipase	38
			(9)

6.	Fatty acid compositions		
7.	Oil compositions		
B. Ex	perim	ents	40
1.	Com	mercial lipase for MAG production	40
2.	Phys	ical and chemical properties of palm olein	40
3.	Selec	ction of commercial lipases for MAG production	40
4.	Selec	ction of support for immobilized lipase	41
5.	Opti	mal condition for enzyme immobilization	41
	5.1	Effect of lipase concentration	41
	5.2	Effect of temperature	42
	5.3	Effect of time	42
6.	Prop	erties of immobilized lipase	42
	6.1	Optimum temperature	42
	6.2	Thermostability	42
7.	Selec	ction of reactor types for MAG production	43
	7.1	Continuous MAG production in CSTR	43
	7.2	Continuous MAG production in PBR	44
8.	Opti	mization of MAG production in solvent system	45
	8.1	Effect of solvent type	45
	8.2	Stability of immobilized lipase in organic solvents	45
	8.3	Effect of water content in glycerol	45
	8.4	Effect of the molar ratio of glycerol to palm olein	45
	8.5	Effect of palm olein concentration in solvent	46
	8.6	Effect of amount of immobilized lipase	46
	8.7	Effect of temperature	46
9.	MAG	G production under optimum condition	46

		Page
10.	Kinetics of glycerolysis of palm olein with glycerol by	47
	soluble and immobilized lipase	
11.	Continuous MAG production in solvent system	47
	11.1 Continuous MAG production in CSTR	47
	11.2 Continuous MAG production in PBR	47
12.	Optimization of continuous MAG production in solvent	48
	system	
	12.1 Effect of molar ratio of glycerol to palm olein	48
	12.2 Effect of water content in glycerol	48
	12.3 Effect of substrate flow rate	48
	12.4 Effect of temperature	48
	12.5 Continuous MAG production under optimum condition	49
13.	Time course of continuous MAG production in solvent	49
	system	
14.	Large scale of continuous MAG production in solvent system	49
15.	Recovery of MAG	49
	15.1 Crystallization at low temperature	49
	15.1.1 Effect of temperature on MAG crystallization	50
	15.1.2 Effect of time on MAG crystallization	50
	15.2 Crystallization using extraction by organic solvent with	50
	temperature control	
	15.2.1 Effect of organic solvent on MAG	50
	crystallization	
	15.2.2 Effect of crude MAG product concentration in	51
	organic solvent on MAG crystallization	
	15.2.3 Effect of temperature on MAG crystallization	51

(11)

		15.2.4 I	Effect of time on MAG crystallization	51			
	15.3	Fractiona	ation by silica gel 60	51			
Chapter 3							
Results and Discussion							
1.	Con	Commercial lipase for MAG production					
2.	Phy	sical and c	chemical properties of palm olein	52			
3.	Selection of commercial lipase for MAG production						
4.	Sele	ction of su	upport to immobilize lipase	60			
5.	Opti	mal condi	ition for enzyme immobilization	63			
	5.1	Effect of	fenzyme loading	63			
	5.2	Effect of	temperature	64			
	5.3	Effect of	fimmobilization time	65			
6.	Prop	Properties of immobilized lipase PS (IM-PS)					
	6.1	Optimun	n temperature	66			
	6.2	Thermos	stability	67			
7.	Continuous glycerolysis			68			
	7.1	Continuo	ous glycerolysis in CSTR	68			
	7.2	Continuo	ous glycerolysis in PBR	68			
8.	Opti	mization	of MAG production in solvent system	70			
	8.1	Effect of	solvent type on MAG production	71			
	8.2	Stability	of IM-PS in acetone/isooctane mixture	73			
		(3:1,v/v)					
	8.3	Effect of	finitial water content on MAG production	75			
	8.4	Effect of	the molar ratio of glycerol to palm olein on	78			
		MAG pr	oduction				
	8.5	Effect of	palm olein cencentration in acetone/isooctane	81			
		mixture	(3:1,v/v) on MAG production				

(12)

				0
	8.6	Effect	of IM-PS loading on MAG production	84
	8.7	Effect	of temperature on MAG production	86
9.	MA	G produ	ction at optimal condition	86
10.	Kine	etics of g	glycerolysis of palm olein with glycerol by lipase	91
	PS a	nd IM-F	PS	
11.	Con	tinuous	MAG production in solvent system	92
	11.1	Contin	uous MAG production in CSTR	92
	11.2	Contin	uous MAG production in PBR	93
12.	Opti	mizatio	n MAG production in PBR	96
	12.1	Effect	of the molar ratio of glycerol to palm olein on	96
		MAG	production	
	12.2	Effect	of the water content in glycerol on MAG	99
		produc	tion	
	12.3	Effect	of substrate flow rate on MAG production	102
	12.4	Effect	of temperature on MAG production in PBR	105
	12.5	Contin	uous MAG production in PBR under optimal	108
		conditi	ons	
13.	Tim	e course	of continuous MAG production in PBR	113
14.	Larg	ge scale	MAG production in PBR	115
15.	Reco	overy of	MAG	117
	15.1	Crystal	lization using temperature control	117
		15.1.1	Effect of temperature on MAG crystallization	117
			from product mixture	
		15.1.2	Effect of time on MAG crystallization from	118
			product mixture	
	15.2	Crystal	lization using extraction by organic solvent with	122
		temper	ature control	

(13)

			-
	15.2.1	Effect of organic solvents on MAG	122
		crystallization	
	15.2.2	Effect of crude MAG product concentration in	122
		isooctane on MAG crystallization	
	15.2.3	Effect of temperature on MAG crystallization in	124
		isooctane	
	15.2.4	Effect of time on MAG crystallization in	125
		isooctane	
15.3	Fraction	nation by silica gel 60	128
Chapter 4			133
Conclusion			133
Suggestions			134
References			135
Appendix			143
Vitae			151

List of Tables

Table		Page
1	Triacylglycerol composition of palm oil	4
2	Major specificities of lipases and their applications	9
3	Industrial application areas for microbial lipases	11
4	Lipases immobilization for MAG production	13
5	Enzymatic MAG production	18
6	Reactor for enzymatic MAG production	26
7	Comercial lipases for glycerolysis of palm olein	53
8	Composition and properties of palm olein	54
9	Hydrolysis activity of immobilized lipase PS on various	61
	supports	
10	Effect of enzyme loading on immobilization lipase PS with	63
	Accurel	
11	Effect of temperature on immobilization lipase PS with	64
	Accurel	
12	Effect of time on immobilization lipase PS with Accurel	65
13	Fatty acid profile of product mixture	111
14	Effect of temperature on MAG crystallization from product	118
	mixture	
15	Effect of time on MAG crystallization from product mixture	119
16	Effect of organic solvents on MAG crystallization	123
17	Effect of crude MAG product concentration in isooctane on	123
	MAG crystallization	
18	Effect of temperature on MAG crystallization in isooctane	124
19	Effect of time on MAG crystallization in isooctane	125
20	Comparison of various methods for MAG recovery	132

List of Figures

Figure		Page
1	Type of reaction catalyzed by lipases	6
2	Hydrolysis or alcoholysis of a triacylglycerol to produce	19
	2-MAG (acyl migration as indicated by the arrow might	
	lead to 1(3)-MAG)	
3	Glycerolysis of a triacylglycerol produces monoacylglycerol	19
4	Esterification of glycerol yield a mixture of 1(3)- and	21
	2-MAG	
5	Enzyme reactor designs	25
6	Schematic diagram for continuous glycerolysis of palm	43
	olein by IM-PS in CSTR	
7	Schematic diagram for continuous glycerolysis of palm	44
	olein by IM-PS in PBR	
8	TLC/FID chromatogram of palm olein	55
9	GC chromatogram of palm olein	56
10	Glycerolysis of palm olein by lipases at 30 °C	58
11	Glycerolysis of palm olein by lipases at 45 °C	59
12	Thermostability of soluble lipase at 45 °C	60
13	The composition of the reaction mixture during glycerolysis	62
	of palm olein with glycerol by lipase PS immobilized on	
	Accurel EP100 (<200 μm)	
14	Effect of temperature on hydrolysis activity of lipase PS and	66
	IM-PS	
15	Thermostability of lipase PS and IM-PS at 45 °C	67
16	Continuous glycerolysis of palm olein with glycerol by	69
	IM-PS in CSTR at 45 °C	

Figure		Page
17	Continuous glycerolysis of palm olein with glycerol by	70
	IM-PS in PBR at 45 °C	
18	Effect of organic solvents on MAG production by IM-PS	72
19	Time course of glycerolysis by IM-PS in acetone/isooctane	73
	mixture (3:1,v/v)	
20	Stability of IM-PS in acetone/isooctane mixture (3:1,v/v)	74
21	Effect of initial water content on MAG production by	76
	IM-PS	
22	Time course of glycerolysis by IM-PS in acetone/isooctane	77
	mixture (3:1,v/v) using 10 %(w/w) water in glycerol	
23	Effect of the molar ratio of glycerol to palm olein on MAG	79
	production by IM-PS	
24	Time course of glycerolysis by IM-PS in acetone/isooctane	80
	mixture $(3:1,v/v)$ using the molar ratio of glycerol to palm	
	olein with 2.7:1	
25	Effect of palm olein concentration on MAG production by	82
	IM-PS	
26	Time course of glycerolysis by IM-PS in acetone/isooctane	83
	mixture (3:1,v/v) using 10 %(w/v) palm olein in	
	acetone/isooctane mixture (3:1,v/v)	
27	Effect of IM-PS loading on MAG production	84
28	Time course of glycerolysis by IM-PS in acetone/isooctane	85
	mixture (3:1,v/v) using 50 %(w/w) IM-PS of palm olein	
29	Effect of temperature on MAG production by IM-PS	87
30	Time course of glycerolysis by IM-PS in acetone/isooctane	88
	mixture (3:1,v/v) at 45 °C	

Figure		Page
31	Time course of glycerolysis by IM-PS in acetone/isooctane	89
	mixture (3:1,v/v) under optimal conditions	
32	Time course of glycerolysis by lipase PS in	90
	acetone/isooctane mixture (3:1,v/v) under optimal	
	conditions	
33	Lineweaver-Burk plot of glycerolysis by lipase PS and	92
	IM-PS in various palm olein concentration in organic	
	solvent system at 45 °C	
34	Continuous MAG production in solvent system by IM-PS in	94
	CSTR	
35	Continuous MAG production in solvent system by IM-PS in	95
	PBR	
36	Effect of molar ratio of glycerol to palm olein on continuous	97
	MAG production by IM-PS in PBR	
37	Time course of continuous MAG production by IM-PS in	98
	PBR using the molar ratio of glycerol to palm olein of 12:1	
38	Effect of water content in glycerol on continuous MAG	100
	production by IM-PS in PBR	
39	Time course of continuous MAG production by IM-PS in	101
	PBR using 10 %(w/w) water in glycerol	
40	Effect of substrate flow rate on continuous MAG production	103
	by IM-PS in PBR	
41	Time course of continuous MAG production by IM-PS in	104
	PBR using substrate flow rate of 0.02 mL/min	
42	Effect of temperature on continuous MAG production by	106
	IM-PS in PBR	

Figure		Page
43	Time course of continuous MAG production by IM-PS in	
	PBR at 45 °C	
44	Continuous MAG production in solvent system by IM-PS in	109
	PBR	
45	TLC/FID chromatogram of product mixture from	110
	continuous MAG production in solvent system by IM-PS in	
	PBR	
46	GC chromatogram of MAG in product mixture from	112
	continuous MAG production in solvent system by IM-PS in	
	PBR	
47	Time course of continuous MAG production in solvent	114
	system by IM-PS in PBR	
48	Large scale of continuous MAG production by IM-PS in	116
	PBR	
49	TLC/FID chromatogram of MAG product after	120
	crystallization in acetone/isooctane mixture (3:1,v/v)	
	at –5 °C for 8 h	
50	GC chromatogram of MAG after crystallization in	121
	acetone/isooctane mixture (3:1,v/v) at -5 °C for 8 h	
51	TLC/FID chromatogram of MAG product after	126
	crystallization in isooctane at 0 °C for 8 h	
52	GC chromatogram of MAG after crystallization in isooctane	127
	at 0 °C for 8 h	
53	Fractionation of crude MAG product by silica gel column	129
	chromatography	

Figure		Page
54	TLC/FID chromatogram of MAG product after fractionation	130
	by silica gel column	
55	GC chromatogram of MAG after fractionation by silica gel	131
	column	
56	Standard curve of albumin bovine serum	147
57	Standard curve of palmitic acid	148
58	GC chromatogram of standard fatty acid mixture	149
59	TLC/FID chromatogram of standard oil compositions	150

List of Abbreviations

μg	=	microgram
μL	=	microlitre
μm	=	micrometre
μmol	=	micromole
μV	=	microvolt
ANOVA	=	Analysis of Varian
AOAC	=	Association of Agriculture Chemists
AOT	=	bis-(2ethylhexyl)sodium sulfosuccinate)
BSTR	=	batch stirred-tank reactors
С	=	degree celsius
C_{E}	=	enzyme concentration
cm	=	centimetre
CRD	=	Completely Randomized Design
$C_{\rm S}$	=	substrate concentration
CSTR	=	continuous stirred-tank reactors
CVL	=	Chromobacterium viscosum lipase
DAG	=	diacylglycerols
DHA	=	docosahexaenoic acid
DMRT	=	Duncan's Multiple Range Test
E	=	enzyme
EPA	=	eicosapentaenoic acid
ES	=	enzyme-substrate complex
FBR	=	fluidized-bed reactors
FFA	=	free fatty acids
FID	=	flame ionization detector
g	=	gram
GC	=	gas chromatography

List of Abbreviations (Continue)

GRAS	=	generally recognized as safe
h	=	hour
HDPE	=	high density polyethylene
ID	=	internal diameter
IM-PS	=	immobilized lipase PS
JAOCS	=	Journal of the American Oil Chemists' Society
k_1	=	first order rate constant
<i>k</i> ₋₁	=	first order rate constant for the decomposition of the ES
		complex
$k_{ m ads}$	=	second order adsorption rate constant
<i>k</i> _{cat}	=	maximum number of substrate molecules reacting per
		active site per second
k _{des}	=	first order desorption rate constant
kg	=	kilogram
Km	=	Michaelis constant
L	=	litre
М	=	molarity
m	=	metre
MAG	=	monoacylglycerols
mg	=	milligram
min	=	minute
mL	=	millilitre
mm	=	milimetre
mM	=	milimolar
mol	=	mole
MR	=	membrane reactor
mV	=	milivolt
MW	=	molecular weight

List of Abbreviations (Continue)

Ν	=	Normality
nm	=	nanometre
OD	=	optical density
000	=	triolein
Р	=	product
PBR	=	packed-bed reactor
PCL	=	Pseudomonas cepacia lipase
PFAD	=	palm oil fatty acid distillate
PFL	=	Pseudomonas fluorescens lipase
PLO	=	1-palmitoyl-2-linoleoyl-3-oleoyl-glycerol
PLP	=	1,3-dipalmitoyl-2-linoleoyl-glycerol
PLSt	=	1-palmitoyl-2-linoleoyl-3-stearoyl-glycerol
PMF	=	palm midfraction
POO	=	1-palmitoyl-2,3-dioleoyl-glycerol
POP	=	1,3-dipalmitoyl-2-oleoyl-glycerol
POSt	=	1-palmitoyl-2-oleoyl-3-stearoyl-glycerol
PP	=	polypropylene
PPP	=	tripalmitin
PVDF	=	polyvinylidene fluoride
Q	=	free fatty acid
rpm	=	revolutions per minute
S	=	second
S	=	substrate
sn	=	stereospecific numbering
SPSS	=	Statistical Package for Social Sciences
TAG	=	triacylglycerols
Tc	=	critical temperature
TLC	=	thin-layer chromatography

List of Abbreviations (Continue)

tot	=	total amount of enzyme
U	=	unit of enzyme
V	=	velocity
v/v	=	volume by volume
Vmax	=	maximum velocity
W/V	=	weight by volume
wt	=	weight