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APPENDICES

APPENDIX A

CALCULATIONS

A1. Oil viscosity determination

Oil viscosity was calculated by ASTM D445 Standard Test Method for kinematics viscosity of transparent and opaque liquids as calculated by equation A 1.1 and A 1.2;

Equation.A 1.1

Kinematic viscosity (mm^2/s) = Viscometer constant x efflux time (second)

Equation.A 1.2

Viscosity (mPa·s) = Kinematic viscosity x Density of oil (g/l)

Example, The viscosity calculation of refined palm oil.

The efflux time is 102 seconds (from the experiment)

Take the efflux time in the equation. A 1.1

Kinematic viscosity (mm^2/s) = 0.01069 (mm^2/s^2) x 6120 (second)

Then, take kinematic viscosity to the equation. A 1.2

Viscosity (mPa·s) = 65.42 x 937.5 (g/l)

Viscosity (mPa·s) = 61,333.88 mPa·s

A2. Calculation of water content

The water content in refined and used frying palm oil was performed by Karl-Fischer tritration (method as describe in chapter 3, section 4.2.3). This result was analyzed by Science Service Department and the calculation as equation A 1.3:

Equation A 2.1

$$\text{Water content \%} = [2 \cdot (\text{mL reagent} \cdot C)]/\text{g sample}$$

Where

C = mg H_2O/mL of Karl Fischer reagent

A3. Molecular weight of oil calculated from molecular weight of triglyceride.

Triglyceride (TAG) is the major composition in oil. Therefore, the molecular weight of triglyceride represents the molecular weight of oil.

To calculate the molecular weight of TAG (i.e. molecular weight of oil), equation A 3.1 was used.

$$MW_{TAG} = 173 + 3\,R_{av} \qquad \text{Equation A 3.1}$$

Where,

MW_{TAG} = Molecular weight of triglyceride

R_{av} = Average value molecular weights of fatty acids

$$= \frac{(\%\text{Fatty acid} \times (MW_{FA} - 45))}{100}$$

% Fatty acid = Percentage of each fatty acid in oil

MW_{FA} = Molecular weight of fatty acid

The example of triglyceride in used frying oil calculation

R_{av} = Average value molecular weights of fatty acids

$$= \frac{(\%\text{Fatty acid} \times (MW_{FA} - 45))}{100}$$

$$= (0.25 \times (214.35 - 45) + (0.73 \times (242.4 - 45)) + (37.36 \times (270.46 - 45)) + (0.98 \times (268.44 - 45)) + (0.98 \times (268.44 - 45)) + (3.97 \times (298.51 - 45)) + (43.99 \times (296.50 - 45)) + (12.20 \times (294.48 - 45)) + (0.26 \times (292.47 - 45)) + (0.24 \times (326.57 - 45))$$

$$= 240.73$$

$MW_{TAG} = 173 + 3 R_{av}$

$= 173 + 3(240.73)$

Therefore molecular weight of TAG in oil = 895.19

A4. Calculation of hydrolytic activities of immobilized lipase

The hydrolytic activity of immobilized lipase was measured with the increasing of *ρ*-nitrophenol in the reaction. The increasing of *ρ*-nitrophenol was measured in spectrophotometer (Zenyth 200rt/25700) at the absorbance at 410 nm (method describe in chapter 3, section 4.3.5). The result of hydrolytic activity of immobilized lipase as shows in Table.C.6 and Table.C.7 was calculated by :

$$Y = 0.19X + 0.031$$

Where

Y = OD at 410 nm

X = μg of *ρ*-nitrophenol

(standard calibration curve of of *ρ*-nitrophenol as shows in Fig. C-5.)

$$\text{Hydrolytic activity} = \frac{X}{MW_{pNP} \cdot W_{im} \cdot t}$$

Where

X = μg of *ρ*-nitrophenol

MW_{pNP} = Molecular weight of *ρ*-nitrophenol (139.11)

W_{im} = Weight of immobilized lipase

A5. Calculation of the percentage of fatty acid methyl ester conversion by GC.

FAMEs were produced as the biodiesel production of transesterification. Samples were taken peiodically and the reaction was followed by GC, using GC 2010 series (Shimadzu, Japan) instrument equipped with a 30 m x 0.25 mm DB-WAX capillary column. Methyl heptadodecanoate was used as the internal standard. GC calibrations were performed relatively to FAME at five different concentration levels in relation to methyl heptadodecanoate (see in Appendex D-1.). All FAMEs were assumed to have four values from major FAME composition in used frying palm oil (as shows in Appendex A-2). Then the FAME conversion (%) results as show in section 4.3 were calculated by

$$\text{FAME conversion (\%)} = \frac{C_{FAME} \times 100}{3 \times C_{FA}}$$

Where

C_{FAME} = Concentration of FAME (M) in the sample used

C_{FA} = Concentration of the initial fatty acid (M) in the used frying palm oil (Appendex A- 6)

A6. Calculation of initial fatty acid in the used frying palm oil.

As the result of fatty acid composition (Appendex B4), the four major fatty acids in used frying palm oil were oleic acid (43.99%), palmitic acid (37.36%), linoleic acid (12.20%) and stearic acid (3.97%). Three grams of used frying palm oil was used in all of the experiment in section 4.3 that had initial fattys acid as follow:

$$\text{oleic acid} = \frac{3 \times 43.99}{100}$$

$$= 1.32 \text{ g or } 4.14 \times 10^{-3} \text{ mole}$$

$$\text{palmitic acid} = \frac{3 \times 37.36}{100}$$

$$= 1.12 \text{ g or } 4.45 \times 10^{-3} \text{ mole}$$

$$\text{linoleic acid} = \frac{3 \times 12.20}{100}$$

$$= 0.36 \text{ g or } 4.02 \times 10^{-3} \text{ mole}$$

$$\text{stearic acid} = \frac{3 \times 3.97}{100}$$

$$= 0.12 \text{ g or } 1.22 \times 10^{-3} \text{ mole}$$

Total fatty acids $= 4.14 \times 10^{-3} + 4.45 \times 10^{-3} + 4.02 \times 10^{-3} + 1.22 \times 10^{-3}$

$=$ 0.01 mole/used frying palm oil 3.2 ml

or

$=$ 3.19 mole/used frying palm oil 1000 ml

Thus

The initial concentration of fatty acids were 3.19 M

A7. Calculation of K_m and V_{max} of the transesterification reaction.

When methanol was used as substrate of the transesterificaion reactionK_m and V_{max} (as shows in Table.4.8 and Table.4.9) were calculated from Lineweaver-Burk plots as follow :

$$V_o = Vmax\ [MeOH]/(Km+[MeOH])$$

or

$$1/V_o = Km/Vmax \cdot 1/[MeOH] + 1/Vmax$$

Where

V_o = Initial velocity of the reaction

K_m = Michalis constant

[MeOH]= Concentration of methanol (M)

So

Slope = Km/Vmax

X - intercept = -1/Km

A8. Calculation in biodiesel quality test

The parameters of biodiesel quality (as shows in Table.4.10) were carried out by HPLC (LC -20 A, Shimadzu) with 30m x 0.53 mm Apollo Sillica 5U column (method as described in chapter 3, section 4.5.1) calculated by follow :

$$\text{Percentage by weight of methyl ester (\%)} = \frac{W_{MES} \times 100}{W_{TAG} + W_{DAG} + W_{MAG} + W_{ME} + W_{FFA}}$$

$$\text{Percentage by weight of triglyceride (\%)} = \frac{W_{TAGS} \times 100}{W_{TAG} + W_{DAG} + W_{MAG} + W_{ME} + W_{FFA}}$$

$$\text{Percentage by weight of diglyceride (\%)} = \frac{W_{DAGS} \times 100}{W_{TAG} + W_{DAG} + W_{MAG} + W_{ME} + W_{FFA}}$$

$$\text{Percentage by weight of monoglyceride (\%)} = \frac{W_{MAGS} \times 100}{W_{TAG} + W_{DAG} + W_{MAG} + W_{ME} + W_{FFA}}$$

$$\text{Percentage by weight of linolenate (\%)} = \frac{W_{L} \times 100}{W_{TAG} + W_{DAG} + W_{MAG} + W_{ME} + W_{FFA}}$$

Where

W_{ME}	=	Weight of methyl ester (g)
W_{MES}	=	Weight of methyl ester in the sample (g)
W_{TAG}	=	Weight of triglyceride (g)
W_{TAGS}	=	Weight of triglyceride in the sample (g)
W_{DAG}	=	Weight of diglyceride (g)
W_{DAGS}	=	Weight of diglyceride in the sample (g)
W_{MAG}	=	Weight of monoglyceride (g)

W_{MAGS} = Weight of monoglyceride in the sample (g)

W_{FFA} = Weight of free fatty acid(g)

W_{FFAS} = Weight of free fatty acid in the sample (g)

W_L = Weight of linolenate (g)

APPENDIX B

PROPERTIES OF REFINED AND USED FRYING OIL

B1. Preparation of used frying oil

Method for frying chicken with safety ("Frying chicken with safety," Food and Drug Administration report (November 2002))

Food and Drug Administration reported the frying chicken with safety method on 24 November 2002 that the temperature and time for frying chicken at the market should be shown in Table B-1

Table B1-1 Frying chicken condition with safety

Chicken's Type	Temperature (^{o}C)	Time (min)
Legs	160-170	15
Breast	170-190	15
Thigh	170-190	15
Wings	160-170	15
Chicken Sausage	150-160	3-5 (Should boiled before frying at 75-80^{o}C)

This condition, chicken must fry under the deep–frying oil.

B2. Color intensity of refined and used frying palm oil.

The color of refined and used frying palm oil was measured by with gray scale of the oil color change photograph into the transillumeter (developed by Vilber Lourmat, France) using Bio-PROFIL program. The higher color intensity represents as the light color. The percentage of color intensity change of used frying palm oil was measured by comparison with refined palm oil (as shows in Table B2-1).

Table B2-1 The color intensity of refined and used frying palm oil was calculated using Gel documentation.

Type of oil	Color [a] intensity value	Percentate of color[b] intensity change
Refined palm oil	204	1.00
the 3 rd cycle of frying palm oil	177	11.76
the 6 th cycle of frying palm oil	162	20.58
the 9 th cycle of frying palm oil	142	30.39
the 12 th cycle of frying palm oil	122	40.19
the 15 th cycle of frying palm oil	108	47.06
the 18 th cycle of frying palm oil	104	49.02
the 21 th cycle of frying palm oil	90	55.88

a: The higher color intensity value represent as the light color, which measured by gel documentation (developed by Vilber Lourmat).

b: The percentage of color intensity change (%) was the relative value of refined palm oil (frying cycle number 0).

B3. Viscosities of refined and used frying palm oil.

Viscosity was determined at room temperature using a standard method (ASTM D445). The viscosities of refined and used frying palm oil were measured by using a viscometer (D2170) with viscosities constant is 0.01069 mm^2/s^2, (cSt/s) (method as described in chapter 3, section 4.2.2). The viscosities of refined and used frying palm oil as shows in Table. B3-1.

Table B3-1 Viscosities of refined and used frying palm oil.

Frying cycles	Viscosity (mPa·s)
	Average
0 (Refined palm oil)	61333.88±601.31
1	62135.63±1251.73
2	65342.63±1837.04
3	67146.56±347.17
4	65543.06±1041.50
5	67547.44±918.52
6	68950.50±918.52
7	67948.31±601.31
8	68148.75±347.17
9	68549.63±601.31
10	68750.06±2276.53
11	69150.94±1202.63
12	68950.50±2276.53
13	66545.25±1837.04
14	67747.88±1932.95
15	68950.50±694.34
16	69351.38±347.17
17	68950.50±347.17
18	69752.25±0.00
19	69351.38±918.52
20	68549.63±601.31
21	67547.44±918.52

B4. Fatty acid composition of refined palm oil and the 21 cyc oil.

B4.1 Analysis of the intermediate products during transesterification reaction of the 21 cyc oil.

The chemical conversion of triglyceride to methyl ester was deterimined using thin layer chromatography (TLC) (chapter 3, section 4.4.2.1). The TLC plate(TLC aluminium sheets 20 x 20 cm,Silica gel 60 F254; Merck,Darmstadt,Germany) was developed by a solvent system containing hexane:ethyl acetate: acetic acid (90:10:2), and will be stained by methanol:sulfuric acid(1:1) . Then , the plate was heated at 110 ^{o}C for 30 minutes (Samukawa *et al.*, 2000). The FAME was converted approximately 98 % (Fig. B4-1). Then the retention factor of intermediate production shows in Table. B4-1 and calculate as follow:

$$\text{Retention factor } (R_f) = \frac{\text{distance mixture component has traveled}}{\text{distance mobile phase has traveled}}$$

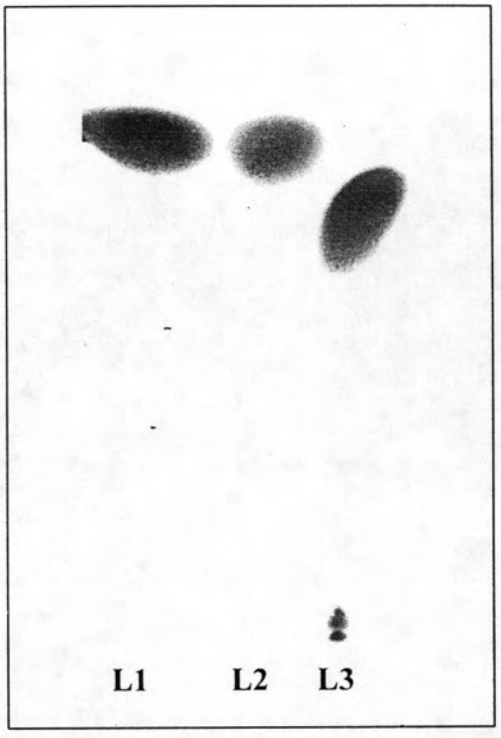

Fig. B4-1 Analysis of the intermediate products during transesterification reaction of the 21 cyc oil oil (condition in chapter 3, section 4.4.2.1). L1: Standard FAME, L2: Sample and L3: Palm oil (Triglyceride).

Table B4-1 The retention factor of the FAME, triglyceride and sample.

No	Sample	R_f
L1	Standard FAME	0.76
L2	Sample	0.75
L3	Palm oil (Triglyceride)	0.67

B4.2 Calculation of each fatty acids composition in oil.

Fatty acids composition in oil (Table.B4-3) were performed by GC using DB-Wax column (length 30 m, internal diameter 0.53 mm, film thickness 0.25 μm). The operating conditions were as follows: injection volumn: 1 μl, column temperature programe: 70°C hold for 3 min, programmed at 20°C/min up to 220°C, programmed at 10°C/min up to 230 final temperature hold for 3 min. The chromatogram of fatty acid methyl ester as shows in Fig. B4-2: The percentage of each fatty acid in oil was calculated as follows :

$$\text{Percentage of fatty acid (\%)} = \frac{\text{Peak area of fatty acid}}{\text{Total peak area of fatty acids}} \times 100$$

The example of fatty acid composition in the 21 st cycle frying oil.

$$\text{Lauric acid (\%)} = \frac{8484 \times 100}{8484+24433+1255570+33263+133343+1478581+410023+8811+7906}$$

$$= 0.25\ \%$$

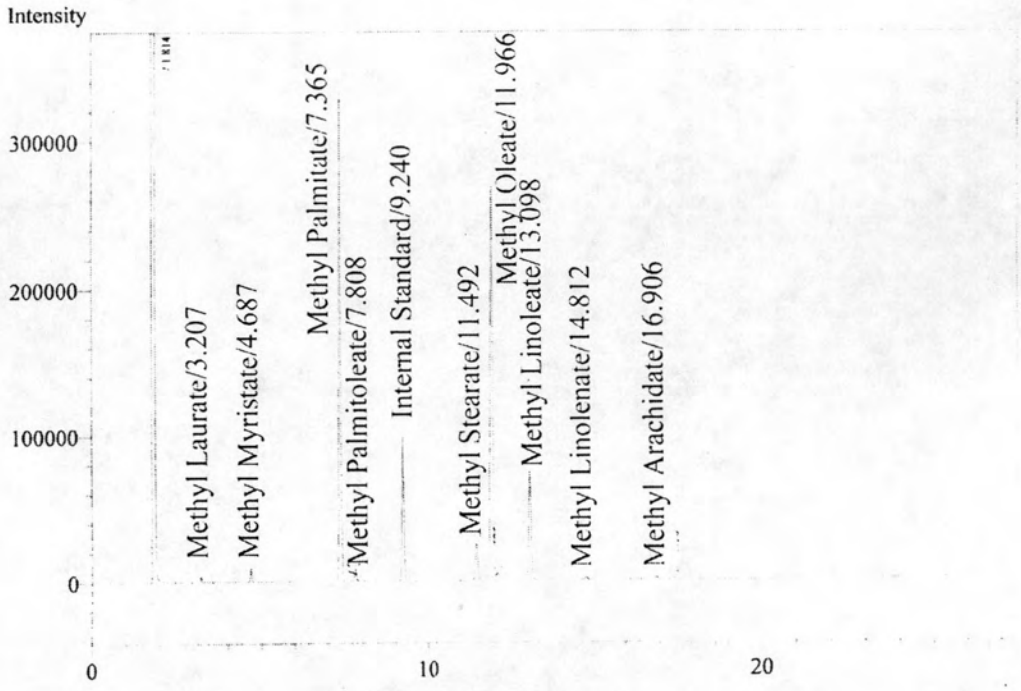

Fig. B4-2 Chromatogram of methyl ester from transesterification of the 21-cyc oil with sodium hydroxide at 50^0C, 300 rpm, 24 hours (method 4.4.2).

Table B4-2 Peak area and peak height of standard fatty acid methyl ester, used in the calculation of fatty acid composition in oil.

Peak	Compound Name	RT	Area	Height
1	Methyl Laurate	3.207	8484	4037
2	Methyl Myristate	4.687	24433	9108
3	Methyl Palmitate	7.365	1255570	331947
4	Methyl Palmitoleate	7.808	33263	8289
5	Internal Standard	9.240	432937	98693
6	Methyl Stearate	11.492	133343	25121
7	Methyl Oleate	11.966	1478581	269011
8	Methyl Linoleate	13.098	410023	75263
9	Methyl Linolenate	14.812	8811	1471
10	Methyl Arachidate	16.906	7906	1232

Table.B4-3 Fatty acid composition of refined palm oil and the 21-cyc oil.

Fatty acid		Fatty acid (%)	
		Refined palm oil	**Used frying palm oil**
Saturated			
Lauric acid	C12:0	0.29	0.25
Myristic acid	C 14:0	0.77	0.73
Palmitic acid	C 16:0	41.76	37.36
Stearic acid	C 18:0	3.83	3.97
Arachidic acid	C 20:0	0.29	0.24
Total Saturated		**46.94**	**42.55**
Unsaturated			
Palmitoleic acid	C 16:1	0.17	0.98
Oleic acid	C 18:1	42.83	43.99
Linoleic acid	C 18:2	9.88	12.20
Linolenic acid	C 18:3	0.17	0.26
Total Unsaturated		**53.05**	**57.43**
Other		0.01	0.02
Total		**100.00**	**100.00**

APPENDEX C

PAHs

C1. Preparation standard calibration curve of PAHs

Naphthalene and benzo[a]pyrene was choosen to be the PAHs' standard and dissolved in acetonitrile. Analyses were performed by HPLC using Hewlett Packard ODS Hypersil (5 μm, 250x4 mm) column. The operating conditions were as follows: injection volumn: 30 μl, flow rate: 1.0 ml/min, pressure: 45-46 bar, wave length = 254 nm and 100% acetonitrile as the mobile phase. The example of the PAHs chromatogram as shows in Fig. C1-1:

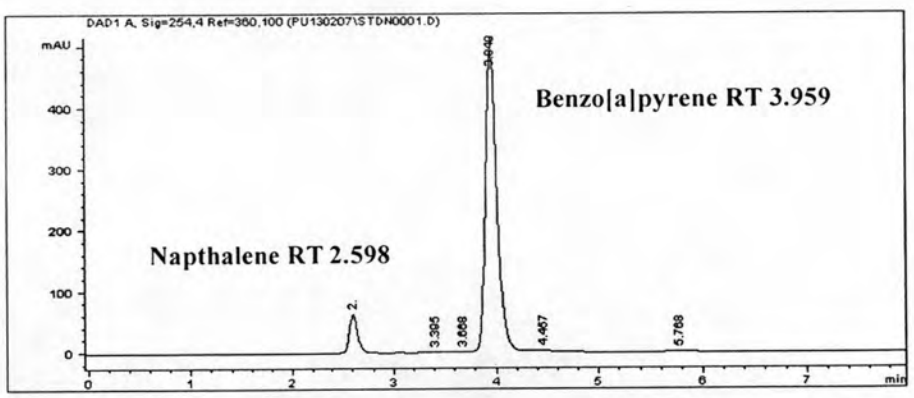

Fig. C1-1 Chromatogram of naphthalene and benzo[a]pyrene standard.

RT of naphthalene was 2.598

RT of benzo[a]pyrene was 3.959

C-1.1 PAHs stock solution 100 mM

PAHs standard in the experiment was prepared in 10 FAMEs. The weight of naphthalene and benzo[a]pyrene standard in stock solutions of 100 mM mixed PAHs standard was given in Table C-1 using auto pipette. The choice of the auto pipette shall be done according to Table C1-1.

Table C1-1 Preparation of 100 mM PAHs stock solution

Substance	M.W.	Volume/weight
Naphthalene	128.17	0.0128 g
Benzo[a]pyrene	252.32	0.0252 g
Acetonitrile	41.05	1000 µl

C1.2 Preparation of PAHs calibration standard curve

The naphthalene and benzo[a]pyrene standard were dissolved in acetonitrile with five different level of concentration (calibration curve of naphthalene shows in Fig.C1-2 a and calibration curve of benzo[a]pyrene shows in Fig. C1-2 b).

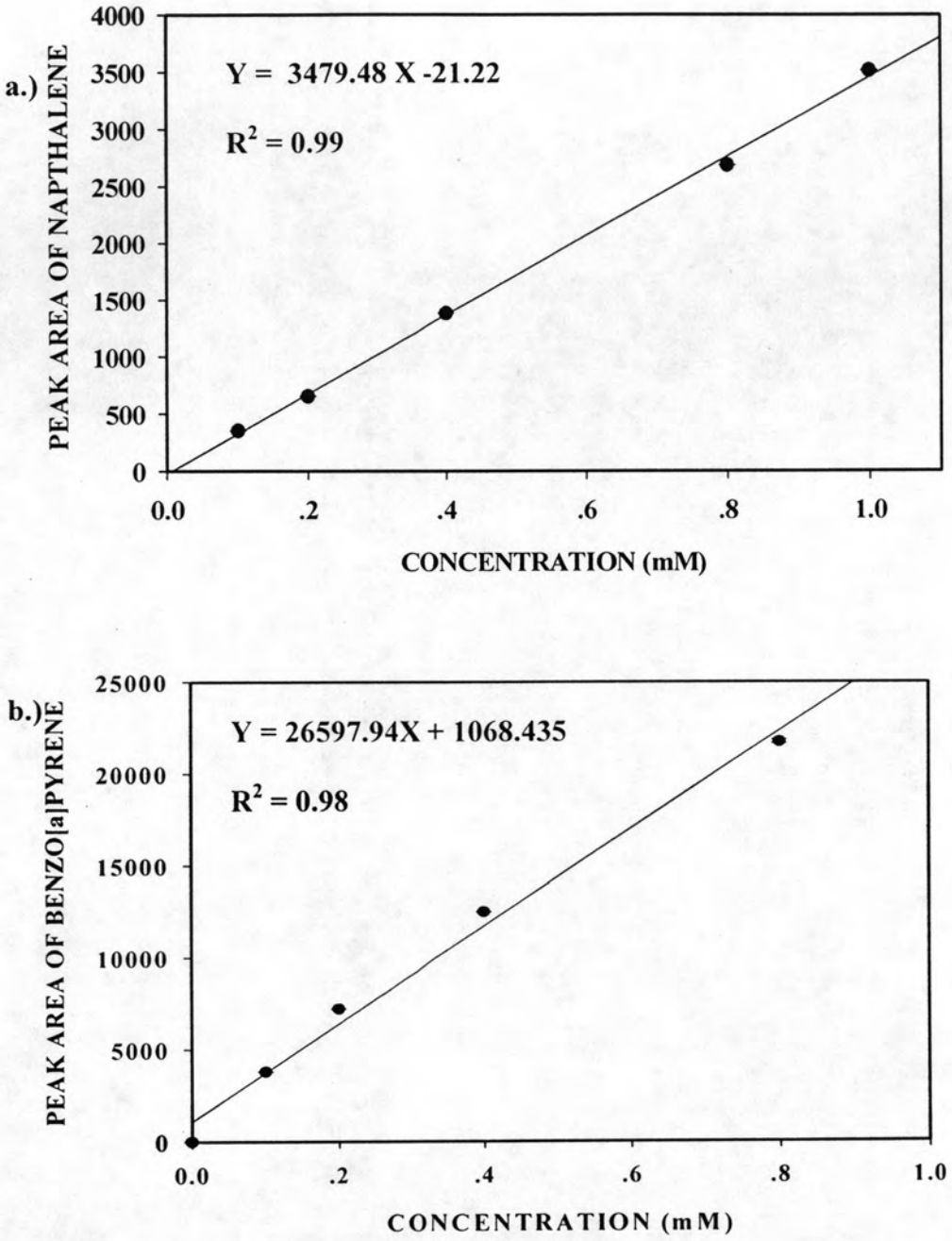

Fig. C1-2 Calibration curve of PAHs

a: Calibration curve of naphthalene

b: calibration curve of benzo[a]pyrene

Table C1-2 Peak area of naphthalene standard.

Concentration of Naphthalene (mM)	Peak area Average(mAU·s)
0.1	352.50±0.46
0.2	655.33±0.51
0.4	1384.03±0.70
0.8	2683.43±0.65
1	3517.33±3.54

Table C1-3 Peak area and peak height of benzo[a]pyrene standard.

Concentration of Benzo[a]pyrene (mM)	Peak area Average (mAU·s)
0.1	3797.07±7.93
0.2	7211.33±8.93
0.4	12489.05±5.09
0.8	21741.63±101.23

C2. Determination of PAHs in refined and used frying palm oil.

PAHs in refined and used frying palm oil were extracted by acetonitrile (method as described in section 3.4.3.1) and performed by HPLC Agilent 1,100 series (Algilent Technologies, Wilmington, DE, USA). Sample was dissolved in acetonitrile (method of PAHs analyses as described in section 3.4.3.3). The PAHs in refined and used frying oil before transesterified was quantified and shows in Table.C2-1.

Table C2-1 The naphthalene concentration in refined and used frying palm oil

Frying cycles	HPLC peak area	Concentration (μM)
	Average (mAU·s)	Average
Refined palm oil	47.39±34.92	197.00±57.58
3	105.47±100.13	364.33±165.31
6	119.97±4.27	405.67±75.07
9	142.91±11.93	472.00±215.79
12	178.79±10.67	575.00±24.55
15	240.88±30.99	753.33±70.86
18	299.72±32.22	922.33±89.24
21	551.46±92.67	1646.00±256.82

C3. Adsorption of naphthalene and benzo[a]pyrene in the immobilized lipase

Naphthalene and benzo[a]pyrene were eluted from the immobilized lipase by liquid-liquid extraction (method describe in section 3.4.3.4) or acetonitrile as the solvent in the reaction. The analyses of PAHs were performed by HPLC Agilent 1,100 series (Algilent Technologies, Wilmington, DE, USA). Sample was dissolved in acetonitrile (method of PAHs analyses as described in section 3.4.3.3).The quantification of naphthalene and benzo[a]pyrene in immobilized lipase shows in Table.C3-1 and Table.C3-2 respectively.

Table C3-1 The adsorption of naphthalene on the immobilized lipase: Lipozyme RM IM and Novozym 435.

Immobilized Lipase	Naphthalene	
	HPLC peak area (mAU·s)	Concentration (μM)
	Average	Average
Lipozyme RM IM	911.70	268.0±1.61
Novozym 435	1531.89	446.4±7.21

Table. C3-2 The adsorption of benzo[a]pyrene on the immobilized lipase: Lipozyme RM IM and Novozym 435.

Immobilized Lipase	Benzo[a]pyrene	
	HPLC peak area(mAU·s)	Concentration (μM)
	Average	Average
Lipozyme RM IM	8370.79	274.5±0.02
Novozym 435	7798.93	253.0±0.39

C4. *ρ*-nitrophenol for hydrolytic activities assay of immobilized enzyme

Activity of immobilized lipase was assayed using 0.5% (w/v) *p*-nitrophenyl palmitate in ethanol as a substrate (chapter 3, section 4.3.5). The increasing in the absorbance at 410 nm means the releasing of *p*-nitrophenol in the enzymatic hydrolysis of *p*-nitrophenylpalmitate. The standard curve of *p*-nitrophenol shows in Fig. C4-1.

Various concentration of *ρ*-nitrophenol was prepared in distilled water. The adsorbance at 410 nm of each solution was determined.

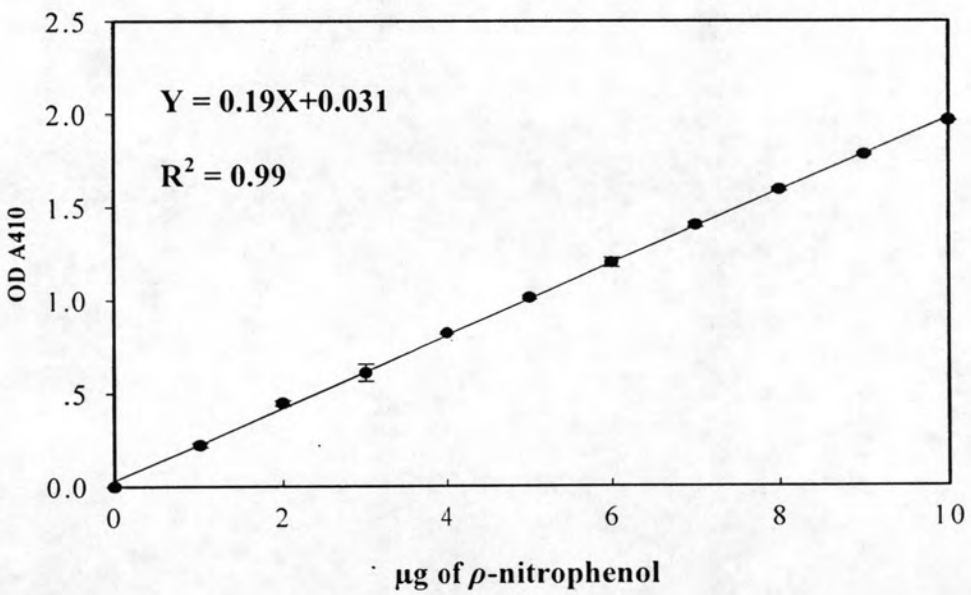

Fig. C4-1 Standard calibration curve of *ρ*-nitrophenol

Table C4-1 Effect of napthalene on hydrolytic activity of immobilized lipase (Lipozyme RM IM and Novozym 435)

	Relative remaining hydrolytic activity						
		By suction			Washing by Hexane		
	Hydrolytic Activity	12 hr	24 hr	48 hr	12 hr	24 hr	48 hr
Lipozyme RM IM	100±0.004	20.97±2.36	19.99±2.21	20.09±2.39	104.36±17.42	110.06±21.35	102.12±43.14
Novozym 435	100±0.005	46.44±2.39	24.26±2.46	18.32±2.27	81.02±12.39	16.42±2.21	16.55±4.13

Table. C4-2 Effect of benzo[a]pyrene on hydrolytic activity of immobilized lipase (Lipozyme RM IM and Novozym 435)

	Relative Hydrolytic Activity						
		By suction			Washing by Hexane		
	Hydrolytic Activity	12 hr	24 hr	48 hr	12 hr	24 hr	48 hr
Lipozyme RM IM	100±0.004	20.65±0.26	22.82±0.25	22.13±0.31	130.00±2.38	90.11±4.98	87.77±0.24
Novozym 435	100±0.005	12.12±2.19	13.39±0.08	12.98±0.11	83.08±0.15	23.28±0.07	18.49±4.45

APPENDIX D

ANALYSIS OF METHYL ESTER

D1. Calibration curve of FAME

D1.1 Internal standard stock solution 50 mM

Methyl heptadodecanoate is the internal standard in the analysis of FAME by GC (condition described in D-1.3). Accurately weigh approximately 0.0142 g of methyl heptadodecanoate in a vial and make up to 1000 µl by hexne as the solvent.

D1.2 FAME stock solution 12 mM

FAMEs standard in the experiment had 10 FAMEs. The volumes of each FAME standard in stock solutions of 12 mM mixed FAMEs standard was given in Table D1-1 using auto pipette. The choice of the auto pipette shall be done according to Table D1-1.

Table D1-1 Preparation of 12 mM FAME stock solution.

Substance	M.W.	Volume/weight
Methyl dodecanoate or methyl laurate	214.35	2.95 µl
Methyl myristate	242.4	3.35 µl
Methyl palmitate	270.46	0.00324 g
Methyl palmitoleate	268.44	3.68 µl
Methyl stearate	298.51	0.00358 g
Methyl oleate	286.50	4.1 µl
Methyl linoleate	294.48	3.98 µl
Methyl linolenate	292.47	3.9 µl
Methyl arachidate	326.62	0.00391 g
Methyl behanate	354.62	0.00425 g
Hexane	86.18	978.04 µl

D1.3 Preparation of FAME calibration standard curve

Prepare daily five calibration solutions by transferring into a series of vials. The volumes of stock solution of FAME standard (D1.2) and of internal standard (D1.1) given in Table D1-1, using auto pipette. The choice of the appropriate auto pipette shall be done according to Table D1-1. After that, FAMEs were performed by GC with condition in section D2 and the calibration curve of FAMEs show in Fig. D2-1.

Table D1-2 Preparation of FAME calibration solutions

Concentration of FAME (mM)	Volume of FAME stock solution (µl)	Volume of hexane (µl)	Volume of internal standard(µl)
0.25	20.83	969.17	10
1.0	83.33	906.67	10
2.5	208.33	781.67	10
5.0	416.67	573.33	10
10.0	833.33	156.67	10

D2 Operation FAME condition by GC

FAME was performed by GC using DB-Wax column (length 30 m, internal diameter 0.53 mm, film thickness 0.25 µm). The operating conditions were as follows: injection volumn: 1 µl, column temperature program: 70°C hold for 3 min, programmed at 20°C/min up to 220°C, programmed at 10°C/min up to 230 final temperature hold for 3 min. The example of the FAME chromatogram as shows in Fig. D2-2:

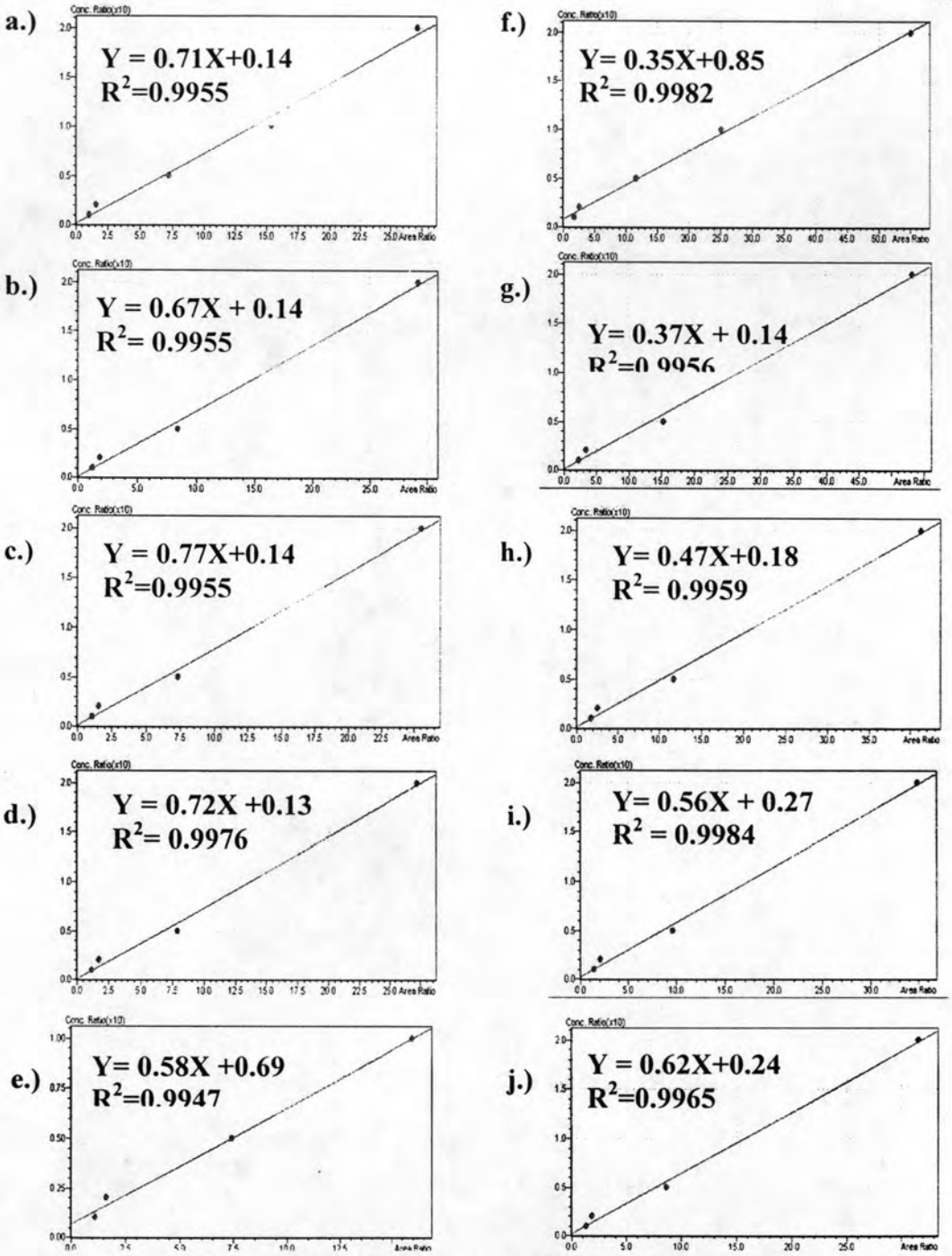

Fig. D2-1 Calibration curve of fatty acid methyl ester

a.) Methyl laurate, b.) Methyl myristate, c.) Methyl palmitate,

d.) Methyl palmitoleate, e.) Methyl stearate, f.) Methyl oleate,

g.) Methyl linoleate h.) Methyl linolenate, i.) Methyl behanate,

j.) Methyl arachidate

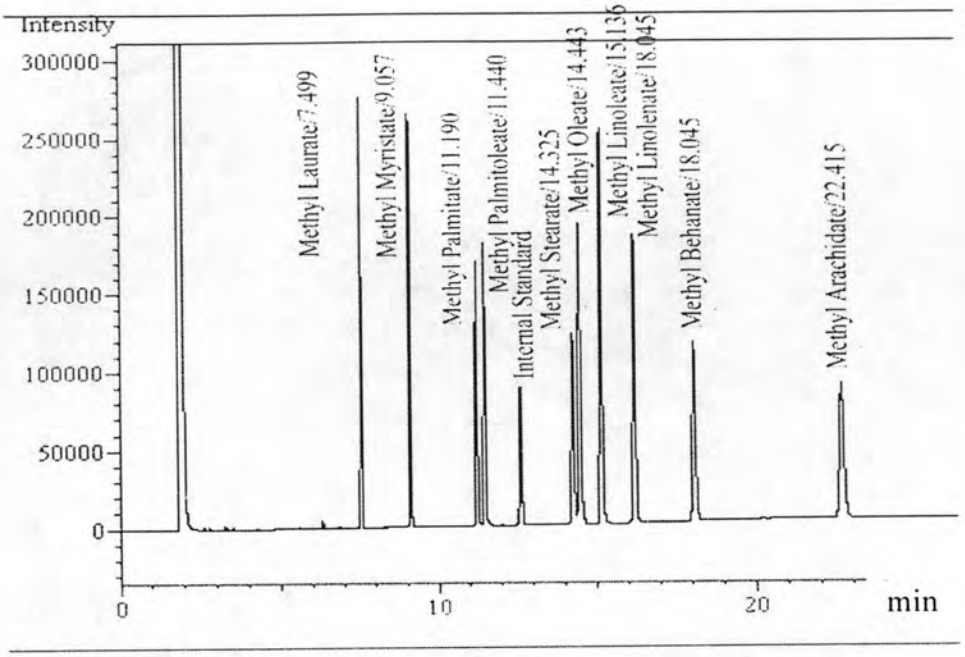

Fig. D2-2 Chromatogram of fatty acid methyl ester standard

D3 Raw data of FAME from the experimental in chapter 3, section 4.4

D3.1 Concentration of FAME in one- step methanolysis.

Methanolysis was carried out in chapter 3, section 4.4.3.1 with the one- step batch methanolysis condition using Lipozyme RM IM and Novozym 435 at 40°C (chapter 3, section 4.4.3.1.1). The substrate from the reaction using Lipozyme RM IM and Novozym 435 were converted to FAME or biodiesel as shows in Table D3-1 and Table D3-2.

Table D3-1 FAME production in one-step methanolysis using 20% Lipozme RM IM as the catalyst.

% Water	Time (hr)	Concentration of FAME (M)					Con[a] (%)
		M. Palmitate	M. Stearate	M. Oleate	M. Linoleate	Total FAME	
Non water	1	0.1838	0.3512	0.4669	0.0810	1.0828	11.31
	2	0.2682	0.3569	0.4965	0.0894	1.2109	12.65
	3	0.4607	0.3697	0.5655	0.1075	1.5034	15.71
	6	0.5829	0.3778	0.6132	0.1204	1.6943	17.70
	8	0.6290	0.3810	0.6288	0.1239	1.7626	18.42
	12	0.7591	0.3914	0.6891	0.1415	1.9810	20.70
	16	0.7552	0.0396	0.6946	0.1467	1.6362	17.10
	24	0.7724	0.3883	0.6851	0.1423	1.9880	20.77
	48	0.7591	0.3914	0.6891	0.1415	1.9810	20.70
5%	1	0.2511	0.3548	0.4875	0.0867	1.1801	12.33
	2	0.4107	0.3654	0.5452	0.1025	1.4237	14.88
	3	0.4139	0.3660	0.5463	0.1026	1.4288	14.93
	6	0.4977	0.3716	0.5800	0.1114	1.5607	16.31
	8	0.6386	0.3804	0.6303	0.1243	1.7736	18.53
	12	0.6668	0.3826	0.6397	0.1271	1.8161	18.98
	16	0.6995	0.3848	0.6606	0.1334	1.8782	19.63
	24	0.7527	0.3899	0.6812	0.1387	1.9625	20.51
	48	0.7660	0.3903	0.6931	0.1421	1.9915	20.81
10%	1	0.4193	0.3667	0.5504	0.1037	1.4401	15.05
	2	0.5098	0.3738	0.8563	0.1125	1.8524	19.36
	3	0.6387	0.3829	0.1554	0.1332	1.3103	13.69
	6	0.6731	0.3840	0.6531	0.1310	1.8413	19.24
	8	0.8308	0.3970	0.7415	0.1567	2.1260	22.22
	12	0.8872	0.3979	0.7428	0.1611	2.1890	22.87
	16	0.9262	0.4053	0.8268	0.1835	2.3417	24.47
	24	0.9275	0.4116	0.8289	0.1873	2.3552	24.61
	48	0.9245	0.4056	0.8514	0.1930	2.3745	24.81
15%	1	0.3194	0.3605	0.5161	0.0948	1.2908	13.49
	2	0.3651	0.3632	0.5324	0.0992	1.3598	14.21
	3	0.4179	0.3672	0.5540	0.1052	1.4442	15.09
	6	0.8513	0.3982	0.7424	0.1572	2.1491	22.46
	8	0.8566	0.3994	0.7923	0.1644	2.2127	23.12
	12	0.9718	0.3996	0.8465	0.1728	2.3906	24.98
	16	1.0778	0.4211	0.8918	0.2026	2.5933	27.10
	24	1.0832	0.4267	0.8919	0.2050	2.6068	27.24
	48	1.1192	0.4199	0.8909	0.1997	2.6297	27.48

a: percentage of FAME conversion (%)

Table D3-1 (cont.)

% Water	Time (hr)	Concentration of FAME (M)					Con[a] (%)
		M. Palmitate	M. Stearate	M. Oleate	M. Linoleate	Total FAME	
20%	1	0.5497	0.3778	0.6058	0.1185	1.6517	17.26
	2	0.6750	0.3870	0.6895	0.1427	1.8942	19.79
	3	0.8794	0.4014	0.7665	0.1637	2.2110	23.10
	6	0.8871	0.4018	0.7735	0.1556	2.2180	23.18
	8	0.8989	0.4030	0.7576	0.1597	2.2193	23.19
	12	0.9260	0.4023	0.7562	0.1594	2.2438	23.45
	16	0.9332	0.4046	0.7610	0.1596	2.2584	23.60
	24	1.0336	0.4130	0.8686	0.1950	2.5101	26.23
	48	1.1705	0.4226	0.9716	0.2270	2.7918	29.17

a: percentage of FAME conversion (%)

Table D3-2 FAME production in one- step methanolysis using 20%Novozym 435 as the catalyst.

% Water	Time (hr)	Concentration of FAME (M)					Con [a] (%)
		M. Palmitate	M. Stearate	M. Oleate	M. Linoleate	Total FAME	
Non water	1	0.1219	0.3484	0.4857	0.0882	1.0443	10.91
	2	0.5719	0.3818	0.7430	0.1636	1.8603	19.44
	3	0.6789	0.3951	0.8733	0.2035	2.1508	22.47
	6	0.7102	0.3951	0.8733	0.2035	2.1820	22.80
	8	0.7247	0.3882	0.7129	0.1595	1.9853	20.74
	12	0.7720	0.4007	0.8870	0.2088	2.2685	23.70
	16	0.7758	0.3921	0.7559	0.1712	2.0950	21.89
	24	0.8382	0.3984	0.8102	0.1873	2.2340	23.34
	48	0.9008	0.4072	0.9027	0.2146	2.4254	25.34
5%	1	0.3727	0.3645	0.5637	0.1119	1.4127	14.76
	2	0.5020	0.3734	0.6226	0.1306	1.6286	17.02
	3	0.5470	0.3769	0.6473	0.1379	1.7091	17.86
	6	0.7763	0.3928	0.7641	0.1731	2.1063	22.01
	8	0.7828	0.3900	0.7643	0.1749	2.1121	22.07
	12	0.8257	0.3961	0.7842	0.1797	2.1857	22.84
	16	0.8781	0.4005	0.8181	0.1882	2.2849	23.88
	24	0.9077	0.4073	0.8229	0.1940	2.3318	24.37
	48	0.9584	0.4069	0.8784	0.2046	2.4483	25.58

a: percentage of FAME conversion (%)

Table D3-2(cont.)

% Water	Time (hr)	Concentration of FAME (M)				Total FAME	Con[a] (%)
		M. Palmitate	M. Stearate	M. Oleate	M. Linoleate		
10%	1	0.4911	0.3733	0.6251	0.1318	1.6213	16.94
	2	0.5237	0.3766	0.7023	0.1538	1.7564	18.35
	3	0.5722	0.3787	0.6619	0.1422	1.7550	18.34
	6	0.7116	0.3889	0.7371	0.1639	2.0016	20.92
	8	0.6672	0.3875	0.7380	0.1642	1.9569	20.45
	12	0.8182	0.3998	0.8556	0.1976	2.2712	23.73
	16	0.8465	0.4009	0.8562	0.1972	2.3008	24.04
	24	0.8525	0.4009	0.8436	0.1935	2.2905	23.93
	48	1.0132	0.4417	1.1680	0.2914	2.9143	30.45
15%	1	0.3646	0.3644	0.5646	0.1124	1.4059	14.69
	2	0.9216	0.4039	0.8256	0.1914	2.3425	24.48
	3	1.1445	0.4196	0.9364	0.2243	2.7248	28.47
	6	1.1922	0.4230	0.9554	0.2308	2.8015	29.27
	8	1.2049	0.4233	0.9633	0.2328	2.8243	29.51
	12	1.2056	0.4249	0.9667	0.2379	2.8351	29.62
	16	1.4112	0.4385	1.0695	0.2634	3.1827	33.26
	24	1.4116	0.4435	1.0699	0.2639	3.1890	33.32
	48	1.4188	0.4442	1.1312	0.2801	3.2743	34.21
20%	1	0.3885	0.3662	0.5867	0.1176	1.4590	15.25
	2	0.3624	0.3641	0.5626	0.1115	1.4005	14.63
	3	0.4073	0.3670	0.5879	0.1185	1.4807	15.47
	6	0.4427	0.3697	0.6039	0.1236	1.5398	16.09
	8	0.4680	0.3712	0.6124	0.1265	1.5781	16.49
	12	0.5244	0.4277	0.6576	0.1346	1.7442	18.23
	16	0.5778	0.3792	0.6644	0.1377	1.7590	18.38
	24	0.5848	0.3774	0.6674	0.1394	1.7690	18.48
	48	0.5872	0.3798	0.6691	0.1433	1.7794	18.59

a: percentage of FAME conversion (%)

D3.2 Concentration of FAME in three- step methanolysis.

Methanolysis was carried out in chapter 3, section 4.4.3.1 with the three- step batch methanolysis condition using Lipozyme RM IM and Novozym 435 at 40°C (chapter 3, section 4.4.3.1.2). The substrate from the reaction using

Lipozyme RM IM and Novozym 435 were converted to FAME or biodiesel as shows in Table D – 6 and Table D-7.

Table D3-3 FAME production in three- step methanolysis using 20%Lipozyme RM IM as the catalyst.

% Water	Time (hr)	Concentration of FAME (M)					Con (%)
		M. Palmitate	M. Stearate	M. Oleate	M. Linoleate	Total FAME	
Non water	1	0.0590	0.0059	0.2761	0.0058	0.3467	3.62
	2	0.1107	0.0111	0.0560	0.0118	0.1895	1.98
	3	0.1693	0.0160	0.0819	0.0172	0.2843	2.97
	6	0.1926	0.0192	0.0902	0.0188	0.3207	3.35
	8	0.3550	0.0343	0.1881	0.0421	0.6195	6.47
	12	0.8046	0.0788	0.5033	0.1131	1.4999	15.67
	14	0.9323	0.0936	0.6556	0.1497	1.8311	19.13
	16	0.9524	0.0905	0.5546	0.1229	1.7203	17.98
	18	0.9789	0.0935	0.5625	0.1244	1.7593	18.38
	24	1.0142	0.0970	0.6142	0.1368	1.8622	19.46
	48	1.1871	0.1133	0.7051	0.1565	2.1620	22.59
5%	1	0.2324	0.0244	0.1111	0.0225	0.3904	4.08
	2	0.3840	0.0425	0.2509	0.0568	0.7341	7.67
	3	0.6517	0.0693	0.3919	0.0858	1.1986	12.52
	6	0.7014	0.0755	0.3820	0.0793	1.2382	12.94
	8	1.0088	0.1009	0.7663	0.1772	2.0532	21.45
	12	1.0523	0.1090	0.7396	0.1668	2.0676	21.61
	14	1.1211	0.1154	0.8367	0.1905	2.2636	23.65
	16	1.1411	0.1169	0.8410	0.1913	2.2904	23.93
	18	1.3364	0.1445	0.6898	0.1414	2.3121	24.16
	24	1.2986	0.1305	0.9505	0.2191	2.5987	27.15
	48	1.5323	0.1541	1.0900	0.2483	3.0248	31.61

a: percentage of FAME conversion (%)

Table D3-3 (cont.)

% Water	Time (hr)	Concentration of FAME (M)					Con[a] (%)
		M. Palmitate	M. Stearate	M. Oleate	M. Linoleate	Total FAME	
10%	1	0.0586	0.0564	0.0283	0.0060	0.1493	1.56
	2	0.4053	0.0371	0.2020	0.0432	0.6874	7.18
	3	0.4540	0.0411	0.2207	0.0472	0.7629	7.97
	6	0.5692	0.1058	0.3327	0.0846	1.0923	11.41
	8	0.6927	0.0708	0.3716	0.3716	1.5067	15.74
	12	1.4070	0.1435	0.9349	0.2095	2.6949	28.16
	14	1.4117	0.1403	0.9788	0.2219	2.7528	28.76
	16	1.4553	0.1456	0.9895	0.2232	2.8136	29.40
	18	1.5958	0.1644	1.0309	0.2294	3.0205	31.56
	24	1.6001	0.1600	1.1552	0.2648	3.1800	33.23
	48	1.6466	0.1674	1.1105	0.2504	3.1750	33.18
15%	1	0.1511	0.0151	0.0727	0.3361	0.5749	6.01
	2	0.5197	0.0597	0.3649	0.0801	1.0243	10.70
	3	0.6617	0.0666	0.3361	0.0703	1.1347	11.86
	6	0.7188	0.0801	0.4391	0.0937	1.3318	13.92
	8	0.7898	0.0833	0.4318	0.0918	1.3968	14.60
	12	1.0762	0.1170	0.8002	0.1797	2.1732	22.71
	14	1.1091	0.1211	0.8467	0.1913	2.2682	23.70
	16	1.1586	0.1239	0.8133	0.1832	2.2789	23.81
	18	1.1946	0.1196	0.8369	0.1894	2.3405	24.46
	24	1.6313	0.1632	1.2270	0.2835	3.3050	34.54
	48	1.8836	0.1876	1.3504	0.3077	3.7292	38.97
20%	1	0.1506	0.1518	0.0732	0.0149	0.3905	4.08
	2	0.5003	0.0666	0.4553	0.1021	1.1242	11.75
	6	0.5162	0.0683	0.4944	0.1104	1.1894	12.43
	8	0.5467	0.0600	0.2962	0.0612	0.9641	10.07
	12	0.8762	0.1052	0.7580	0.1720	1.9114	19.97
	14	1.1509	0.1372	1.0169	0.2308	2.5358	26.50
	16	1.1736	0.1393	1.0429	0.2381	2.5939	27.10
	18	1.5012	0.1505	1.1010	0.2521	3.0047	31.40
	24	1.7014	0.1706	1.2791	0.2947	3.4458	36.01
	48	1.5184	0.1517	1.1546	0.2681	3.0927	32.32

a: percentage of FAME conversion (%)

Table D3-4 FAME production in three-step methanolysis using 20% Novozym 435 as the catalyst.

% Water	Time (hr)	Concentration of FAME (M)					Con [a] (%)
		M. Palmitate	M. Stearate	M. Oleate	M. Linoleate	Total FAME	
Non water	1	80.1000	9.5100	98.4100	23.9400	211.9600	2.21
	2	111.7350	13.4850	157.9250	38.7500	321.8950	3.36
	3	220.3200	27.2750	208.7450	51.7850	508.1250	5.31
	6	280.8450	30.0500	249.9850	62.2750	623.1550	6.51
	8	446.2200	46.1850	360.5150	90.3500	943.2700	9.86
	12	1334.3350	140.9700	1200.9700	277.4350	2953.7100	30.86
	16	1411.5350	143.7200	1368.2150	313.8300	3237.3000	33.83
	24	1551.6950	200.7150	1340.4700	312.0650	3404.9450	35.58
	48	1641.5400	171.0450	1635.0350	381.1900	3828.8100	40.01
5%	1	1707.3900	177.9250	1574.9800	368.5350	3828.8300	40.01
	2	1722.6000	183.7000	1724.5000	285.5500	3916.3500	40.92
	3	146.2900	16.4550	118.4050	29.6200	310.7700	3.25
	6	169.2550	19.6150	144.7000	35.9800	369.5500	3.86
	8	264.9750	27.0400	181.5750	46.0450	519.6350	5.43
	12	316.9050	32.2150	218.8100	55.6950	623.6250	6.52
	16	386.6750	39.4200	267.6950	68.2400	762.0300	7.96
	24	1388.9300	138.1350	962.3500	232.6450	2722.0600	28.44
	48	1577.3300	156.8500	1096.7100	339.2000	3170.0900	33.13
10%	1	1795.1950	178.6900	1288.9150	303.1900	3565.9900	37.26
	2	1954.2100	194.7450	1449.1450	334.6100	3932.7100	41.09
	3	2325.3350	231.0000	1634.0750	389.0100	4579.4200	47.85
	6	2630.4950	259.5850	1843.9300	441.8350	5175.8450	54.08
	8	124.0050	14.8300	106.3800	26.8350	272.0500	2.84
	12	198.9850	21.7600	140.0250	35.1550	395.9250	4.14
	16	262.4250	27.0450	179.9400	46.0800	515.4900	5.39
	24	268.2100	27.5400	181.1200	46.0550	522.9250	5.46
	48	289.8800	31.4750	199.2700	54.8200	575.4450	6.01
15%	1	366.8400	40.3100	286.9900	71.8850	766.0250	8.00
	2	856.9400	87.7300	600.1500	146.8500	1691.6700	17.68
	3	1271.3550	126.9350	883.5650	214.3250	2496.1800	26.08
	6	1637.7800	162.6650	1130.4700	271.5400	3202.4550	33.46
	8	2004.8500	198.0650	1391.0100	331.4150	3925.3400	41.02
	12	2451.1450	242.4050	1770.6100	414.1450	4878.3050	50.97
	16	120.8800	15.2050	110.7850	27.8150	274.6850	2.87
	24	302.0050	30.9250	207.1550	52.2900	592.3750	6.19
	48	390.0650	39.3450	266.2600	67.3400	763.0100	7.97

a: percentage of FAME conversion (%)

Table D3-4 (cont.)

% Water	Time (hr)	Concentration of FAME (M)					Con[a] (%)
		M. Palmitate	M. Stearate	M. Oleate	M. Linoleate	Total FAME	
20%	1	430.7950	44.2500	301.1500	76.3500	852.5450	8.91
	2	519.3550	60.2700	437.4550	109.1800	1126.2600	11.77
	3	1148.2700	148.2700	1014.9100	247.4750	2558.9250	26.74
	6	1239.2350	122.8700	848.0500	205.6500	2415.8050	25.24
	8	1457.9800	143.9000	996.9550	240.9500	2839.7850	29.67
	12	1829.9850	182.2100	1330.2200	309.4400	3651.8550	38.16
	16	1919.5050	188.7000	1324.7850	317.7100	3750.7000	39.19
	24	2149.7550	212.3000	1503.4800	357.6050	4223.1400	44.13
	48	76.7300	9.8750	70.8500	17.7550	175.2100	1.83

a: percentage of FAME conversion (%)

D3.3 Concentration of FAME in continuous-flow methanolysis.

Methanolysis was carried out in chapter 3, section 4.4.3.1 with the continuous - flow methanolysis condition using Lipozyme RM IM and Novozym 435 at 40°C (chapter 3, section 4.4.3.1.3). The substrate from the reaction using Lipozyme RM IM and Novozym 435 were converted to FAME or biodiesel as shows in Table D3–5 and Table D3-6.

Table D3-5 FAME production in continuous-flow methanolysis using 20% Lipozyme RM IM as the catalyst.

% Water	Time (hr)	Concentration of FAME (M)					Con[a] (%)
		M. Palmitate	M. Stearate	M. Oleate	M. Linoleate	Total FAME	
Non water	1	0.8255	0.0903	0.6529	0.1694	1.7381	18.16
	2	1.1881	0.1291	0.9235	0.2400	2.4808	25.92
	3	1.5785	0.1706	0.8271	0.1891	2.7653	28.90
	6	1.4761	0.1615	1.0695	0.2723	2.9794	31.13
	8	1.5393	0.1678	1.0034	0.2484	2.9587	30.92
	12	1.6544	0.1827	1.2451	0.3200	3.4021	35.55
	16	2.3128	0.2531	1.0034	0.2484	3.8176	39.89
	24	1.8568	0.2038	1.4375	0.3746	3.8727	40.47
	48	1.9327	0.2094	1.4671	0.3791	3.9883	41.68

a: percentage of FAME conversion (%)

Table D3-5 (cont.)

% Water	Time	Concentration of FAME (M)					Con[a] (%)
		M. Palmitate	M. Stearate	M. Oleate	M. Linoleate	Total FAME	
5%	1	0.9256	0.1014	0.7098	0.1838	1.9205	20.07
	2	1.0624	0.1149	0.6286	0.1957	2.0016	20.92
	3	1.4402	0.1517	0.7622	0.1769	2.5310	26.45
	6	1.5071	0.1517	0.7622	0.1769	2.5978	27.15
	8	1.3988	0.1507	0.9628	0.2442	2.7566	28.80
	12	1.4092	0.1532	0.9788	0.2497	2.7909	29.16
	16	1.4294	0.1540	0.9141	0.2273	2.7249	28.47
	24	1.4984	0.1606	0.9712	0.2432	2.8733	30.02
	48	1.7812	0.1860	0.8831	0.2005	3.0507	31.88
10%	1	0.5487	0.0607	0.4292	0.1114	1.1501	12.02
	2	0.8641	0.0948	0.6501	0.1670	1.7760	18.56
	3	1.2357	0.1362	0.7370	0.1764	2.2853	23.88
	6	1.7978	0.1948	0.3166	0.0198	2.3290	24.34
	8	1.7975	0.1967	1.2285	0.3056	3.5282	36.87
	12	1.2688	0.1399	0.9395	0.2397	2.5880	27.04
	16	1.3066	0.1423	0.9399	0.2385	2.6273	27.45
	24	1.4808	0.1634	0.9417	0.2293	2.8152	29.42
	48	1.6403	0.1791	0.8859	0.2050	2.9103	30.41
15%	1	0.7764	0.0856	0.6145	0.1596	1.6360	17.10
	2	0.9656	0.1057	0.7069	0.1802	1.9583	20.46
	3	0.9997	0.1370	0.7591	0.1826	2.0784	21.72
	6	1.1035	0.1207	0.8253	0.2117	2.2612	23.63
	8	1.2485	0.1370	0.7591	0.1826	2.3272	24.32
	12	1.2582	0.1369	0.8703	0.2177	2.4832	25.95
	16	1.3632	0.1485	1.0556	0.2721	2.8395	29.67
	24	1.4226	0.1580	1.0598	0.2487	2.8891	30.19
	48	1.4920	0.1616	1.0553	0.2659	2.9747	31.08
20%	1	0.4627	0.0518	0.3555	0.0920	0.9619	10.05
	2	0.6311	0.0691	0.4983	0.1287	1.3272	13.87
	3	0.8181	0.0911	0.6173	0.1583	1.6848	17.61
	6	1.4079	0.1540	0.9090	0.2210	2.6919	28.13
	8	1.4183	0.1550	0.8714	0.2102	2.6549	27.74
	12	1.6106	0.1759	0.8880	0.2071	2.8816	30.11
	16	1.4871	0.1617	1.0560	0.2656	2.9704	31.04
	24	1.5275	0.1661	1.1174	0.2841	3.0951	32.34
	48	1.5275	0.1661	1.1174	0.2841	3.0951	32.34

a: percentage of FAME conversion (%)

Table D3-6 FAME production in continuous-flow methanolysis using 20% Novozym 435 as the catalyst.

% Water	Time (hr)	Concentration of FAME (M)					Con [a] (%)
		M. Palmitate	M. Stearate	M. Oleate	M. Linoleate	Total FAME	
Non water	1	0.6034	0.3809	0.6672	0.1445	1.7960	18.77
	2	0.7072	0.3874	0.7234	0.1625	1.9804	20.69
	3	0.7815	0.3928	0.7688	0.1752	2.1182	22.13
	6	0.9311	0.4065	0.8812	0.2077	2.4266	25.36
	8	1.0047	0.4219	1.1590	0.2865	2.8720	30.01
	12	1.0932	0.4152	0.9367	0.2253	2.6703	27.90
	16	1.1893	0.4349	1.1590	0.2998	3.0830	32.22
	24	1.7320	0.4706	1.4555	0.3768	4.0349	42.16
	48	1.8371	0.4706	1.4555	0.3847	4.1479	43.34
5%	1	0.3711	0.3642	0.5673	0.1141	1.4167	14.80
	2	0.4330	0.3685	0.5951	0.1226	1.5192	15.87
	3	0.5499	0.3770	0.6546	0.1412	1.7227	18.00
	6	0.9198	0.4043	0.8336	0.1966	2.3543	24.60
	8	1.0396	0.4112	0.8925	0.2138	2.5570	26.72
	12	1.2198	0.4344	1.1901	0.2957	3.1400	32.81
	16	1.2418	0.4285	1.1537	0.2535	3.0776	32.16
	24	1.5837	0.4415	1.2587	0.2783	3.5622	37.22
	48	1.5861	0.4541	1.2715	0.3232	3.6349	37.98
10%	1	0.3762	0.3655	0.5714	0.1148	1.4280	14.92
	2	0.3970	0.3662	0.5788	0.1171	1.4591	15.25
	3	0.4461	0.3702	0.6087	0.0706	1.4956	15.63
	6	0.4969	0.3764	0.6246	0.1315	1.6295	17.03
	8	1.0225	0.4105	0.8818	0.2098	2.5246	26.38
	12	1.4416	0.4409	1.0881	0.2721	3.2427	33.88
	16	1.5386	0.4675	1.2834	0.2569	3.5463	37.06
	24	1.5358	0.4590	1.2977	0.3267	3.6193	37.82
	48	1.5352	0.4589	1.2782	0.2612	3.5334	36.92
15%	1	0.3805	0.3656	0.5735	0.1152	1.4348	14.99
	2	0.3762	0.3652	0.5710	0.1135	1.4258	14.90
	3	0.4070	0.3670	0.5860	0.1192	1.4793	15.46
	6	0.6335	0.3837	0.6978	0.1533	1.8683	19.52
	8	0.8074	0.4095	0.9547	0.2271	2.3986	25.06
	12	0.9428	0.4093	0.8455	0.1989	2.3966	25.04
	16	1.0906	0.4192	0.9598	0.2304	2.7001	28.21
	24	1.4355	0.4440	1.1414	0.2827	3.3036	34.52
	48	1.4751	0.4449	1.1318	0.2829	3.3347	34.84

a: percentage of FAME conversion (%)

Table D3-6 (cont.)

% Water	Time (hr)	Concentration of FAME (M)					Con [a] (%)
		M. Palmitate	M. Stearate	M. Oleate	M. Linoleate	Total FAME	
20%	1	0.2776	0.3585	0.5272	0.1013	1.2647	13.22
	2	0.3885	0.3661	0.5798	0.1175	1.4519	15.17
	3	0.4951	0.3742	0.6357	0.1347	1.6397	17.13
	6	0.7161	0.3917	0.7611	0.1710	2.0398	21.31
	8	0.8660	0.4003	0.8135	0.1884	2.2681	23.70
	12	1.2290	0.4392	1.1572	0.2870	3.1124	32.52
	16	1.3101	0.4326	1.0316	0.2535	3.0279	31.64
	24	1.1680	0.4249	0.9780	0.2375	2.8084	29.35
	48	1.4429	0.4095	0.9547	0.2271	3.0342	31.71

a: percentage of FAME conversion (%)

D3.4 Dosage of immobilized lipase: FAME concentration

D3.4.1 Dosage of Lipozyme RM IM in continuous–flow methanolysis

Methanolysis was carried out in section 3.4.4.3.1 with the continuous - flow methanolysis condition using Lipozyme RM IM at 40°C (chapter 3, section 4.4.3.2). The substrate from the reaction using Lipozyme RM IM and were converted to FAME or biodiesel as shows in Table D3–7.

Table D3-7 FAME production in continuous-flow methanolysis by 5, 10, 20, 30 and 40% Lipozyme RM IM as the catalyst.

% enzyme	Time (hr)	Concentration of FAME (M)					Con [a] (%)
		M. Palmitate	M. Stearate	M. Oleate	M. Linoleate	Total FAME	
5	1	0.1725	0.3504	0.4617	0.0792	1.0638	11.12
	2	0.1741	0.3506	0.4624	0.0795	1.0666	11.14
	3	0.1819	0.3512	0.4653	0.0801	1.0784	11.27
	6	0.1904	0.3520	0.4714	0.0821	1.0960	11.45
	8	0.1934	0.3528	0.4686	0.0810	1.0958	11.45
	12	0.1977	0.3521	0.4739	0.0830	1.1068	11.57
	16	0.2083	0.3531	0.4795	0.0825	1.1235	11.74
	24	0.2740	0.3574	0.4987	0.0830	1.2131	12.68
10%	1	0.2608	0.3567	0.4910	0.0868	1.1952	12.49
	2	0.2722	0.3576	0.4949	0.0879	1.2126	12.67
	3	0.2834	0.3583	0.4996	0.0892	1.2305	12.86
	6	0.2854	0.3579	0.5014	0.0905	1.2352	12.91
	8	0.3133	0.3600	0.5177	0.0955	1.2865	13.44
	12	0.3264	0.3615	0.5140	0.0934	1.2952	13.53
	16	0.3561	0.3631	0.5237	0.0959	1.3388	13.99
	24	0.3627	0.3638	0.5272	0.0962	1.3499	14.11
20%	1	1.2660	0.1300	0.6050	0.1370	2.1380	22.34
	2	0.9390	0.1050	0.7200	0.1900	1.9540	20.42
	3	1.2050	0.1260	0.6450	0.1550	2.1310	22.27
	6	1.1900	0.1595	0.8975	0.2225	2.4695	25.80
	8	1.2025	0.1650	0.9450	0.2290	2.5415	26.56
	12	1.4200	0.1550	1.0950	0.2850	2.9550	30.88
	16	1.5473	0.3657	1.4110	0.3370	3.6610	38.25
	24	1.6689	0.4459	1.4670	0.3560	3.9378	41.15
30%	1	1.6563	0.4593	1.2843	0.3261	3.7259	38.93
	2	1.6982	0.4617	1.3154	0.3352	3.8105	39.82
	3	1.7026	0.4617	1.3160	0.3360	3.8163	39.88
	6	2.0389	0.4864	1.4990	0.3906	4.4148	46.13
	8	2.1258	0.4936	1.5424	0.4028	4.5647	47.70
	12	2.1171	0.4929	1.4182	0.3566	4.3848	45.82
	16	2.3696	0.5128	1.3675	0.3269	4.5768	47.82
	24	2.3785	0.5149	1.4235	0.4364	4.7533	49.67
40%	1	1.9172	0.0956	0.4786	1.1340	3.6254	37.88
	2	1.9438	0.4793	1.4504	0.3764	4.2499	44.41
	3	2.1212	0.4929	1.5070	0.3896	4.5107	47.13
	6	2.3696	0.5128	1.3650	0.3269	4.5743	47.80
	8	2.3140	0.5064	1.6467	0.4338	4.9009	51.21
	12	2.4645	0.5182	1.5783	0.4013	4.9623	51.85
	16	2.8293	0.5493	1.6522	0.4127	5.4435	56.88
	24	2.8683	0.6049	1.6522	0.412695	5.538095	57.87

a: percentage of FAME conversion (%)

D3.4.2 Dosage of Novozyme 435 in continuous–flow methanolysis

Methanolysis was carried out in section 3.4.4.3.1 with the continuous - flow methanolysis condition using Novozym 435 at 40 °C (chapter 3, section 4.4.3.2). The substrate from the reaction using Lipozyme RM IM and were converted to FAME or biodiesel as shows in Table D –10.

Table D3-8 Table D3-7 FAME production in continuous-flow methanolysis by 5, 10, 20, 30 and 40% Novozym 435 as the catalyst

% enzyme	Time (hr)	Concentration of FAME (M)					Con [a] (%)
		M. Palmitate	M. Stearate	M. Oleate	M. Linoleate	Total FAME	
5%	1	0.1115	0.3460	0.4450	0.0757	0.9782	10.22
	2	0.1112	0.3460	0.4420	0.0746	0.9738	10.18
	3	0.4808	0.3487	0.4565	0.0790	1.3649	14.26
	6	0.1482	0.3489	0.4567	0.0790	1.0327	10.79
	8	0.1571	0.3492	0.4626	0.0809	1.0499	10.97
	12	0.1608	0.3494	0.4583	0.0793	1.0477	10.95
	16	0.2259	0.3535	0.4977	0.0879	1.1649	12.17
	24	0.2662	0.3576	0.5012	0.0924	1.2175	12.72
10%	1	0.2154	0.3535	0.4805	0.0864	1.1358	11.87
	2	0.2224	0.3537	0.4796	0.0858	1.1415	11.93
	3	0.2757	0.3580	0.5077	0.0947	1.2361	12.92
	6	0.7224	0.3587	0.5297	0.0945	1.7053	17.82
	8	0.8445	0.3595	0.5492	0.0937	1.8468	19.30
	12	0.9260	0.3597	0.5691	0.0939	1.9487	20.36
	16	1.0811	0.3626	0.5742	0.0966	2.1144	22.09
	24	1.2004	0.4033	0.6628	0.1226	2.3891	24.96
20%	1	0.8604	0.4003	0.7337	0.1653	2.1596	22.57
	2	1.2920	0.4312	0.9868	0.2419	2.9519	30.85
	3	1.3172	0.4324	1.0463	0.2578	3.0537	31.91
	6	1.4949	0.4460	1.1564	0.2893	3.3866	35.39
	8	1.2223	0.4277	1.3415	0.2524	3.2439	33.90
	12	1.5444	0.4473	1.1138	0.2818	3.3873	35.39
	16	1.6947	0.4612	1.2722	0.3226	3.7507	39.19
	24	1.8778	0.4736	1.3550	0.3484	4.0549	42.37

a: percentage of FAME conversion (%)

Table D3-8 (cont.)

% enzyme	Time (hr)	Concentration of FAME (M)					Con [a] (%)
		M. Palmitate	M. Stearate	M. Oleate	M. Linoleate	Total FAME	
30%	1	0.9422	0.4063	0.8040	0.1863	2.3388	24.44
	2	1.3618	0.4375	1.0891	0.2680	3.1564	32.98
	3	1.4139	0.4404	1.0841	0.2690	3.2075	33.52
	6	1.4912	0.4474	1.1878	0.2957	3.4221	35.76
	8	1.7706	0.4649	1.2299	0.3154	3.7808	39.51
	12	1.9172	0.4786	1.1340	0.2588	3.7887	39.59
	16	1.9733	0.4829	1.4344	0.3691	4.2597	44.51
	24	2.3683	0.4549	1.6022	0.3627	4.7882	50.03

a: percentage of FAME conversion (%)

D3.5 Temperature and methanol content on methanolysis: FAME convertion.

Methanolysis was carried out in chapter 3, section 4.4.3.1 with the continuous - flow methanolysis condition using Lipozyme RM IM was varied at 40°C, 50°C and 60°C (chapter 3, section 4.4.3.3). The FAME from this method at at 40°C, 50°C and 60°C using Lipozyme RM IM as shows in Table D3–9, Table D3-10, Table D3-11 respectively. Beside the using of Novozym 435 as catalyst at 40 °C, 50°C and 60°C, FAME conversion shows in Table D3-12, Table D3-13 and Table D3-14 respectively. Moreover, kinematic parameter in the reaction using Lipozyme RM IM and Novozym 435 show in Table D3-15 and Tabel D3-16 respectively.

Table D3-9 Velocity and FAME production in continuous-flow methanolysis using 20% Lipozyme RM IM as the catalyst at 40°C.

Concentration of methanol (M)	V (mM/hr)	V (mM/hr·g)	1/V (hr·g/mM)	Total FAME		FAME Conversion (%)	
				16 hr	24 hr	16 hr	24 hr
0.5	0.38	0.69	1.45	0.60	1.09	6.29	11.37
1	0.64	1.06	0.95	1.59	1.36	16.65	14.17
2	0.97	1.61	0.62	2.79	3.20	29.18	33.42
3	1.71	2.86	0.35	3.23	3.71	33.75	38.77
4	0.99	1.65	0.61	3.86	3.99	40.37	41.69
5	0.65	1.08	0.93	2.86	3.77	29.83	39.39
6	0.59	0.98	1.02	2.15	2.51	22.49	26.26
7	0.46	0.76	1.31	2.00	3.12	20.85	32.59
8	0.31	0.51	1.96	1.83	2.21	19.16	23.04
9	0.24	0.40	2.48	2.18	2.25	22.78	23.50

Table D3-10 Velocity and FAME production in continuous-flow methanolysis using 20% Lipozyme RM IM as the catalyst at 50°C.

Concentration of methanol (M)	V (mM/hr)	V (mM/hr·g)	1/V (hr·g/mM)	Total FAME		FAME Conversion (%)	
				16 hr	24 hr	16 hr	24 hr
0.5	0.32	0.54	1.87	0.87	1.84	9.08	19.27
1	0.63	1.06	0.95	2.98	1.99	31.15	20.75
2	0.97	1.62	0.62	2.53	2.62	26.44	27.38
3	1.71	2.86	0.35	3.54	3.66	37.01	38.20
4	0.99	1.65	0.61	3.84	3.98	40.14	41.60
5	0.65	1.08	0.92	3.76	4.00	39.30	41.76
6	0.59	0.98	1.02	2.71	3.76	28.36	39.31
7	0.46	0.76	1.31	2.63	3.22	27.51	33.66
8	0.31	0.51	1.96	2.53	3.01	26.48	31.41
9	0.24	0.40	2.48	2.16	2.98	22.61	31.10

Table D3-11 Velocity and FAME production in continuous-flow methanolysis using 20% Lipozyme RM IM as the catalyst at 60°C.

Concentration of methanol (M)	V (mM/hr)	V (mM/hr·g)	1/V (hr·g/mM)	Total FAME		FAME Conversion (%)	
				16 hr	24 hr	16 hr	24 hr
0.5	0.21	0.35	2.89	0.73	0.52	7.66	5.40
1	0.37	0.62	1.61	1.67	2.30	17.49	24.02
2	0.97	1.62	0.62	2.79	3.20	29.18	33.42
3	1.71	2.86	0.35	3.23	4.71	33.78	49.22
4	1.05	1.75	0.57	3.86	4.99	40.37	52.14
5	0.65	1.08	0.93	2.86	4.78	29.83	49.94
6	0.59	0.98	1.02	2.15	2.51	22.49	26.26
7	0.46	0.76	1.31	2.00	3.12	20.85	32.59
8	0.31	0.51	1.96	1.83	2.18	19.16	22.78
9	0.24	0.40	2.48	2.21	2.25	23.04	23.50

Table D3-12 Velocity and FAME production in continuous-flow methanolysis using 20% Novozym 435 as the catalyst at 40°C.

Concentration of methanol (M)	V (mM/hr)	V (mM/hr·g)	1/V (hr·g/mM)	Total FAME		FAME Conversion (%)	
				16 hr	24 hr	16 hr	24 hr
0.5	1.80	3.01	0.33	1.48	1.48	15.44	15.44
1	1.64	2.74	0.37	1.63	1.65	17.03	17.21
2	3.14	5.24	0.19	2.94	3.46	30.73	36.16
3	3.43	5.72	0.17	3.47	4.21	36.27	44.02
4	3.06	5.10	0.20	4.27	4.43	44.59	46.25
5	2.59	4.32	0.23	3.81	4.27	39.79	44.65
6	2.66	4.43	0.23	3.23	3.44	33.72	35.90
7	3.12	5.20	0.19	2.98	2.97	31.10	31.01
8	2.42	4.03	0.25	2.31	2.67	24.14	27.88
9	2.09	3.48	0.29	1.86	2.13	19.42	22.27

Table D3-13 Velocity and FAME production in continuous-flow methanolysis using 20% Novozym 435 as the catalyst at 50°C.

Concentration of methanol (M)	V (mM/hr)	V (mM/hr·g)	1/V (hr·g/mM)	Total FAME		FAME Conversion (%)	
				16 hr	24 hr	16 hr	24 hr
0.5	1.73	2.89	0.35	1.42	1.42	14.79	14.83
1	2.04	3.40	0.29	1.65	1.84	17.29	19.25
2	2.57	4.28	0.23	1.98	2.06	20.73	21.57
3	2.78	4.63	0.22	2.35	2.40	24.55	25.05
4	4.00	6.66	0.15	3.42	3.56	35.75	37.21
5	3.37	5.61	0.18	3.93	4.18	41.03	43.67
6	2.99	4.99	0.20	3.24	3.15	33.83	32.95
7	3.01	5.02	0.20	2.45	2.47	25.60	25.79
8	2.75	4.58	0.22	2.84	3.02	29.67	31.56
9	2.54	4.23	0.24	2.30	2.48	24.00	25.92

Table D3-14 Velocity and FAME production in continuous-flow methanolysis using 20% Novozym 435 as the catalyst at 60°C.

Concentration of methanol (M)	V (mM/hr)	V (mM/hr·g)	1/V (hr·g/mM)	Total FAME		FAME Conversion (%)	
				16 hr	24 hr	16 hr	24 hr
0.5	2.07	3.45	0.29	1.77	1.82	18.51	19.05
1	2.69	4.48	0.22	2.42	2.61	25.26	27.24
2	4.34	7.23	0.14	3.66	3.67	38.21	38.38
3	4.77	7.95	0.13	3.65	3.83	38.13	40.04
4	3.54	5.89	0.17	3.13	3.72	32.75	38.90
5	2.14	3.56	0.17	1.72	1.84	18.02	19.27
6	3.39	5.64	0.18	2.92	2.94	30.48	30.72
7	3.16	5.27	0.19	2.70	2.98	28.24	31.18
8	2.62	4.37	0.23	2.39	2.67	25.01	27.93
9	2.61	4.35	0.23	2.56	2.59	26.76	27.02

Table D3-15 Kinetic parameter in the transesterification using Lipozyme RM IM.

Temperature	V_{max} (mM/hr·g)	K_m (M)
40°C	6.90	4.76
50°C	10.90	9.62
60°C	21.66	14.08

Table D3-16 Kinetic parameter in the transesterification using Novozym 435.

Temperature	V_{max} (mM/hr·g)	K_m (M)
40°C	3.09	2.06
50°C	3.85	2.58
60°C	28.84	14.71

D3.6 Stability of immobilized lipase

Methanolysis was carried out in chapter 3, section 4.4.3.1 with the continuous-flow methanolysis condition using Lipozyme RM IM at 40°C (chapter 3, section 4.4.3.4). Then immobilized lipase was washed by 10 ml of hexane. The FAME conversion and residual activity from this method by using Lipozyme RM IM and Novozym 435 as shows in Table D3–17 and Table D3-18, respectively.

Table D3-17 FAME conversion and residual activity of Lipozyme RM IM

Wash No.	Concentration of FAME (M) Total FAME	Con[a] (%)	Residual activity(%)
1	0.68	9.62	100.00
2	0.60	8.54	88.76
3	0.54	7.70	80.03
4	0.47	6.69	69.53
5	0.45	6.36	66.12
6	0.40	5.65	58.73
7	0.26	3.63	37.72
8	0.17	2.46	25.59
9	0.17	2.43	25.30
10	0.68	9.62	100.00

a: percentage of FAME conversion (%)

Table D3-18 FAME conversion and residual activity of Novozym 435

Wash No.	Concentration of FAME (M) Total FAME	Con[a] (%)	Residual activity(%)
1	0.8161	8.53	86.52
2	0.7395	7.73	78.40
3	0.6993	7.31	74.14
4	0.7076	7.39	75.01
5	0.6995	7.31	74.15
6	0.6901	7.21	73.16
7	0.6558	6.85	69.52
8	0.6645	6.94	70.45
9	0.6613	6.91	70.11
10	0.5927	6.19	62.84

a: percentage of FAME conversion (%)

BIOGRAPHY

Miss Parnuch Hongsawat was born on the 26th of November (1983) in Bangkok, Thailand. She received her Bachelor's degree of Science in Biochemistry from the Department of Biochemistry, Faculty of Science, Chulalongkorn University in 2003. She began her studies in the Environmental Management program, Graduate school, Chulalongkorn University in 2004 and completed Master's degree program in 2007.