CHAPTER IV

RESULTS

4.1 Properties of used frying oil

During the frying process, the chemical reaction of fats and oils are stimulated by heat. This leads to formation of new compounds, which are harmful to human body. The visible changes taking place in a fat or oil during frying is color change. On the other hand, the changing of the viscosity and water in the oil is affected on the quality of oil (Goburdhun *et al.*, 2000).

In this experiment, the refined palm oil was heated to 170°C at the beginning and chicken breasts were fried for 15 min. After 15 min, the oil sample was collected and the frying operation was carried out for a new cycle with pieces of chicken breast. Refined palm oil was subjected to 21 cycles of frying. Then, the oil properties, viscosity and water content of the frying oil were determined and compared with those of the refined palm oil.

4.1.1 The color of refined and used frying palm oil

Frying cycle	0	3	6	9	12	15	18	21
Color intensity value[a]	204	177	162	142	122	108	104	90
Color intensity change (%)[b]	1.00	11.76	20.58	30.39	40.19	47.06	49.02	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).

Fig. 4.1 The color change of refined palm oil and used frying palm oil at 3, 6, 9, 12, 15, 18 and 21 frying cycles.

Figure. 4.1 shows that the oil color became darker when the refined palm oils was fried for 3, 6, 9, 12, 15, 18 and 21 cycles. For comparison, the color intensity was calculated in gray scale using a gel documentation (Vilber Lourmat, France). It was found that the color intensity of oil was increased 56 folds after palm oil was used for 21 cycles (Fig. 4.1).

4.1.2 Viscosities of refined and used frying palm oil

Viscosity is the unit specifying the resistance to flow, therefore viscosity relates to the quality of oil. The high viscosity affect the utilization of this oil as an alternative fuel oil (for biodiesel production engines). After 21 cycles of frying, the viscosity of frying palm oil was determined. A viscometer D2170, U-tube type with a viscosmeter constant of 0.01069 mm^2/s^2 was used to measure oil viscosity, according to American Standard Test Method D445 (ASTM D445). The result shows that viscosity of refined palm oil was gradually increased with increasing of frying cycle. The viscosity of oil increased by 10% (from 61,333.88 mPa·s to 67,547.44 mPa·s) of the 21-cyc oil (Fig. 4.2), probably due to high molecular weight compound formed during the frying process.

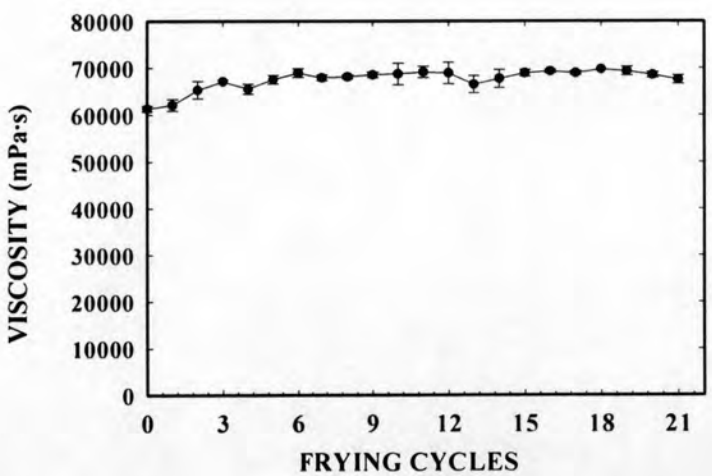


Fig. 4.2 The change in the viscosities of the frying oil in the increasing of the frying cycles. The data are means of three independent experiments.

4.1.3 Water content of refined and used frying palm oil

In general, a fried food becomes tougher as frying time increasing. Mass transfer during frying consists of moisture loss and oil absorption. Different of moisture in the frying oils represents different quality of oils. In this study, after 21 cycles of frying, water content of oil decreased by 37.5 % (from 0.08% to 0.05 %) (Table 4.1).

Table 4.1 Water content of refined and the 21-cyc oil by Karl Fischer method AOAC, 2005.

Type of oil	Water Content (%)
Refined palm oil	0.08
The 21-cyc oil	0.05

4.1.4 Fatty acid composition of used frying oil

Fatty acids bound to glycerin as triglycerides, vary in carbon chain length and in the number of unsaturated bonds (double bonds). Fatty acid compositions are different among various types of oils. In biodiesel production, type of fatty acid has significant effect on the transesterification of glycerides with alcohols. Fatty acid composition of refined palm oil and used frying oil in each frying cycles was measured through the fatty acid methyl ester from the transesterification of oil with 1% sodiumhydroxide and methanol (section 3.4.4).

The result shows that palm oil composes of unsaturated fatty acid in higher level than saturated fatty acid having four major fatty acids i.e. oleic acid, palmitic acid, linoleic acid and stearic acid, respectively (Fig. 4.3). Afer frying, the fatty acid composition percentage changed in that the unsaturated fatty acid level was slightly increased, while satturated fatty acid level was slightly decreased (4% differences) (Fig. 4.4). Nonetheless, the total unsaturated fatty acid is still the main fatty acid composition.

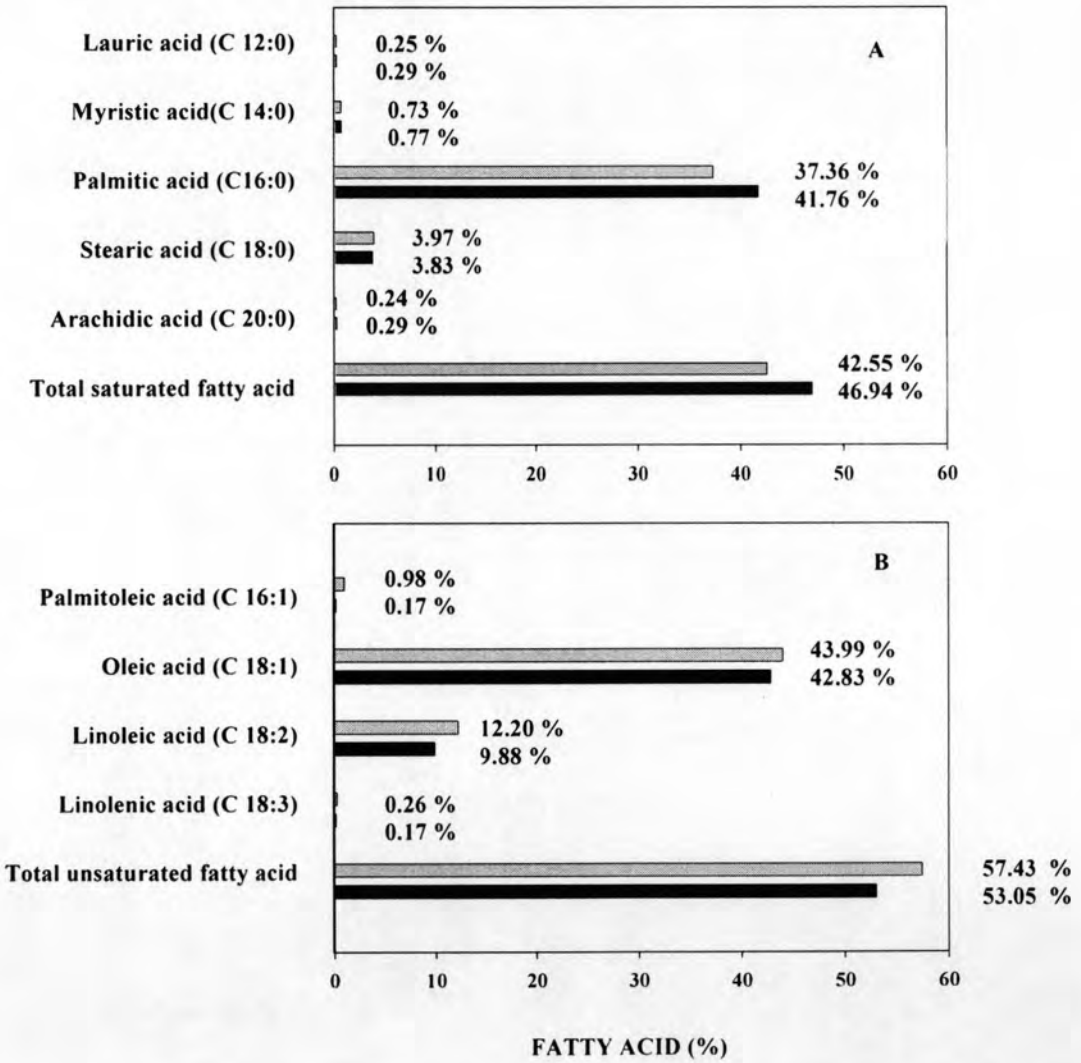


Fig. 4.3 Fatty acid composition of refined palm oil ( ) and the 21-cyc oil ( ). A) saturated fatty acid and B) unsaturated fatty acid.

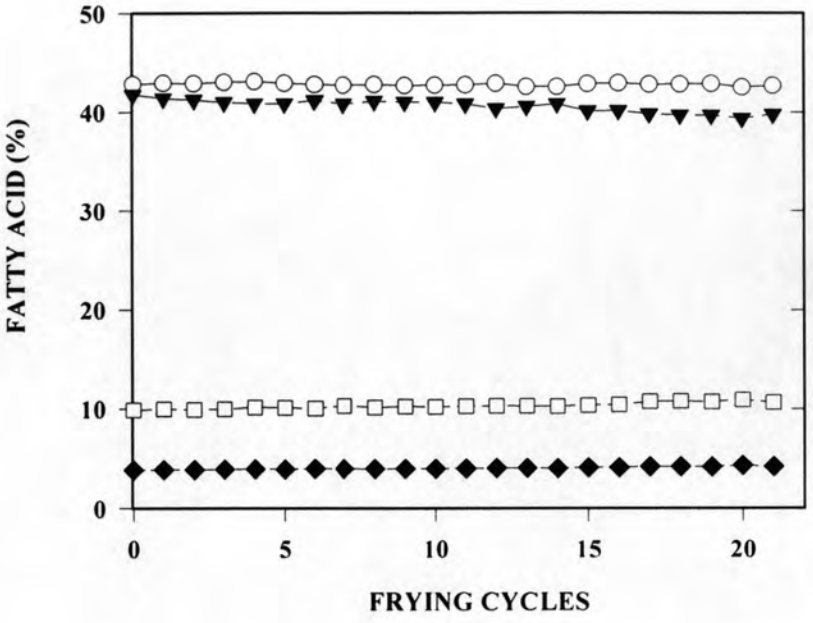


Fig. 4.4 The change in the fatty acid profile of used frying oil with increasing of frying cycles. Four major fatty acids: oleic acid (⊖); palmitic acid (▼); stearic acid (⊟); linoleic acid (◆). Open symbols are unsaturated fatty acid, while closed symbols are saturated fatty acid.

4.2 Identification of PAHs in oil

PAHs are group of high lipophilic compounds that have been the subject of much concern in recent years due to their toxic potential. Edible oils and fats can be heavily contaminated with these xenobiotic substances. Frying process is known as one of food processes causing elevation level of PAHs in food. The example of the chromatogram of PAHs in the 21-cyc oil shows in Fig. 4.7.

4.2.1 Extraction of PAHs from oil

PAHs in the 21-cyc oil were extracted with the liquid partition method (section 3.4.3). Although cyclohexane with dimethyl sulphoxide (DMSO) are the most common solvents used for liquid-liquid extraction of PAHs from oils and fats, acetonitrile was chosen in this study because this solvent produce less emulsions than the other, easier to separate from the oil samples and gave the hightest recovery percentage of PAHs (Table 4.2).

Table 4.2 Napthalene and benzo[a]pyrene recovery by acetonintile extraction.

Extraction solvent(s)	**Percentage of napthalene recovery (%)**	**Percentage of benzo[a]pyrene recovery (%)**
	Average	**Average**
Cyclohexane DMSO-water (9:1)	16.09±5.19	15.92±3.81
Acetonitrile	37.28±3.59	15.31±1.82

Although the PAHs recovery percentage in this experiment seemed low, but the recovery range was in the average recovery range reported so far (e.g the recovery percentage of naphthalene of Moet *et al.* (2000) gave 31.9% by liquid–liquid extraction). More discussion details are in section 5.2.1.

4.2.2 Type and concentration of PAHs in used frying oil

The 21-cyc oil was mixed with 100 % acetonitrile (1:1 v/v), and rotated at room temperature for 48 hr. Then, the extraction samples were examined whether there were PAHs presented using HPLC and GC-MS (section 3.4.3)

The result from mass spectrometry of the 21-cyc oil showed m/z of 128 (Fig. 4.5A). This molecular weight is similar to that of naphthalene (Fig. 4.5B). Whereas benzo[a]pyrene was not detected in the 21-cyc oil by the GC-MS.

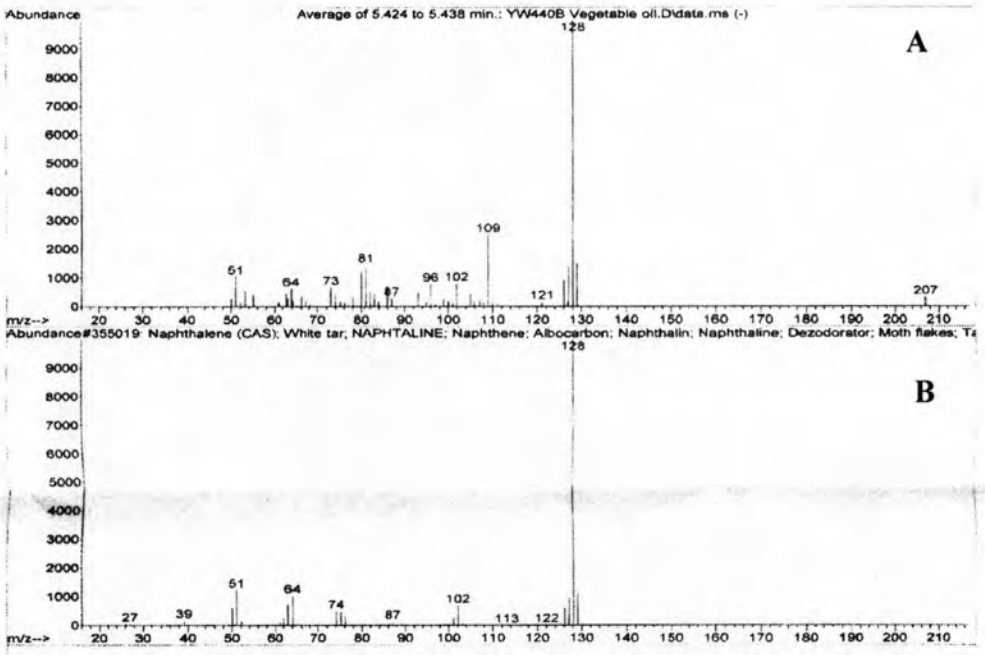


Fig. 4.5 Detection and analysis of PAHs in used frying palm oil using GC – MS (Science Service Department). A.) Analysis of used frying palm oil. B) Standard naphthalene (molecular mass of 128).

In this study, the result showed that concentration of naphthalene increased in concentrate with increasing of frying cycle as shown in Fig. 4.6. and the example chromatogram of PAHs in the 21-cyc oil as shown in Fig. 4.7.

Napthalene formed in frying palm oil increased from 197±57.58 μM to 1646±256.82 μM after 21 cycle of frying at 170°C.

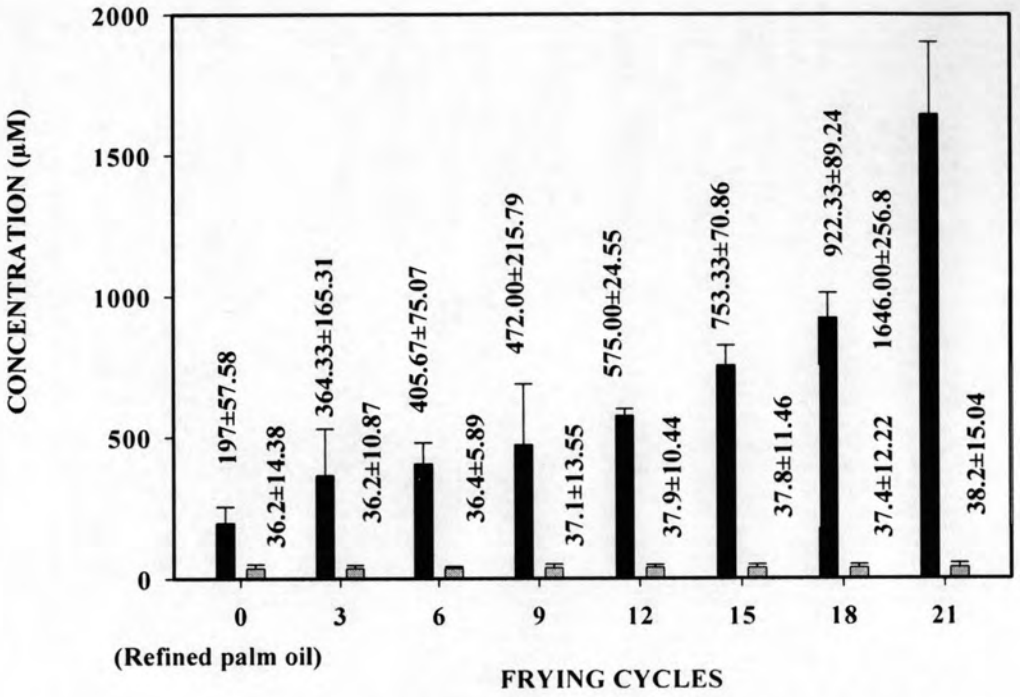


Fig. 4.6 Increase of naphthalene (■) and benzo[a]pyrene (■) in oil during each frying cycle.

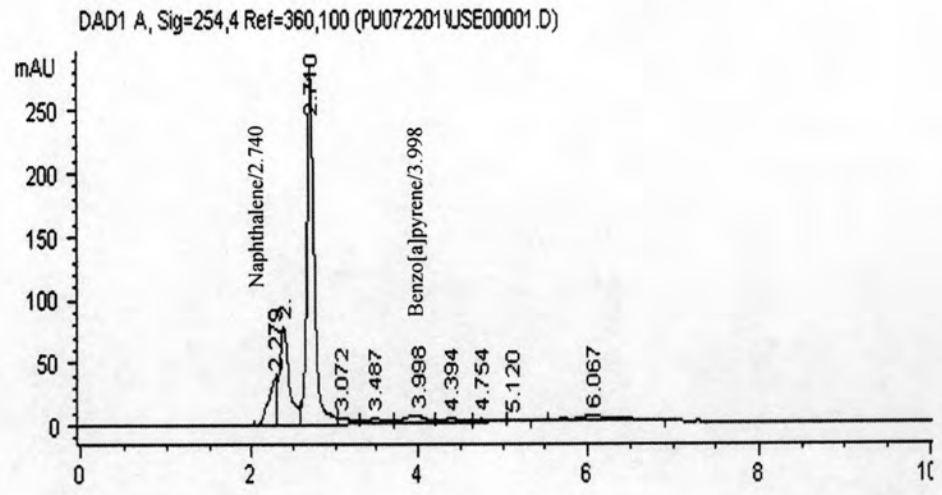


Fig. 4.7 Chromatogram of PAHs in the 21-cyc oil.

4.2.3 Adsorpsion of PAHs on immobilized lipase

Utilization of used palm oil for lipase-based biodiesel production is one of the several approaches for management of frying oil waste (more discussion details in section 5.2.3). Used frying palm oil containing PAHs was used in transesterification. The biological transesterification was catalyzed by a heterogenous catalyst, immobilized lipase. Since PAHs are lipophilic compound which tends to absorb on solid surface, one of your hypothesizes is to determine if immobilized lipase was adsorbed by PAHs produced in used frying palm oil in the transesterification (Table 4.3) and if the adsorption affect the reaction efficiency.

To examine the adsorption, the reaction consisting of immobilized lipase and 2.0 μmole naphthalene or benzo[a]pyrene dissolved in acetonitrile (2ml) were mixed together for 48 hr. Then, PAHs in acetonitrile was analyzed as described in details in section 3.4.3. The results showed that PAHs (naphthalene and benzo[a]pyrene) was adsorbed onto immobilized lipase (Fig. 4.8 and Table 4.3).

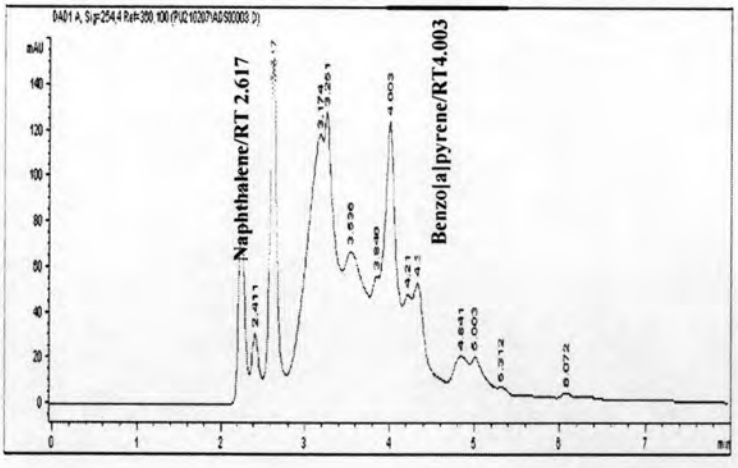


Fig. 4.8 Naphthalene and benzo[a]pyrene was adsorbed onto Lipozyme RM IM and analyzed by HPLC (section 3.4.3).

Table 4.3 The adsorption of napthalene and benzo[a]pyrene in the immobilized lipase; Lipozyme RM IM and Novozym 435.

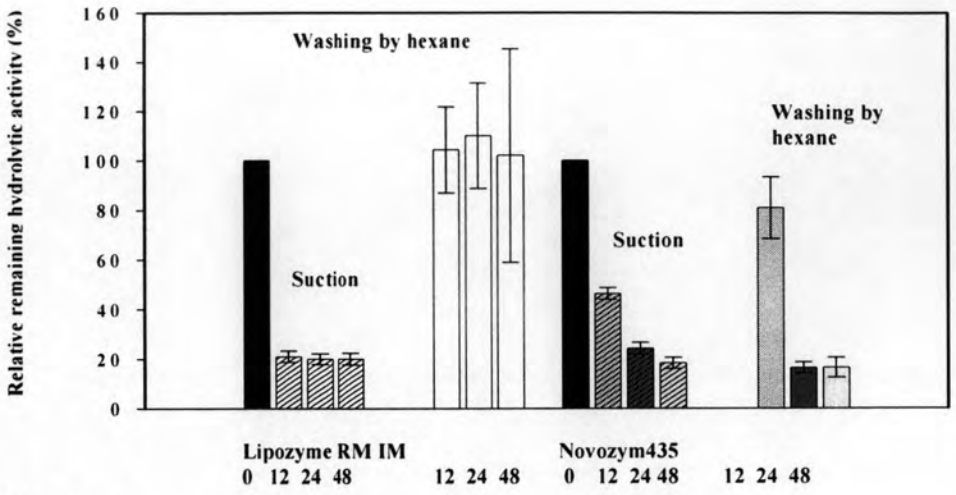
Immobilized lipase	Napthalene (μmole/g lipase)	Benzo[a]pyrene (μmole/g lipase)
Lipozyme RM IM	1.96±0.002	1.96 ± 0.018
Novozym 435	1.93± 0.007	1.96± 0.387

4.2.4 Effect of PAHs on hydrolytic activities of immobilized lipase

As the results in section 2.3, the effect of naphthalene and benzo[a]pyrene adsorption on hydrolytic activity of immobilized lipases (Lipozyme RM IM and Novozym 435) was determined. The experiment was designed by mixing of immobilized lipase (0.2 g), water (12 ml) and methanol (8 ml) (section 3.4.3). In presence of naphthalene and benzo[a]pyrene, hydrolytic activity of Lipozyme RM IM decreased by 77.2-77.9 % and 79.0-80.01 % relative remaining hydrolytic activity (Fig. 4.9), while that of Novozym 435 decreased by 53.6-81.7 % and 86.6-87.9 %, respectively (Fig. 4.9). Then, the Lipozyme RM IM and Novozym 435 were washed by hexane. When naphthalene on the immobilized lipase was washed by hexane, the hydrolytic activity of Lipozyme RM IM was remained. While that of Novozym 435 decreased by 18.98 %, 83.58% and 83.45% at 12, 24 and 48 hours, respectively. Therefore, hexane was reduced the decreasing of the relative remaining hydrolytic activity of Novozym 435 (Fig. 4.9a).

Naphthalene

a.)



Benzo[a]pyrene

b.)

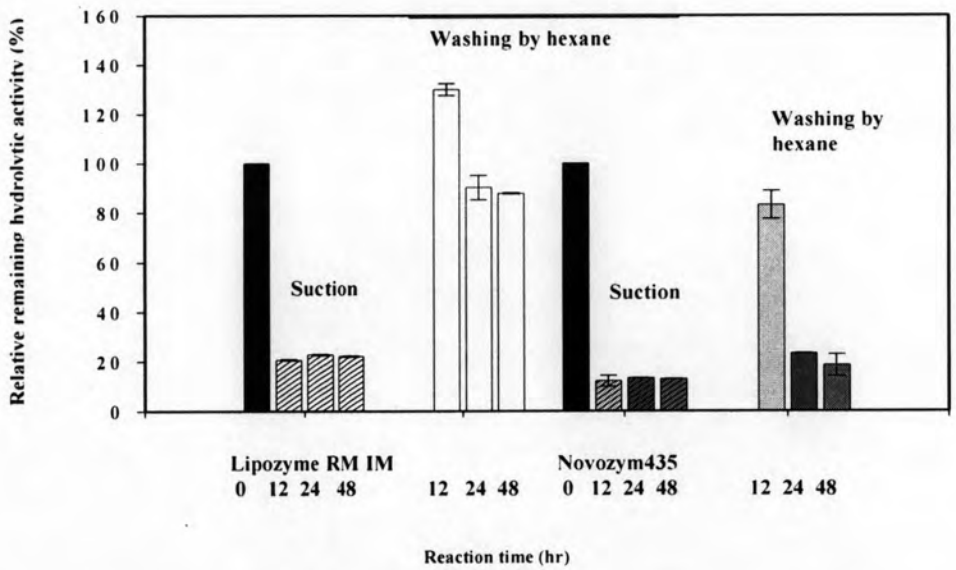


Fig. 4.9 Effect of naphthalene (a) and benzo[a]pyrene (b) on hydrolytic activity of Lipozyme RM IM and Novozym 435. After the reaction between immobilized lipase and PAHs, the relative remaining hydrolytic activity in the reaction when immobilized lipase was washed by: suction (▨), washed by hexane (□) and non-washed (■).

4.3 Utilization of used frying palm oil for lipase-based biodiesel production

Alkaline-catalyzed transesterification processes are normally used for biodiesel production. In recent year, the increasing in consciousness for environmental pollution creates the concept of zero emission. A complete recycling of used frying oil attracts considerable attention. Therefore, the enzymatic transesterification process has been an interesting process for many countries. However, there are several factors affecting biodiesel production using enzymatic transesterification as described in section 4.3.1-4.3.5.

4.3.1 Methanolysis type of used frying oil

In general, lipases efficiently catalyze the transesterification reaction when the substrates (oil or triglyceride and methanol) dissolve in each other. Since the solubility of methanol in triglyceride is normally low and the excess of methanol leads to the deactivation of the immobilized lipase. Thus, ratio of triglyceride and methanol well as the methanol feeding into the transesterification were the two most important parameters among others in the reaction.

To achieve the effective methanol feeding, three types of methanolysis were investigated: one-step methanolysis, three-step methanolysis and continuous-flow methanolysis.

One-step batch methanolysis of used frying oil

One-step batch methanolysis was the adding of methanol into the reaction in one-step (described in chapter 3, section 4.4.3.2). In the presence of water (20%w/w), the highest FAME production using 20 % (w/w) of Lipozyme RM IM with used oil was 29.2 % (Fig. 4.11a), while the reaction with Novozym 435 gave the highest percentage of FAME conversion of 34.2% in the reaction with 15%(w/w) water(Fig. 4.11b).

Three-step batch methanolysis of used frying oil.

Three–step batch methanolysis was the methanolysis of oil in which 1/3 molar equivalent of methanol was internal added at 0, 8, and 16 hours during the reaction. (Fig. 4.11c and Fig. 4.11d). In the presence of water (15%w/w), the highest FAME production using 20 % (w/w) of Lipozyme RM IM with used oil was 38.9 % (Fig. 4.11c), while the reaction with Novozym 435 gave the highest percentage of FAME conversion of 50.9% in the reaction with 5%(w/w) water (Fig. 4.11d).

Continuous-flow methanolysis of used frying oil

Continuous-flow methanolysis was a batch reaction in which methanol was slowly and continuously feed to the final concentration of 3 molar. The FAME production was increased by the low flow of methanol in the reaction.

The highest FAME production was approximately 40 % FAME conversion when the methanol added continuously in the reaction at the minimum flow rate (0.018 ml/min) (Fig. 4.10). FAME production using 20% (w/w) of Lipozyme RM IM and Novozym 435 by continuous-flow were approximately 30 % and 40 % FAME conversion (Fig. 4.11e and Fig. 4.11f).

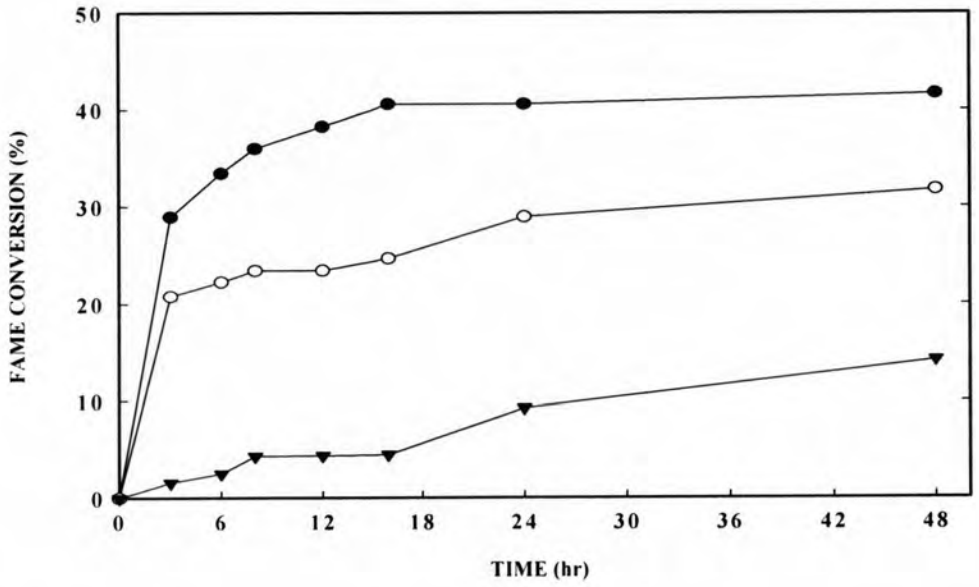


Fig. 4.10 Time courses of fatty acid methyl ester production using 20 % (w/w) of Lipzyme RM IM with the 21-cyc oil in continuous-flow methanolysis with various flow rates of methanol feeding (described in section 3.4.5.2):

0.018 ml/min (-●-); 0.083 ml/min (-⊖-) and 0.159 ml/min (-▼-)

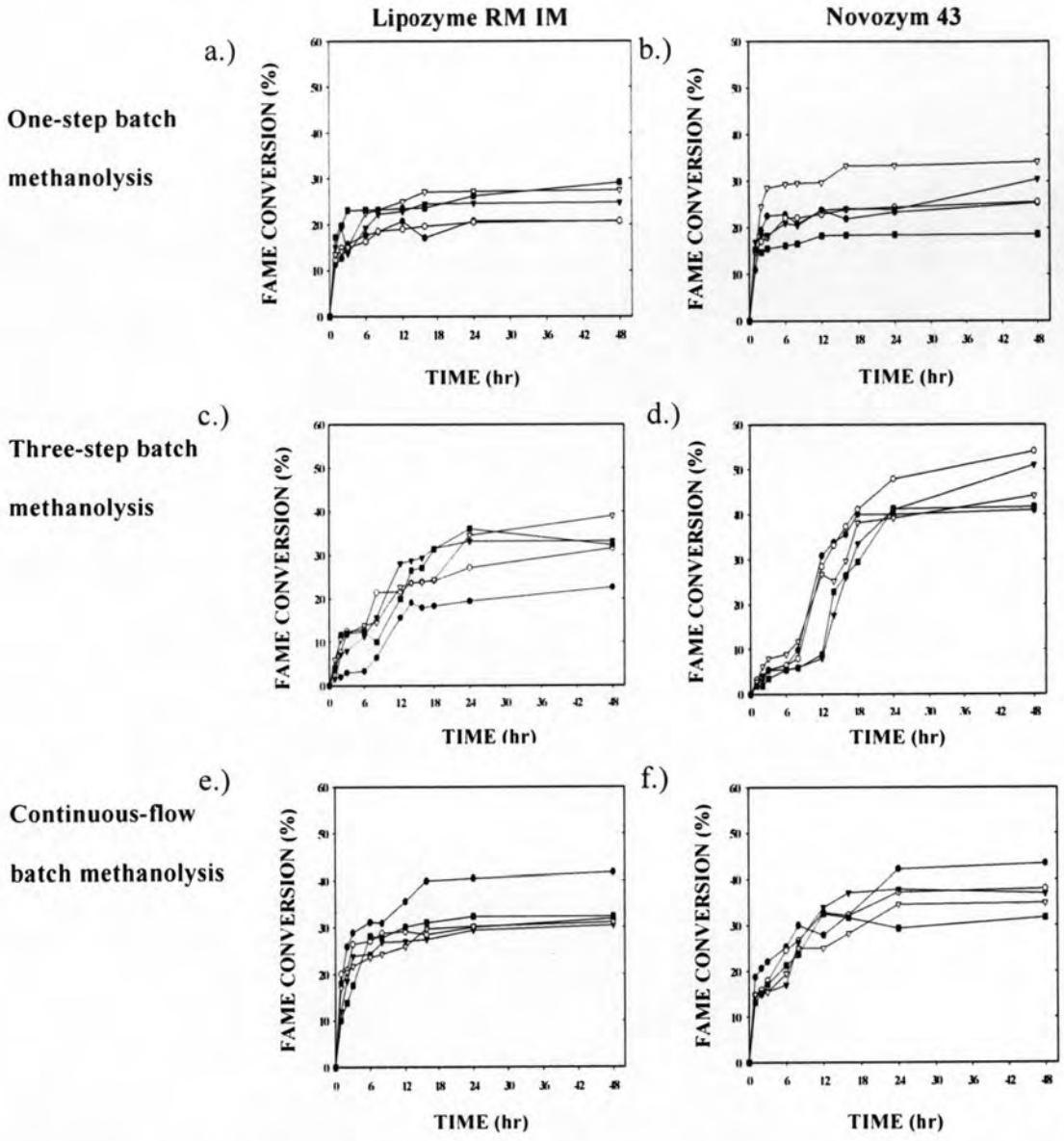


Fig. 4.11 Time courses of fatty acid methyl ester production using 20 % (w/w) (under the reaction conditions as described in section 3.4.5.2) of Lipozyme RM IM and Novozym 435 with the 21-cyc oil.

a.) Lipozyme RM IM with one-step methanolysis

b.) Novozym 435 with one-step methanolysis

c.) Lipozyme RM IM with three-step methanolysis

d.) Novozym 435 with three-step methanolysis

e.) Lipozyme RM IM with continuous-flow methanolysis

f.) Novozym 435 with continuous–flow methanolysis.

The water content in the reaction was: non water (●), 5% (w/w), (⊖), 10%(w/w)(▼), 15%(w/w)(▽) and 20%(w/w)(⊟).

4.3.2 Effect of water content in used frying oil on its methanolysis

Water content affected the transesterification, the excess water deactivated the fatty acid methyl ester production in one–step batch methanolysis, three-step batch methanolysis and continuous-flow methanolysis using Lipozyme RM IM (Fig. 4.12a, Fig. 4.12c and Fig. 4.12e, respectively). On the other hand, the FAME production increased up to 15 wt % of water for one-step batch and three-step batch methanolysis using Lipozyme RM IM (Fig. 4.12a and Fig. 4.12c). For comparison, in one-step and three-step batch methanolysis using Novozym 435 as the catalyst, the FAME conversion increased up to the highest percentage of conversion with 15 % and 10 % water in the reaction, respectively (Fig. 4.12b and Fig. 4.12d). On the other hand, in continuous-flow methanolysis the water did not necessary in the reaction when using Novozym 435 (Fig. 4.12f).

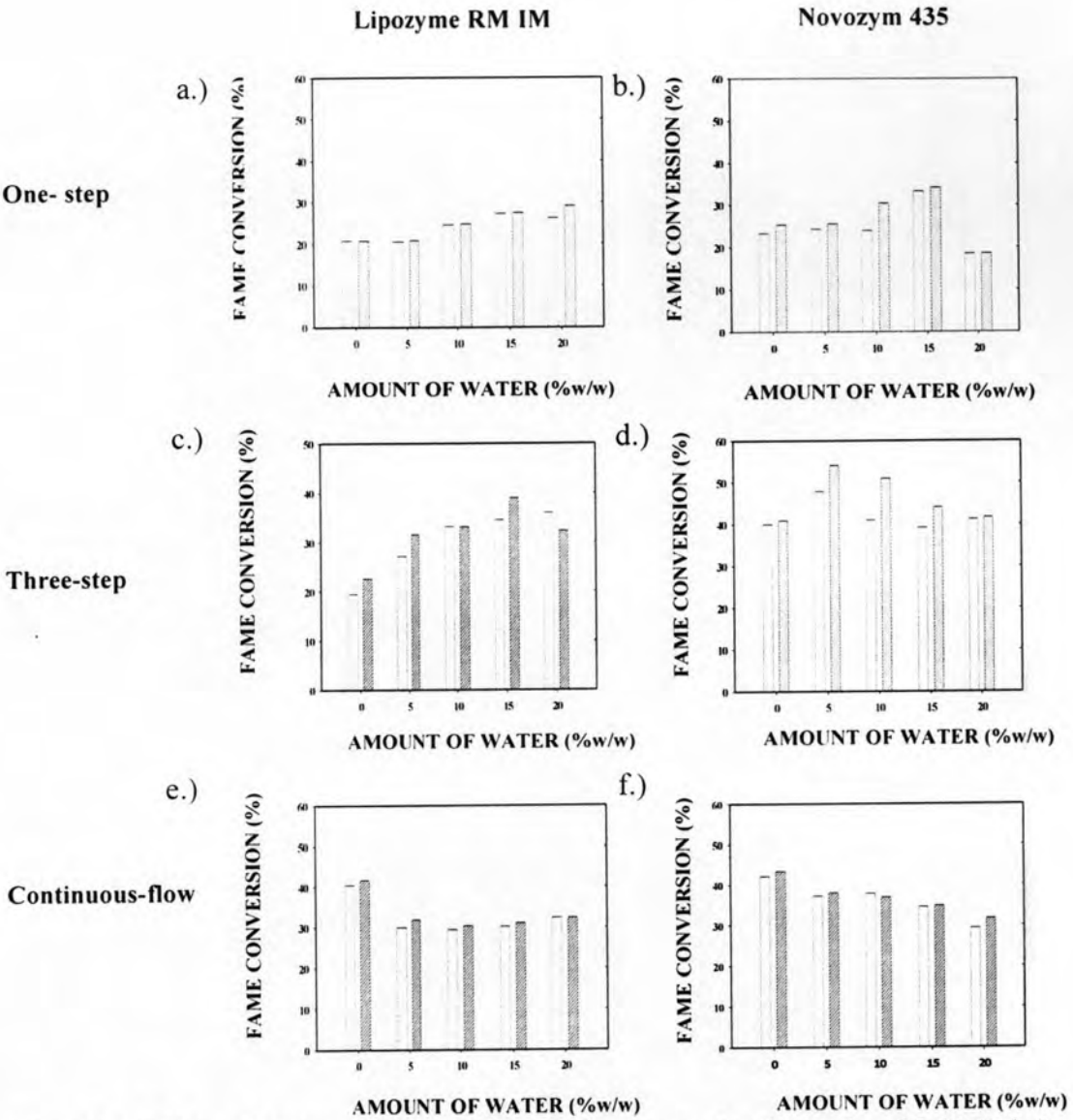


Fig. 4.12 Effect of water to fatty acid methyl ester production using 20 % (w/w) of immobilized lipase by weight of the 21 cyc-oil (fixed condition in section 3.4.5.1 and method describe in section 3.4.5.3) at 24 hours (□) and 48 hours (▨). a.) Lipozyme RM IM with one-step methanolysis, b.) Novozym 435 with one-step methanolysis, c.) Lipozyme RM IM with three-step methanolysis, d.) Novozym 435 with three–step methanolysis, e.) Lipozyme RM IM with continuous-flow methanolysis, f.) Novozym 435 with continuous–flow methanolysis.

4.3.3 Effect of immobilized lipase dosage in used frying oil on its methanolysis.

Immobilized lipase was used as heterogeneous catalysts. Thus, the reaction mixture was consisted of oil, immobilized lipase, water and methanol. Generally, lipase activity depends on the available surface area at the oil-water interface. Thus, the fatty acid methyl ester production depended on the amount of immobilized enzyme. The enzyme dosage of 20-40 % (w/w) did not increase with the ratio of Lipozyme RM IM and Novozym 435 (Fig. 4.13a and Fig. 4.13b)

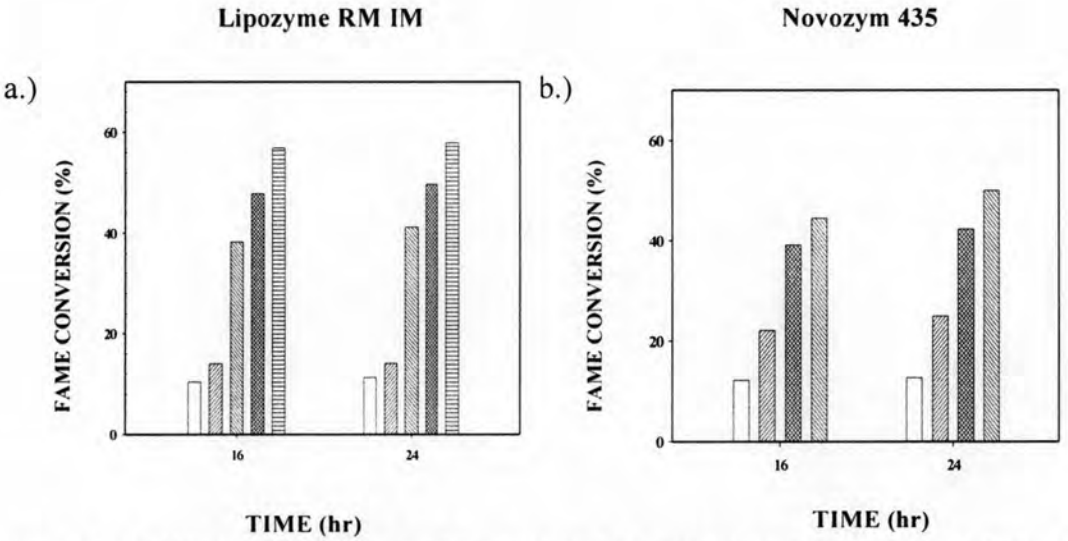


Fig. 4.13 Efffect of the dosage of Lipozyme RM IM (a) and Novozym 435 (b) to fatty acid methyl ester production of the 21 cyc-oil (fixed condition in section 3.4.5.1) in continuous-flow methanolysis with 0.018 ml/min (method describe in section 3.4.5.4). Percentage of dosage of immobilized lipase in the reaction: 5% (▭), 10 % (▨), 20%(▧), 30% (▩) and 40% (▤).

4.3.4 Effect of substrate molar ratio in used frying oil on its methanolysis

One of the most important variables affecting the fatty acid methyl ester production is the substrate molar ratio (oil: methanol molar ratio). When Lipozyme RM IM was used in the reaction, the velocity of the reaction was highest when the oil: methanol mole ratio was 1:3 at the bottom of the graph at 40°C, 50°C and 60°C (Fig. 4.14a). This result came from the substrate inhibition in the reaction (more discuss in section 5.3.4). On the other hand, the highest velocity of the reaction at 40°C, 50°C and 60°C in Novozym 435 when the oil: methanol molar ratio was 1:3 like the Lipozyme RM IM (Fig. 4.14).

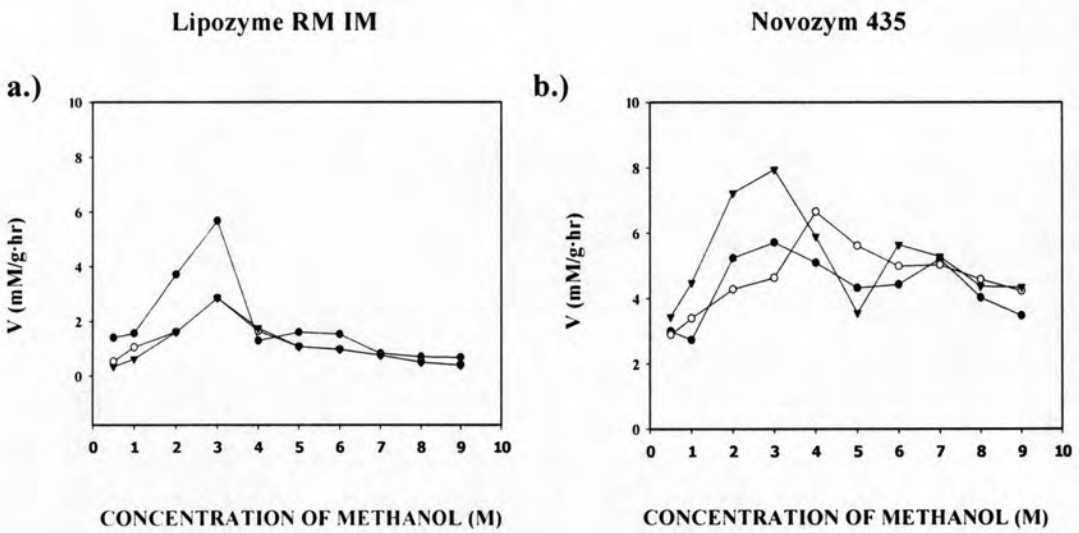


Fig.4.14 Efffect of substrate molar ratio in methanolysis using Lipozyme RM IM (a) and Novozym 435 (b) to fatty acid methyl ester production of the 21-cyc oil (fixed condition in section 3.4.5.1) in continuous-flow methanolysis with 0.018 ml/min (method describe in section 3.4.5.5) at 40°C (-●-), 50°C (-⊖-and 60°C (-▼-).

4.3.5 Effect of temperature on the initial velocity of tranesterification reaction

The maximum velocity and Michaelis constant of transesterification using Lipozyme RM IM and Novozym 435 were increased by the heat in the reaction (Fig. 4.15 and Fig. 4.16).

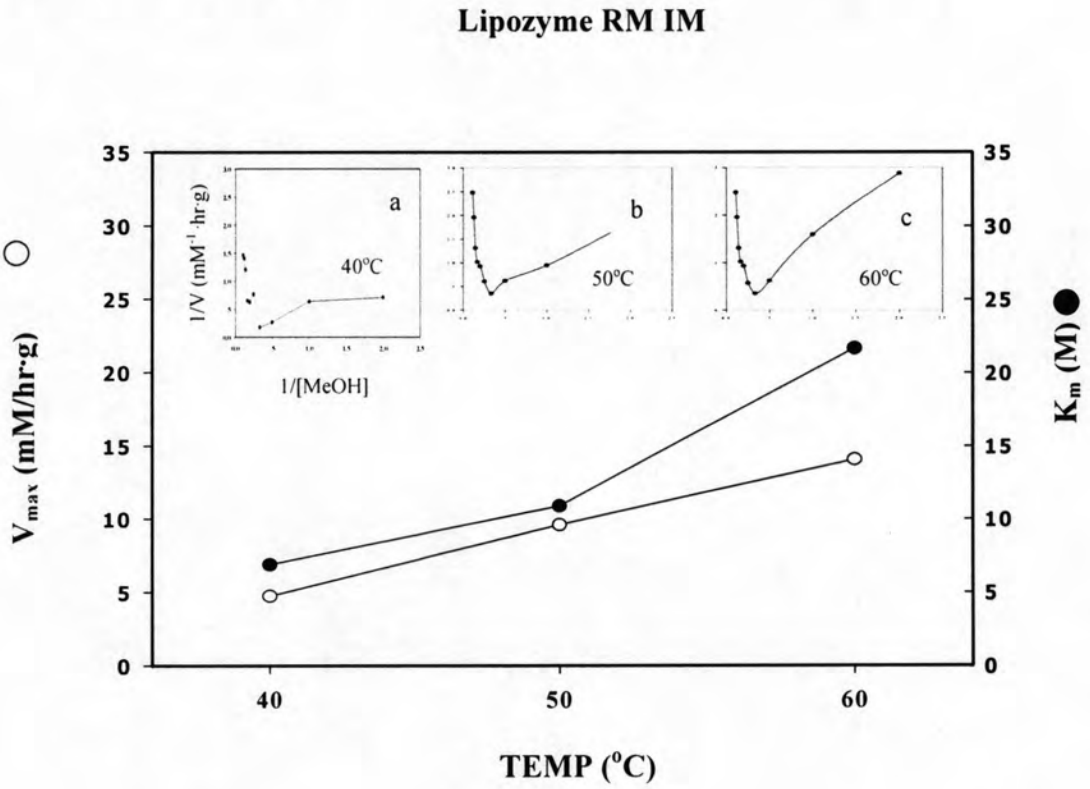


Fig. 4.15 Efffect of temperature in methanolysis using Lipozyme RM IM (fixed condition in section 3.4.5.1) in continuous-flow methanolysis with 0.018 ml/min (method describe in section 3.4.5.5) to initial velocity at 40°C (a), 50°C (b) and 60°C (c), maximum velocity (-⊖-) and michaelis constant (-●-).

Novozym 435

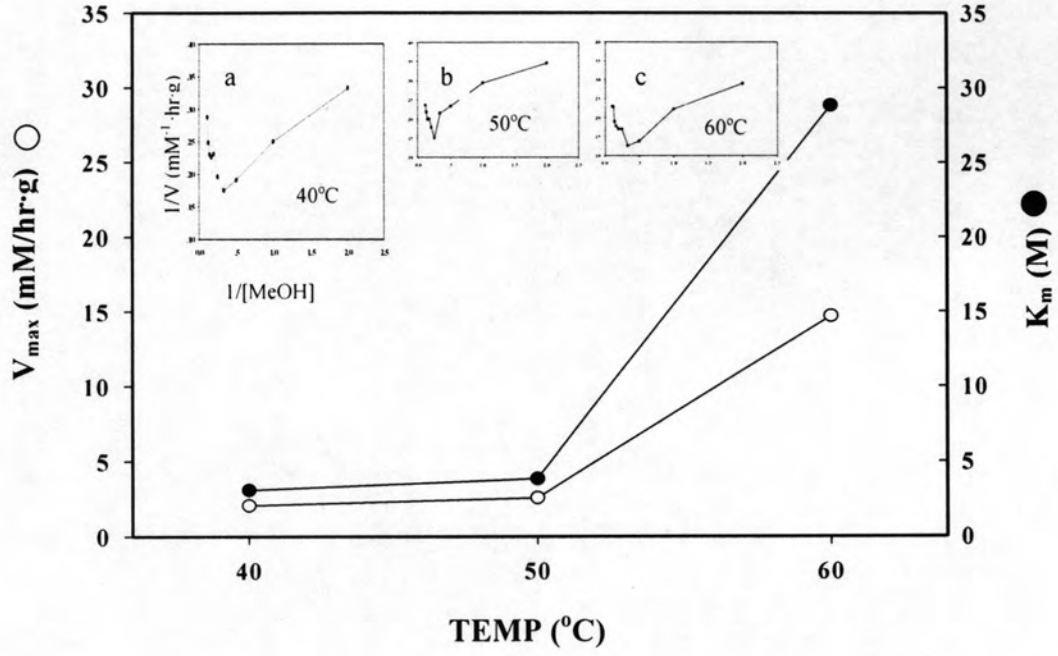


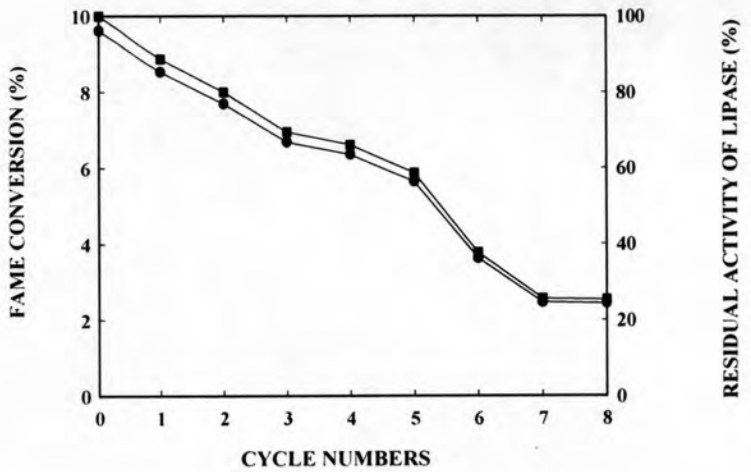
Fig. 4.16 Efffect of temperature in methanolysis using Novozym 435 (fixed condition in section 3.4.5.1) in continuous-flow methanolysis with 0.018 ml/min (described in section 3.4.5.5) to initial velocity at 40°C (a), 50°C (b) and 60°C (c), maximum velocity (-○-) and Michaelis constant (-●-).

4.3.6 The stabilities of immobilized lipase

During repeatedly used of immobilized lipase, glycerol layer gradually formed on the surface of the enzymatic support, which was caused the loss of lipase activity of Lipozyme RM IM and Novozym 435 (Fig. 4.17a and Fig. 4.17b).

a.)

Lipozyme RM IM



b.)

Novozym 435

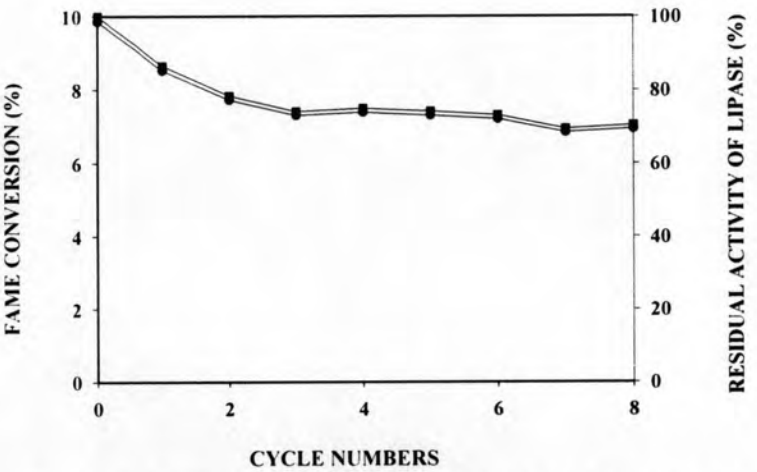


Fig. 4.17 Operational stability of Lipozyme RM IM (a) and Novozym 435 (b) using methanol as the acyl acceptor. The reaction was the mixture of used frying oil at the 21-cyc oil with 20% Lipozyme RM IM by weight of oil (fixed condition in section 3.4.5.1) with one-step of methanolysis at 40°C. Fatty acid methyl ester conversion (—●—) and Residual activity of lipase (%)(—■—).