CHAPTER V

DISCUSSIONS

Biodiesel has been used as alternative energy source in many countries, which the chemical production process was used wildly. Although biodiesel can be successfully produced by chemical approach, there are several associated problems, such as glycerol recovery and removal of inorganic salts (Shimada *et al.*, 2002). Use of enzymatic catalysts in transesterification of oils for biodiesel production addresses these problems and offers an environmentally more attractive option. According to Mangesh *et al* (2006), significant factors which affect the cost of biodiesel production are feedstock cost, plant size, and separation of glycerine by-product. Therefore, utilization of used frying oil as a starting feedstock seems more attractive as it is cheaper than the refined oil. However, a small amount of water and free fatty acids contained in used frying oil could lead to the generation of fatty acid alkaline salt (soaps) in the reaction. Hence, disposal of the resulting alkaline water creates other environmental concerns. Moreover, the frying conditions may cause the harmful compound in the used frying oil. The objectives of this research are 1) to salvage of used frying palm oil for biodiesel production using immobilized lipase and 2) to determine the PAHs contamination and the effect of PAHs on biodiesel production efficiency.

5.1 Properties of used frying oil

Frying conditions affected the physical and chemical properties of oil as well as the qualities of fried food. In this study, the following properties of oil were determined: color change, viscosity, water content, fatty acid composition and PAHs accumulation.

5.1.1 The color change of used frying oil

The refined palm oil was subjected to frying at 170°C for 15 minutes for 21 cycles of frying. The change of oil color was visually determined as one of the parameters for oil quality. The oil color became darker as the color intensity was increased 55.9 folds after 21 frying cycles (section 4.1.1). The change of oil color after processing of frying depends on type of oil. Maskan *et al.* (2003) reported the color change in frying sunflower oil. In their research, sunflower oil was used to fry potato at 170°C for 15 minutes for 50 cycles. The color intensity of oil determined by HunterLab Colorflex (A-60-1010-615 Model Colorimeter, HunterLab, Reston, Va.) increased by 10 folds. Their color intensity were expressed as L (whiteness or brightness/darkness), a (redness/ greenness) and b (yellowness/blueness) at any time, respectively and calculated the total color difference at various time. Due to the different in the calculation method, so their color intensity was different from this experiment. Moreover, the color intensity of sunflower oil after chicken was fried as the previous condition, increased 8 folds. The lower value of color change was caused by the different in fatty acid

composition between sunflower and palm oil and by the different in fried food between the potato and chicken. Thus, the color change was also caused by the presence of a high amount of the polyunsaturated fatty acids of oil such as linolenic acid and linoleic acid (Li *et al.*, 2007).

Goburdhun *et al.* (2000) reported the color change potato at 180°C for 7 minutes for 21 cycles of frying. The color intensity of oil increased by 1.8 folds. Darkening of the oil color is a result of hydrolysis, cyclization, polymerization, isomerization and oxidation of oil (Seebun *et al.*, 2000). The leaching of pigments from the chicken into the oil and the formation of the brown pigment (melanoidin in maillard reaction) also lead to the color change (Seebun *et al.*, 2000). As change in oil color during frying depends on degrees of oil hydrogenation and frying time, which relates to changes of other properties.

5.1.2 Viscosities of used frying oil

Viscositiy is the one of the quality indexs of oils. When oil was heated, the viscosity increased as a result of 1) polymerization of non–volatile decomposition products in the oil (Goburdhun *et al.* 2000), 2) an increasing of the average chain length of fatty acids in the triglycerides, and 3) an increasing and accumulation of non–volatile decomposition products in the oil (Goburdhun *et al.*, 2000). As shown in Fig. 4.2, the viscosity of 21-cyc oil increased by 10 % relative to that of refined palm oil. The slight increase of viscosity of the used oil could be due to the increasing of the average chain length of fatty acids in the oil (Fig. 4.3). This result agrees with a report from Maskan *et al.* (2003) that the viscosity value

of sunflower oil after 50th frying increased by 7 % (from 0.83 mPa·s (initial value) to 0.89 mPa·s).

5.1.3 Water Content of refined and used frying oil.

During frying oil, moisture content was an important factor in the determination of the oil's uptake (Goburdhun *et al.*, 2000). When the frying cycles increased, the moisture decreased by 0.3 % (Table. 4.1). The reduction of water content was occured by the evaporation of water during the frying and the water content was replaced by the release of fat from the chicken. Heat can cause melting of the chicken's fat that leaches out into the frying oil. Goburdhun *et al.* (2000), compared fat content between frying potato and chicken and they founded that chicken had released fat (2.4%) into the frying oil while the potato absorbed fat (6.9%) during frying.

5.1.4 Fatty acid composition of used frying oil

The fatty acid composition profile in each oil varies among various types of vegetable oils. In this study, fatty acid composition of refined and used frying palm oil were determined (section 3.4.4). Fig. 4.3 shows that the amounts of saturated and unsaturated fatty acids of refined and used frying palm oil. The four major fatty acids were oleic acid (43.99%), palmitic acid (37.36%), linoleic acid (12.20%), and stearic acid (3.97%). These fatty acids were bounded to the glycerin as triglycerides. After frying oil, the total unsaturated fatty acid increased

by 4.38 % and saturated fatty acid decreased by 4.39%. Although total fatty acid composition change was not much, but it was significant compared to the literature described below.

When the oil was fried, the unsaturated fatty acid was generally decreased since the double bond was broken down to be the saturated fatty acid (Leung *et al.*, 2006). Sulieman *et al.* (2006), reported that the saturated fatty acids was increased by 0.33, 0.80 and 1.4 % after 8 hours of frying in sunflower oil, cotton seed oil and palm oil, respectively.

The fatty acid composition is different in various types of oil. Dermirbas *et al.* (2003) reported that the major fatty acid in palm oil, soybean oil, olive oil and cotton oil were palmitic acid (C 16:0) 42.6 %, linoleic acid (C 18:2) 56.2%, oleic (C 18:1) 74.7% and linoleic acid (C 18:2) 57.4 %, respectively. The highest fatty acid value in refined palm oil and used frying palm oil were 42.83 % oleic acid and 43.99% oleic acid, respectively (Fig. 4.3).The difference of fatty acid composition affects the properties of oil. Therefore, it affects biodiesel property. Although the cetane number of highly saturated fatty acid oil is higher than that of the highly unsaturated oil (Ma *et al.*, 1999), high saturated fatty acid oil tends to crystallize at unacceptably high temperatures (Ma *et al.*, 1999).

Therefore, while saturated fatty acid oil give biodiesel with high cetane number, the unsaturated fatty acid oil such as palm oil (refined and used oil) gives biodiesel with a better cold flow.

5.2 Identification of PAHs in oil

PAHs are a group of aromatic compounds that have been more concern in recent years due to their toxic potential. They are known as highly stable contaminants present in many foods including fried food (Giorgia *et al.*, 2006). In this study, PAHs in refined and used frying palm oil were determined and their effect on lipase-based transesterification was investigated.

5.2.1 Extraction of PAHs from oil

The 21-cyc oil was contaminated by PAHs because of their specific characteristics, which are a significant problem for their extraction from oil. Difficulties with their extraction from oil increase as the affinity of PAHs to oil components increases. PAHs in the 21-cyc oil were extracted by liquid-liquid extraction method by 100 % acetonitrile. In this experiment, acetonitrile was chosen because this solvent produced less emulsions than the other and easier to separate from the oil sample. The recovery of naphthalene and benzo[a]pyrene seem low, but the recovery range was in the average recovery range reported so far as the previous works (Table 4.2).

Moret *et al.* (2000) determined PAHs in edible fats and oils using dimethylformamide/water (9:1 v/v). The recovery ranged of PAHs between 42.5-92.2%, depending on molecular masses, except for naphthalene (31.9%) probably due to its volatility. Therefore, solid phase extraction or SPE purification was used

and naphthalene can get 63 % recovery by using dimethylformamide/cyclohexane (9:1 v/v) or liquid-liquid partition.

Vazquez *et al.* (2000) reported the presence of PAHs in edible oils and fats. The ultrasonic bath and acetonitrile (100%) were used in their extraction method. Then, the percentage recovery of benzo[a]pyrene was 81 %. The impurities were used purified by silica in the method.

Barranco *et al.* (2003) optimized the extraction procedure by using hexane to increase the recovery of low molecular mass PAHs (PAHs with 2-4 rings) with solid phase extraction, which can get the recovery at ~ 90 %. Hexane was reduced losses of low PAHs by volatilization during the evaporation step.

Therefore, the factors that affect to the recovery of naphthalene and benzo[a]pyrene in oil were the volalization of each PAHs and the solubility of PAHs in the extraction solvent.

5.2.2 Type and amount of PAHs

The presence of PAHs has been reported in various types of vegetable oils (Barranco *et al.*, 2003). For example, benzo[a]pyrene was found in palm oil (1.1 ppb), olive oil (0.5 ppb), coconut oil (1.7 ppb) and sunflower oil (3.6 ppb). In this study, naphthalene and benzo[a]pyrene were detected in refined palm oil at 197±57.58 μM (0.19±0.58 ppb) and 36.2±14.38 (0.04±0.14 ppb), respectively (Fig.4.6). Naphthalene accumulated and increased by 88 % to 1646±256.82μM (1.6± 0.25ppb) after 21 cycles (Fig.4.6).

Giorgia *et al.* (2005) reported that, after 60 kg of potato was fried in palm oil/sunflower oil (60/40 v/v) at 180°C for 2.5 hours, naphthalene was increased to 2.6 ppb (0.043 ppb per kg potato). Naphthalene was increased 0.4 ppb in palm oil/soybean oil (50/50) after frying 60 kg of potato at 180°C for 2.5 hours. Janoszka *et al.* (2004) reported the benzo[a]pyrene was found in chicken breast (1.5 ppb/kg), pork chop (4.1 ppb/kg) and beef collar (3.8 ppb/kg). As this result, type of oil and type of fried food affected the occurrence of PAHs in oil.

5.2.3 Effect of PAHs on hydrolytic activities of immobilized lipase

As the used frying palm oil was subject for biodiesel production using immobilized lipase, effect of PAHs presented and increasingly formed during frying on biodiesel production efficientcy was investigated. According to the result in chapter 4, section 2.3, there was significant amount of naphthalene adsorbed onto surface of immobilized lipase, Lipozyme RM IM (1.96±0.002 μmole/g lipase) and Novozym 435 (1.96±0.018 μmole/g lipase) (Table 4.3). PAHs adsorbed onto the immobilized lipase affected the hydrolytic activities of immobilized lipase. To test this assumption, 2.0 μmole naphthalene was dissolved in the mixture of methanol (3.0 ml) and water (7.0 ml) and immobilized lipase (0.2 g) (section 3.4.3). When naphthalene was adsorbed onto the surface of lipozyme, the hydrolytic activity of Lipozyme RM IM decreased by 79.03 %, 80.01 % and 79.91 % after mixing for 12, 24 and 48 hours, respectively (Fig. 4.9a).

Gong *et al.* (2007) reported that the PAHs adsorption on the activated carbon was affected by adsorption capacity of oil or the solubility of PAHs in oil. Thus, the solubility of PAHs in each solvent phase was involved in the adsorption of PAHs onto the immobilized lipase in our experiment. Mackay *et al.* (1992) reported that the solubility of naphthalene and benzo[a]pyrene were 31 and 0.0038 mg/l. Moreover, the Log K_{ow} of naphthalene and benzo[a]pyrene were 3.37 and 5.91, respectively. That mean both of PAHs were dissolved in the oil phase and the support of the immobilized lipase more than the water and methanol phase. Whereas, PAHs were found in the oil phase and the immobilized lipase supported.

When PAHs on the Lipozyme RM IM was washed by hexane, the PAHs (naphthalene and benzo[a]pyrene) were removed from the support of them. Thus, the hydrolytic activity was recovered. This result, confermed that the PAHs affected the hydrolytic activity of immobilized lipase. In contrast with the hydrolytic activity of Novozym 435 could not recover by hexane.

Kojima *et al.* (2004) reported that the high hydrophobicity of the solvent remained the stability of immobilized lipase. Therefore, the hydrophobicity of hexane (3.5) was higher than other solvent when compared with chloroform (2.0), dimethylsulfoxid (-1.3), ethanol (-0.24) and butanol (0.8). Thus, hexane was chosen to be the washing solvent in this experiment because of its hydrophobicity maintaining the hydrophobicity of the immobilized lipase.

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

Immobilized lipase was used as a catalyst in biodiesel process. In this research, we used the used frying oil in transesterifiction with methanol (methanolysis) and immobilized lipase (Lipozyme RM IM and Novozym 435) as the catalyst. The important parameters in methanolysis were studied as follow:

1) Three types of methanolysis (one-step methanolysis, three-step methanolysis and continuous-flow methanolysis).

2) Amount of water in methanolysis (non-water, 5 wt%, 10 wt%, 15 wt% and 20 wt%).

3) Dosage of immobilized lipase (5 wt%, 10 wt%, 20 wt%, 30 wt% and 40 wt%).

4) Temperature (40°C, 50°C and 60°C).

5) Methanol to oil mole ratio (0.5:1, 1:1, 2:1, 3:1, 4:1, 5:1, 6:1, 7:1, 8:1 and 9:1).

6) Stability of immobilized lipase.

5.3.1 Methanolysis type of used frying oil

The efficiency of fatty acid methyl ester (FAME) formation from methanolysis depends on several important factors. The methanolysis type is one of them. In this study, three types of methanolysis were compared: one-step addition, three-step addition and continuous-flow addition. The highest of FAME conversion (%) in one-step methanolysis, three-step methanolysis and continuous-

flow methanolysis with Lipozyme RM IM were 29.17 %, 33.23% and 41.68 %, respectively (Fig. 4.11a, Fig. 4.11c and Fig. 4.11e). The continuous-flow methanolysis was the best method among three types of methanolysis which was gradually increased 8.45% from three-step methanolysis. Similarly to the transesterification of Lipozyme RM IM, the highest FAME conversion (%) in one-step methanolysis, three-step methanolysis and continuous-flow methanolysis when using Novozym 435 were 25.58 %, 54.08 % and 43.34%, respectively (Fig. 4.11b, Fig. 4.11d and Fig. 4.11f). As this result, the three-step methanolysis was the best method among three types of methanolyses when using Novozym 435.

Beko *et al.* (2002) compared the one-step, two-step, three-step, four-step methanolysis and continuous-flow methanolysis using Novozym 435 with sunflower oil. Their result showed that the continuous-flow methanolysis gave the highest at 97% conversion. When using three-step methanolysis, the conversion (%) was approximately 40% conversion. Beside this result, the continuous-flow methanolysis was the efficiency method in this experiment. However, the other reports showed that three-step methanolylsis could get the FAME conversion (%) approximately 100 % conversions. As the result of Du *et al.* (2005) reported that soybean oil was transesterified with Lipozyme TL using three-step methanolysis at 40°C. The FAME conversion was 90% conversion in third step. Similarly to Shimada *et al.* (2002) reported that the highest FAME conversion (%) was 90.4 % from the transesterification of waste oil using Novozym 435 in three-step methanolysis at 30°C.

The excess methanol deactivated the immobilized lipase activity (Shimada *et al.*, 2002). Thus, lower methanol-addition in three-step methanolysis

could reduce the negative effect of methanol on the lipase activity. In their studies, three-step methanolysis was used to activate the immobilized lipase (Novozym 435) in transesterification of waste oil. The second and third step increased 1 and 2 folds from the first step, respectively. Similar to the FAME conversion when using three-step methanolysis, the second and third step with immobilized lipase (Lipozyme RM IM and Novozym 435) increased 1 and 2 folds from the first step, respectively (Fig. 4.11c and Fig. 4.11 d).

The comparison of the FAME conversion between Novozym 435 and Lipozyme RM IM. Novozym 435 gave the FAME conversion (%) more than 10% conversion of Lipozyme RM IM in continuous methanolysis. This result was explained by the different support of the immobilized lipase between Lipozyme RM IM and Novozym 435. The different of the immobilized lipase support's chemical structure affected to the adsorpsion of the glycerol. Shimada et al. (2002) reported that the FAME production was affected from the remaining of glycerol in the reaction. When the support was adsorbed with glycerol, the immobilized lipase was deactivated in the reaction. Thus, the support of Lipozyme RM IM was the macroporous anion (duolite) resin. Therefore, the glycerol was adsorbed into the porous more than the support of Novozym 435(the support of Novozym 435 was anion resin bead).

In the continuous methanolysis, the FAME conversion was affected by the flow rate of the methanol feeding. The FAME conversion with flow rate at 0.018, 0.083 and 0.159 ml/min were 42%, 30% and 14%, respectively (Fig. 4.10).The flow rate at 0.018 ml/min was increased the FAME conversion with approximately 2 folds and 1 fold from 0.083 and 0.159 ml/min, respectively (Fig.

4.10) because of the longer contact time between the oil and immobilized lipase. This also agree with a report published by Chulalaksananukul. (2001) reported that lower flow rate gave higher conversion, when using the continuous method in column reactor with an immobilized lipase. The percentage of conversion was about 27.8 %, 24.0 %, 17.6 %, 15.0 % and 13.7 % for flow rate at 6.0, 10.5, 17.2, 23.2 and 27.6 ml/hr, respectively. Royon *et al.* (2007) reported that FAME conversion obtained with the continuous reactor was 95%, 74%, 60% and 53% at flow rate 9.6, 12, 14 and 18 ml/hr. The methyl ester conversion increased 42% when the flow rate increased at 8.4 ml/hr. Thus, the methyl ester conversion was affected by flow rate of the reaction.

In conclusion, the continuous methanolysis with the flow rate at 0.018 ml/min was chosen to use in this study because of the highest percentage of FAME conversion by Lipozyme RM IM and Novozym 435.

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

The water content had the positive and negative effects in the methanolysis. In continuous methanolysis using Lipozyme RM IM and Novozym 435, water did not affect the activity of them (Fig. 4.12e and Fig. 4.12f). In contrast, the Lipozyme RM IM and Novozym 435 in one-step methanolysis required 20% and 15 % water added into the reaction to maintain a specific water activity of the immobilized lipase during operation (Fig. 4.12a and Fig. 4.12b). Whereas, the result of three-step methanolysis using Lipozyme RM IM and Novozym 435 required 15% and 5 % water in the reaction, respectively (Fig.

4.12c and Fig. 4.12d). This result was similarly to the report by Yang *et al.* (2006) which showed that 15% water content maintained a specific water activity of Novozym 435 and related to the interaction of water with the hydrophilic groups located on enzyme molecule surface. The hydrogen bond interaction changed inside enzyme and leaded to an open the conformation form.

Yuji *et al.* (2002) reported that the adding of water in the reaction was decreased the FAME conversion approximately 10% and 5% in Lipozyme RM IM and Novozym 435, respectively. Similarly to the reported of Shimada *et al.*, (1999), Oda *et al.*, (2005), and Du *et al.*, (2005) showed that water in the reaction decreased the reaction rate when soybean oil was transesterified with Novozym 435. Moreover, the higher amount of the water decreased the rate of methanolysis and the continuous methanolysis was not necessary to add the water in the reaction (Fig. 4.12e and Fig. 4.12f).

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

The criteria about the optimum quantity of immobilized lipase depend on the effective of the FAME conversion in the reaction. For example, when the amount of immobilized lipase increased in 2 folds, the FAME conversion should increase 2 folds too. Moreover, the optimum of the quantity of immobilized lipase depended on the adsorption of enzyme support. When the reaction used high amount of immobilized lipase, the substrate can adsorb too

much in the support of enzyme. That lead to the support of the immobilized enzyme was swelled by the substrate.

In this experiment, the FAME conversion from 10% (w/w) and 20% (w/w) of Lipozyme RM IM in the reaction were about 13 % and 22%, respectively, which increased about 1 fold. While the FAME conversion from 30% (w/w) and 40% (w/w) of Lipozyme RM IM were not increased with 3 folds and 4 folds from 10% (w/w) of enzyme (Fig. 4.13a). Therefore, the using of Lipozyme RM IM with 30% and 40% in this experiment were not chosen in the reaction. Similarly to Novozym 435 the amount of Novozym 435 with 30% (w/w) was not effective amount in this reaction because of the FAME conversion were not increased along the increased with the amount ratio of the Novozym 435 (Fig. 4.13b). Moreover, the using of 40% (w/w) Novozym 435 was adsorped by the substrate. Thus, the transesterification of used frying oil with 40 % (w/w) of Novozym 435 could not process. Therefore, the optimum quantity of the immobilized lipase was 20% (w/w) when using Novozym 435 in this experiment.

Xu *et al.* (2003) reported that the optimum quantity of Lipozyme TL IM was 4 % (w/w) in two-step methanolysis of soybean oil at 30°C. In three-step methanolysis of soybean oil as the same previous condition, the optimum quantity was 10 % (w/w) of Lipozyme TL IM. Due to lipase activity depended on the available surface area. Thus, rate of the reaction depended upon the amount of enzyme, which limited by the available surface area of the immobilized lipase. This result was not similarly to the reported of Kose *et al.* (2002). The report showed that the optimum quantity of Novozym 435 in the transesterification of soybean oil and cotton seed oil was 30 % (w/w) at 40°C.

5.3.4 Kinetic and mechanism of reaction

Used frying palm oil and methanol were used as the substrates in the transesterification reaction. The maximum velocity of the reaction were 6.9, 10.90 and 21.66 mmole/hour·g to (Fig. 4.15) when Lipozyme RM IM in the reaction at 40°C, 50°C and 60°C. The maximum velocity of the reaction increased about 4.0 and 13.70 mmole/hour·g when the temperature was increased 10°C and 20°C, respectively. In the same condition, when the used frying palm oil was transesterified using Novozym 435 as a catalyst, the maximum velocity of the reaction at 40°C, 50°C and 60°C were 3.90, 3.85 and 28.84 mmole/hour·g, respectively (Fig. 4.16).

When compared between Lipozyme RM IM and Novozym 435 at 40°C and 50°C, the maximum velocity of Lipozyme RM IM was higher than Novozym 435 3.09 and 7.05 mmole/hour·g, respectively (Fig. 4.15 and Fig. 4.16). This result suggested that the transesterification at 40-50°C using Lipozyme RM IM was faster than using Novozym 435. However, the maximum velocity of Novozym 435 at 60°C was higher than Lipozyme RM IM for 7.18 mmole/hour·g. Thus, the transesterification of Novozym 435 at 60°C was faster than Lipozyme RM IM. This result was quite similar to Al- Zuhair *et al.* (2005) which reported that the maximum velocity of transesterification using Lipozyme RM as the catalyst at 50°C was 5.41 mmole/hour·g. Moreover, Heinsman *et al.* (2001) reported that the maximum velocity of esterification with ethanol using Novozym 435 as the catalyst at 35°C and 45°C were 1.85 and 2.93 mmole/hour·g. When the

temperature of the reaction increased 10°C, the maximum velocity of the reaction increased 1.08 mmole/hour·g.

The other factor in the reaction was the molar ratio of the methanol in the reaction. When the methanol to oil molar ratio was three molar in the reaction with Lipozyme RM IM and Novozym 435 at 40°C, 50°C and 60°C (Fig.4.14a) and Fig. 4.14b), the maximum velocity was the highest value in this reaction. Except for the using of Novozym 435 at 50°C, the optimum methanol to oil molar ratio was four molar. This was caused by the immobilized lipase was deactivated by the excess of methanol in the reaction (Kumar *et al.*, 2007). As the result of Chen *et al.* (2003) reported that the optimum molar ratio of methanol to oil was three to one molar ratio in transesterification using Novozym 435 at 30°C with soybean oil.

Besides, the Michaelis constant in the reaction of Lipozyme RM IM and Novozym 435 at 40°C, 50°C and 60°C were 4.76, 9.62, 14.08 and 2.06, 2.58, 14.71 M, respectively (Fig. 4.15 and Fig. 4.16). In contrast, Heinsman *et al.*, (2001) reported that the Michaelis constant were 0.018, 0.042 M in the esterification of ethanol at 35°C, 45°C, respectively. When the Michaelis constant was increased in the reaction, suggesting that the contact between lipase and substrate in the reaction was not suitable.

Thus, the temperature and the concentration of the methanol in the reaction affected to the optimal conditions for the lipase catalyzed transesterification. In this study, the optimum condition for transesterification using lipase catalyzed was the transesterification at 60°C and used three to one methanol to oil molar ratio.

5.3.5 The stabilities of immobilized lipase

During the repeated use of immobilized lipase, the enzyme lost activity in the transesterification reaction. After that, the immoblilized lipase was washed by hexane. Until the 5th cycles' usage of Lipozyme RM IM the residual activity of enzyme decreased to 50 % or the half life of the Lipozyme RM IM was 5 cycles of usage in the reaction (Fig. 4.17a). After the 8th cycles usage, the residual activity of lipase decreased to 25 % (Fig. 4.17a), which was affected by the glycerol in the reaction. Du *et al.* (1982) reported that the relative activity of lipase decreased when the glycerol dissolved in the reaction. Then, soybean oil was transesterified with Lipozyme TL IM and washed by isopropanol, 50% residual acitivity decreased at the 8th cycles.

In this experiment, the residual activity of Novozym 435 decreased to 50 % when the cycle's usage was more than 10 cycles or half life of Novozym 435 was more than 10 cycles of usage in the reaction (Fig. 4.17b). This result also agrees with a report of Yang *et al.* (2006). The report showed that the residual activity decreased to 50 % when the cycle's usage was more than 10 cycles (the 15th cycles) in the immobilized lipase from *Candida* sp (the same genus as Novozym 435) with soybean oil. On the other work, soybean oil was transesterifed using Novozym 435 as the catalyst and methanol as the acyl donor. The residual activity of Novozym 435, which Novozym 435 was not washed by any solvent, decreased to 50 % when the cycles were at the 5th cycle of usage. As the same experiment, soybean oil was transesterified using Novozym 435 as the catalyst and methyl acetate as the acyl donor. More than 100 cycles, the residual

activity of Novozym 435 did not decrease from the initial activity. This effect was caused by no glycerol produed in the reaction when used methyl acetate as the acyl donor. Thus the glycerol has to eliminate from immobilized lipase during transesterification for biodiesel production.

5.3.6 PAHs in biodiesel

One of the raw material of biodiesel is used frying oil. Thus, the accumulation of PAHs in used frying oil affect to the properties of biodiesel. In the combustion, biodiesel containing PAHs can cause air pollution. This study show the valuable data about the adsorption of PAHs on immobilized lipase (enzymatic transesterification). When immobilized lipase was washed by hexane, PAHs was released in hexane. That lead to the easier management of PAHs in the environment.

Lin *et al.*, 2006 reported that PAHs content in palm-biodiesel was close to zero, so a high fraction of biodiesel blends resulted in a lower PAHs emission.