World Academy of Science, Engineering and Technology
International Journal of Chemical and Molecular Engineering
Vol:5, No:4, 2011

Biodiesel Fuel Production by Methanolysis of Fish Oil Derived from the Discarded Parts of Fish Catalyzed by *Carica papaya* Lipase

P. Pinyaphong, P. Sriburi and S. Phutrakul

***Abstract*—**In this paper, naturally immobilized lipase, *Carica papaya* lipase, catalyzed biodiesel production from fish oil was studied. The refined fish oil, extracted from the discarded parts of fish, was used as a starting material for biodiesel production. The effects of molar ratio of oil: methanol, lipase dosage, initial water activity of lipase, temperature and solvent were investigated. It was found that *Carica papaya* lipase was suitable for methanolysis of fish oil to produce methyl ester. The maximum yield of methyl ester could reach up to 83% with the optimal reaction conditions: oil: methanol molar ratio of 1: 4, 20% (based on oil) of lipase, initial water activity of lipase at 0.23 and 20% (based on oil) of *tert*-butanol at 40°C after 18 h of reaction time. There was negligible loss in lipase activity even after repeated use for 30 cycles.

***Keywords*—**biodiesel fuel production, methanolysis, fish oil, *Carica papaya* lipase.

I. Introduction

BIODIESEL, monoalkyl esters of vegetable oils or animal fats, can be synthesized from edible, non-edible and waste oil [1]. Biodiesel is attractive because it is a non-toxic, biodegradable and renewable energy source [2]. Production of biodiesel from vegetable oil and animal fats has drawn more attention because of the increasing awareness of environmental pollution and short supply of fossil fuels [3]. At present, the industrial-scale production of biodiesel is performed chemically, using alkali as catalyst. Chemical methods give high conversion ratio of triacylglycerols (TAG) to methyl esters (biodiesel) in short times (4-10 h) [4]. However, chemical transesterification are connected with some drawbacks as for example, excessive methanol requirement, high energy consumption, difficulty in glycerol recovery, disposal of fatty acid alkaline salts (soaps) creating other environmental concerns and high water consumption during washing in the purification steps [2]. Moreover, a by-product, glycerol, is also obtained and this must be separated due to its high viscosity, and as it is usually contaminated with alkaline catalysts, its purification to provide an added value to the alkaline process is not easy [5].

P. Pinyaphong is with the Department of Chemistry, Uttaradit Rajabhat University, Uttaradit, Thailand 53000 (corresponding author to provide phone: 66 81 8861587; fax: 66 55 411096 ext 1312; e-mail: porntippapinyaphong@yahoo.com).

P. Sriburi is with the Chemistry Department, Chiang Mai University, Chiang Mai, Thailand 50200 (e-mail: pensiri@chiangmai.ac.th).

S. Phutrakul is with theChemistry Department, Chiang Mai University, Chiang Mai, Thailand 50200 (e-mail: scchi007@chiangmai.ac.th).

Enzymatic method can overcome those problems which allow mild reaction conditions and no chemical waste is produced [6]. Several studies on lipase-catalyzed alcoholysis of vegetable oils and animal fats with primary and secondary alcohols in a solvent or a solvent-free system have been reported [7]-[8]. Owing to the toxicity and flammability of organic solvents, and the easiness of product recovery, enzymatic alcoholysis in a solvent-free system is preferable. Recently, it has been claimed that enzymatic technology has been applied to the industrialization with a capacity of 20,000 ton/year in China, and this is the first industrial scale with lipase as the catalyst in the whole world to date [9]. Immobilized *Candida Antarctica* lipase was found to be the most effective for the methanolysis of oil and fat [10]-[11]. However, its cost is prohibitively high for this purpose. In this paper, some efforts have been made to explore the possibility to use a relatively cheap lipase from *Carica papaya* latex. The particulate part of *C. papaya* latex possesses lypolytic activity with 1(3)-regiospecificity [12] as *Rhizopus oryzae* lipase which was an effective catalyst in methanolysis for biodiesel synthesis [13]-[14]. *C. papaya* lipase (CPL) has potential as a biocatalyst in lipid transformation [15]. CPL washing with iso-propanol produced 69.8% methyl ester from methanolysis of triolein [16].

In fact, the price of raw oil is the most dominant factor in the cost of biodiesel fuel, and determines the competitiveness of biodiesel with fossil fuel in the fuel market [17]. To search for a low-cost raw material with adequate fuel characteristics for biodiesel production is an important step towards establishing a successful biodiesel industry. The annual production quantity of discarded parts of fish in Thailand amounted 34,144 tons each year. The discarded parts of fish including viscera, eyes, fins, tails and maw is frequently ground into fishmeal for pet food and ferment as fertilizer. In this study, the extraction of crude fish oil from the discarded parts of fish for use as raw oil for the production of biodiesel is considered to reduce cost of biodiesel production and also adding value of the discarded parts of fish. Thus, the present paper discussed the enzymatic synthesis of biodiesel from the refined fish oil extracted from the discarded parts of fish with CPL and different synthesis conditions, such as molar ratio of oil: methanol, amount of lipase, solvent, temperature of reaction, and initial water activity of enzyme, were optimized for further industrialization.

Therefore, it was refined by a set of pre-treatment processes. This process included the absorption of the fish residue by active clay, winterizing at 4°C for 120 min, centrifuging at 3,000 rpm for 10 min to remove the solid impurities, washing with water by 5% by volume of distilled water for 15 min, and heating to 105°C for 30 min [17]. Some properties of the refined fish oil including the acid value and the moisture content were verified. The refined fish oil was then used to produce biodiesel.

A. Preparation of CPL

Papaya latex was obtained by making a longitudinal incision on the unripe fruit (70-100 days) of Thai papaya tree. The latex was scraped and collected in plastic bottle and then was stored at -20°C until be usable. Defrosted hard latex at room temperature and then centrifuged at 9,500×g for 15 min. The insoluble particulate was washed with distillate water and lyophilized for using as CPL. The water activity of CPL was determined by a Thermoconstanter TH200 Novasina (Novasina, Switzerland), respectively. The hydrolysis activity of CPL on refined fish oil was investigated by colorimetric method [18].

B. Determination of the fatty acid profiles of glycerides in refined fish oil

The refined fish oil (40 mg) was weighted into a small Erlenmeyer flask and then 3 ml of 0.5 M methanolic sodium hydroxide was added. The mixture was heated over a steam bath in hood until a homogeneous solution was obtained. For saponification reaction, BF_3-methanol (5 ml) was added to the reaction mixture and then boiled for 2 to 3 min. The solution was cooled and transferred into a separator funnel containing 25 ml of hexane and 20 ml of saturated NaCl solution. The solution was gently shake well and allowed the layers to separate. The hexane layer, containing the fatty acid methyl esters, was dried with about 1 g of anhydrous $MgSO_4$ and filtered into a small vial. The solution was concentrated on the steam bath until the volume was reduced to 0.5 ml. This solution of fatty acid methyl esters was analyzed by gas chromatography-mass spectrometry as described in E.

C. Production of the biodiesel from the refined fish oil

To improve activity of the enzyme, CPL (0.04 g) which natural immobilized enzyme was put in iso-propanol (30 ml) for 3 h. After solution was drawn, the enzyme was put in the refined fish oil (30 ml) for 1 h before used as biocatalyst in methanolysis of the refined fish oil. Experiments were conducted to study the best condition for biodiesel production such as oil: methanol molar ratio, temperature, enzyme dosage, initial water activity of enzyme and solvent.Due to the methanolysis reaction is reversible, an increase in the amount of one of the reactants will cause in higher ester yield and at least 3 molar equivalents of methanol are required for the complete conversion methyl ester. First, the oil: methanol molar ratio in methanolysis of the refined fish oil was investigated at 1: 3, 1: 4, 1: 5 and 1: 6 at 45°C for 24 h. An aliquot of 50 µl of reaction medium was taken at 6, 12, 18 and 24 h and added with 450 µl of chloroform for gas chromatography (GC) analysis. In order to investigate the effect of temperature on enzymatic methanolysis for biodiesel production, the reactions consisted of the refined fish oil and methanol in molar ratio of 1: 4 and were conducted at 30, 40, 50 and 60°C for 24 h. To study the effect of enzyme dosage, the CPL (5-25 wt% of oil used) was added into the reaction mixture that consisted of the refined fish oil and methanol in molar ratio of 1: 4. All of reactions were carried out at 40°C for 24 h. For effect of initial water activity of enzyme, before starting of the reaction, CPL was pre-equilibrated with the water vapor of saturated salt solution. Pre-equilibration was done at 25°C for 5 days. The saturated salt solution used were prepared with LiCl (water activity, a_w = 0.11), CH_3COOK (a_w = 0.23), $MgCl_2$ (a_w = 0.33) and $Mg(NO_3)_2$ (a_w = 0.53). A water activity of the bio-catalyst was determined using a Thermoconstanter TH 200 Novasina. The enzyme 20 wt% of original oil with various a_w was added to the reaction mixture consisted of the refined fish oil and methanol in molar ratio of 1: 4. The reaction was carried out at 40°C for 18 h. To investigate an effect of organic solvent, the refined fish oil was mixed with methanol in molar ratio of 1: 4 and various organic solvent (20 wt% of original oil) such as *tert* –butanol, hexane, heptane and diethyl ether were added in the substrate mixture and followed by adding CPL (20 wt% of original oil, a_w = 0.23) into the reaction mixtures. All of mixtures were incubated in orbital shaker fitted with temperature of 40°C for 18 h.The stability of CPL was also investigated. After the first use, the enzyme was recovered by centrifugation and was used in next reaction, which composed of new substrates. The enzyme was used for 30 runs and the yield of methyl ester was determined for every runs after 18 h.

E. Analytical method

The fatty acid composition of glycerides in the refined fish oil was determined by a gas chromatograph (GC 6850, Agilent Technologies) fitted with a capillary column (HP-1MS, 30 m × 0.25 mm, 0.25 µm thickness) and equipped with mass spectrometer (MSD 5973 (EI), Agilent Technologies). The chromatographic conditions were as follow: on temperature of MS Quadrupole and MS Source were 150°C and 230°C, respectively, helium as a carrier gas at flow rate 1.0 ml/min, and an injector temperature of 250°C. Separations were made using the following oven temperature profile: initial temperature 140°C, programmed to 240°C at 10°C/min, and final temperature held for 15 min.

The methyl ester in the reaction mixture was analyzed by GC as follows: Hewlett-Packard 6890 gas chromatograph (Wilmington, Germany) equipped with a WCOT fused silica capillary column (Chrompack, 7483), 25 m × 0.25 mm i.d. The chromatographic conditions were as follows: on column injection, flame ionization detector at 370°C, helium as a carrier gas at 16 psi, and an injector temperature of 360°C.

Separations were made using the following oven temperature profile: initial temperature 180°C, programmed to 220°C at 7°C/min and to 350°C at 20 °C/min, and final temperature held for 15 min. The yield of methyl ester was defined as the mol ratio of methyl ester to initial the refined fish oil multiplied 100.

F. Analysis of the fuel properties

The heating value of the biodiesel was analyzed by an oxygen bomb calorimeter. The Kinematic viscosity in centi-Stokes (cSt) of the fuel was measured by a capillary viscosity meter in a water tube at a constant temperature of 40°C. The acid number, which is expressed as mg KOH/g, was determined by titration with 0.01 N potassium hydroxide for the mixture of tested fuel and chemical reagents until the appearance of the color pink. A Pensky-Martens closed-cup tester for flash point was used for the measurement of the flash point of the fuel.

II. Results And Discussion

A. Characterisation of the refined fish oil

The fatty acid contents of the refined fish oil were shown in Table I. The saturated and unsaturated fatty acid represented about 40.57% and 59.41%, respectively. The saturated fatty acids were comprised mainly of palmitic acid (C16:0), while the unsaturated acids were comprised mainly of oleic acid (C18:1). The quantity of saturated fatty acid was nearby the quantity of unsaturated fatty acid. Thus, the biodiesel from the refined fish oil have a higher cloud point, cetane number and better stability [19].

The acid value of the refined fish oil was determined to be 10.12 mg KOH/g oil which was higher than the maximum acid value of 2 mg KOH/g oil for alkaline transesterification [20]. The moisture contents were 0.10% which was far below the values that could cause biodiesel yield decrease [21].

B. Lipase activity and physical properties of CPL

Fresh papaya latex contained 62±35 u of lipase/g of latex. High speed centrifugation was required to separate the particulate part from the latex. This part of latex possessed all lipase activities which were found to be 284±55 u of lipase/g of particulate fraction, whereas no activities were found in a clear solution. The lipase activity of dried lyophilized CPL on the refined fish oil hydrolysis was 425±40 u of lipase/g of lyophilized particulate. The water activity of crude CPL was 0.395. The crude CPL was used as biocatalyst in further methanolysis of the refined fish oil without purification fraction.

TABLE I
FATTY ACID PROFILE AND CONTENT OF THE REFINED FISH OIL

Type	Fatty acid	Content (g/100g oil)
Saturated	Lauric acid (C12:0)	0.12
	Myristic acid (C14:0)	3.08
	Plamitic acid (C16:0)	27.35
	Margaric acid (C17:0)	0.37
	Stearic acid (C18:0)	8.94
	Arachidic acid (C20:0)	0.71
	Total	40.57
Unsaturated	Palmitoleic acid ($C16:1^{\triangle 9}$)	5.36
	γ-Linolenic acid ($C18:3^{\triangle 6,9,12}$)	0.58
	α-Linolenic acid ($C18:3^{\triangle 9,12,15}$)	0.34
	Linoleic acid ($C18:2^{\triangle 6,9}$)	14.13
	Oleic acid ($C18:1^{\triangle 9}$)	35.87
	Eicosapentaenoic acid ($C20:5^{\triangle 5,8,11,14,17}$)	0.40
	Docosahexaenoic acid ($C20:6^{\triangle 4,7,10,13,16,19}$)	2.73
	Total	59.41

C. Biodiesel production from methanolysis of the refine fish oil

The molar ratio of the refined fish oil: methanol ranged from 1: 3 to 1: 6 was studied to evaluate the effect of methanol on CPL lipases activity. As shown in Fig. 1, when the molar ratio of oil: methanol was increased from 1:3 to 1:4, yield of methyl ester increased from 49 to 51%, indicating that CPL was tolerant to methanol presence within this range and maintained its activity. This phenomenon also was reported that combined use of Lipozyme TL IM and Novozym 435 catalyze the methanolysis of rapeseed oil could produce the highest biodiesel yield of 95% under the reaction medium that consisted of methanol: oil molar ratio 4: 1 [6]. However, when the molar ratio of oil: methanol was increased to 1: 6, significant reduction in conversion was observed. It might be explained that the enzyme was inactivated by contact with high concentration of insoluble methanol. Therefore, the molar ratio of the refined fish oil: methanol at 1: 4 was used in this study.

Reaction temperature has a significant influence on the activity and stability of enzyme. Higher temperature can activate the substrate molecules, reduce the viscosity of reaction and lead to a higher reaction rate [22]. However, lipase can be deactivated by higher temperature and also methanol may be loss through evaporation. Therefore, the effect of temperature (30 to 60°C) on the yield of methyl ester was investigated.

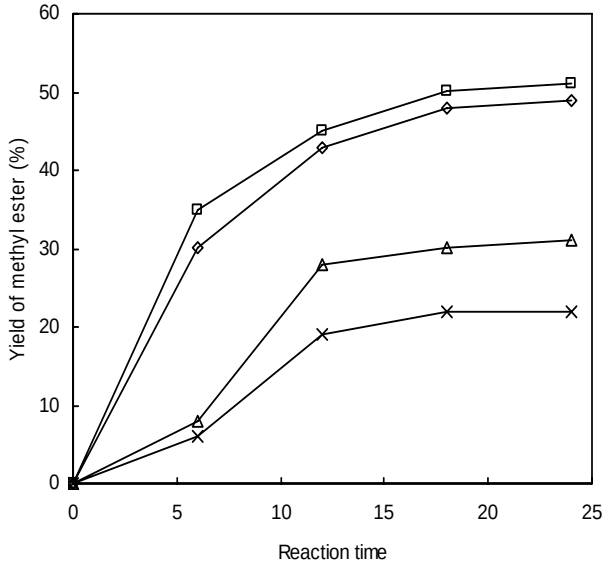

Fig. 1 Effect of oil: methanol molar ratio at 1: 3 (◊), 1: 4 (□), 1:5 (△) and 1: 6 (×) on methanolysis of the refined fish oil. Reaction condition: 40 mg lipase, 45 °C and 200 rpm

When temperature was controlled at 30 to 40°C, the yield of methyl ester increased from 30 to 56% with increasing temperature (Fig. 2). The phenomenon could be explained that the viscosity of the reaction may be reduced with increasing the temperature. When the temperature was increased from 40 to 60°C, the yield of methyl ester decreased whereas an increase in the free fatty acids was observed (data not shown). This is due to enzymatic loss of activity. This behavior was declared that more favorable hydrolysis reactions occurred at higher temperature. Therefore, reaction temperature of 40°C was selected in our study.

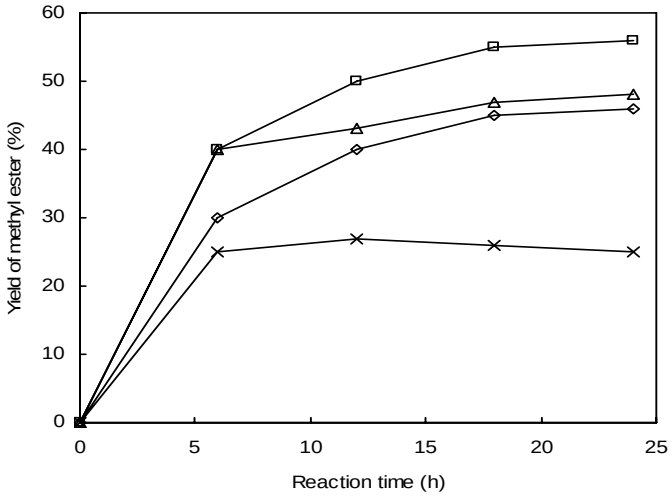

Fig. 2 Effect of temperature on methanolysis of the refined fish oil. Conditions of reactions were carried out in a mixture 30 °C (◇), 40 °C (□), 50 °C (△) and 60 °C (×)

The effect of CPL dosage on the methanolysis of the refined fish oil for biodiesel production was presented in Fig. 3. The yield of methyl ester was enhanced by increasing lipase dosage. When the amount of lipase was increased up to 20% (based on oil weight), the highest yield of methyl ester was 66% at 18 h. Obviously, more lipase showed abundant activated sites and sufficient mass contact, consequently the methyl ester yields were higher. When the CPL dosage was more than 20% (based on oil weight), this influence was not evident and the yield was nearly constant at about 66%. Consequently, 20% of CPL was used as catalyst in the next experiment. CPL was known as a lipase with 1,3-positional specificity as same as commercial lipase Lipozyme TL IM. From comparison of two lipases, methanolysis of the refined fish oil catalyzed by CPL produced methyl ester less than the methanolysis of rapeseed oil catalyzed by Lipozyme TL IM [6].

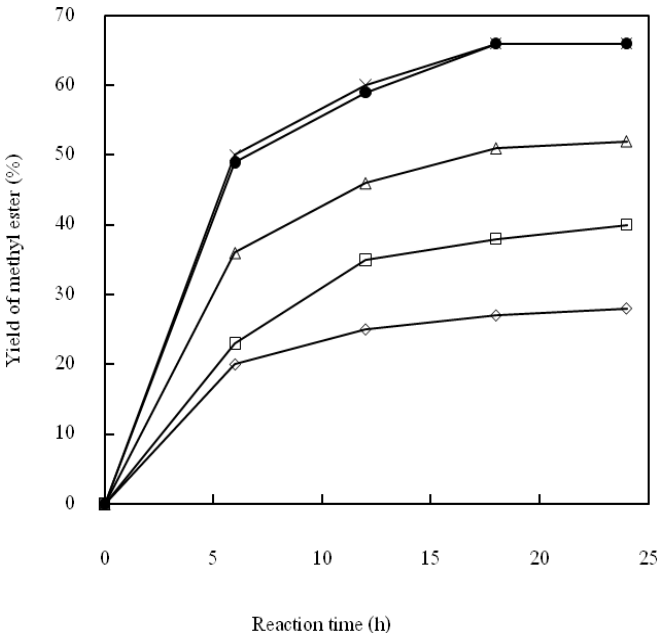

Fig. 3 Effect of CPL dosage on the methanolysis of the refined fish oil. Reaction condition: oil/methanol molar ratio 1:4; lipase dosage based on the oil weight when ◊, □, △, ● and × represented 5%, 10%, 15%, 20% and 25% of enzyme dosage respectively

It is well known in all studies of enzymes in organic media that the amount of water associated with the enzyme is a key determinant of the properties that enzyme exhibits such as activity, stability and specificity [1]. The effect of initial water activity (a_w) on methanolysis was investigated through CPL and it was pre-equilibrated separately at desired water activity using saturated salt solution (from 0.11-0.53). As shown in Fig. 4, yield of methyl ester was highest when initial water activity of enzyme was reduced to 0.23. When the initial water activity of enzyme more than 0.23 would cause the dramatically decrease of methyl ester yield. It can be

explained that the minimal amount of water that associated with enzyme is necessary for acquisition and maintenance of enzyme's catalytically active conformation in methanol. However, too much water facilitates enzyme aggregation, which leads to a decrease in enzyme activity. A similar effect was observed in the methanolysis reaction of soybean oil using lipase from *Mucor miehei* [23].

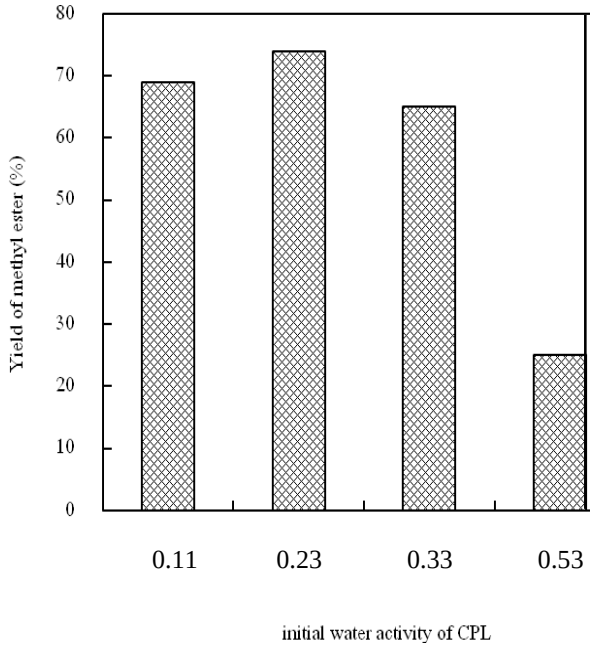

Fig. 4 Effect of initial water activity on methanolysis of the refined fish oil. Reaction condition: oil/methanol molar ratio 1:4; 20% (by weight of oil) of lipase dosage, 40°C, 18 h and 200 rpm

To reduce the inhibition of lipase by methanol, adding various organic solvents including *tert*-butanol, *n*-hexane, *n*-heptane and diethyl ether into reaction mixture were investigated. The result was shown in Fig. 5. The methyl ester yield was highest when *tert*-butanol was added into the reaction mixture. It can be explained that the presence of *tert*-butanol could improve the solubility of methanol in the reaction mixture, so lipase still maintained high activity even with all methanol present in the system [6]. Moreover, the negative effects caused by glycerol can be eliminated by the ability of *tert*-butanol to dissolve glycerol [8],[24].

It has been demonstrated that the cost of lipase accounts for a larger part in the total cost of biodiesel production, and one of the main advantages of an immobilized lipase is that it can be used repeatedly over an extended period of time [25]. To investigate the stability of CPL, the enzyme was reused directly without any treatment after 18 h reaction in each cycle. The operational stability of this lipase was studied in medium without *tert*-butanol and in *tert*-butanol medium. The result was shown in Fig. 6. There was no obvious loss in methyl ester yield even after lipase being reused for 30 cycles for reaction in *tert*-butanol medium.

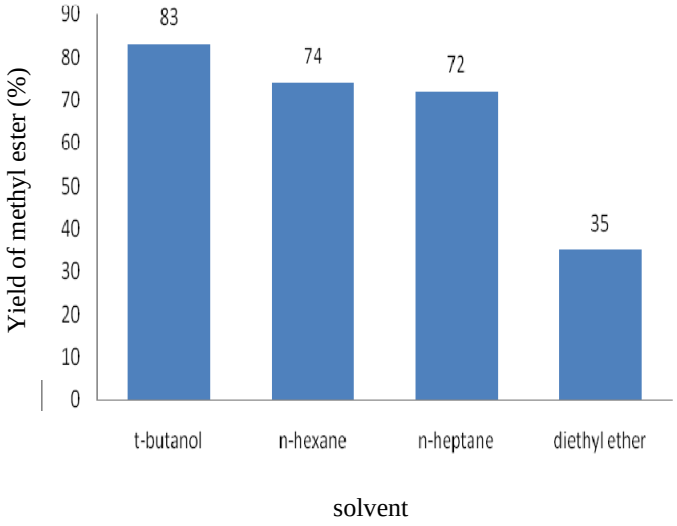

Fig. 5 Effect of organic solvents on methanolysis of the refined fish oil. Reaction conditions: oil/methanol molar ratio 1:4; 20% (by weight of oil) of lipase dosage (a_w = 0.23), 40°C, 18 h and 200 rpm

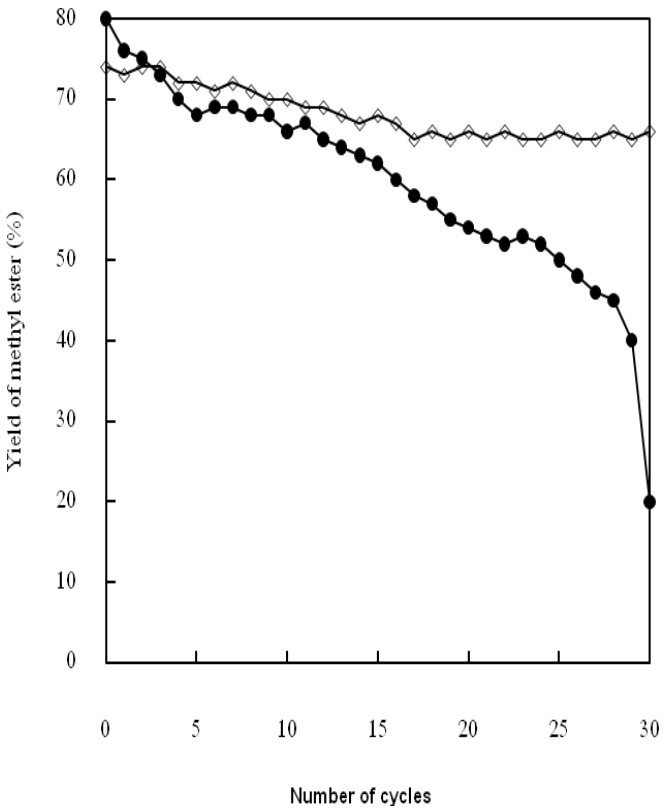

Fig. 6 Stability of CPL for repeated cycle. Reaction conditions: oil/methanol molar ratio 1:4; 20% (by weight of oil) of lipase dosage (a_w = 0.23), 40°C, 200 rpm and 18 h each cycle. When (◇) represented the *tert*-butanol medium and (●) represented the solvent-free medium.

While the traditional medium without *tert*-butanol, the methyl ester yield was decreased sharply. The yield was only 20% in the thirtieth cycles, indicating that the lipase activity was loss and it was not suitable for further reaction. This phenomenon may be explained that by-product glycerol could be solubilized in *tert*-butanol medium and was not adsorbed onto surface of the lipase which contributed a lot to improve lipase stability. While in solvent-free medium, glycerol has been found to be adsorbed onto the surface of the immobilized lipase which led to the quite short operational life of the lipase [6].

D. Fuel properties of the refined fish oil methyl ester

The fuel properties of the refined fish oil methyl ester were shown in Table II. The present results showed that the acid value of the refined fish oil biodiesel was higher than that of the commercial biodiesel produced from waste cooking oil. Since greater water content of the crude fish oil will cause a larger acid number for a biodiesel [17]. The viscosity of biodiesel from the refined fish oil has relatively closer to the commercial biodiesel from waste cooking oil. The flash point of the refined fish-oil biodiesel was more than 120^{o}C as shown in Table II, which was lower than the flash point of the commercial biodiesel from waste cooking oil. This is probably due to the refined fish oil, which was made from discarded parts of a mixture of fish, contained traces of methanol or volatile impurities [17]. The heating value of the refined fish oil biodiesel was higher than that of commercial biodiesel from wasted cooking oil. Hence, the refined fish oil biodiesel contained long chain (C20-C22) fatty acids while commercial biodiesel from wasted cooking oil composed of unsaturated fatty acids of C18 carbon chains such as oleic acid and linoleic acid [17].

TABLE II
FUEL PROPERTIES OF METHYL ESTERS OF THE REFINED FISH OIL

Fuel property	REFINED FISH OIL BIODIESEL	Commercial biodiesel from waste cooking oil [17]
Acid number (mg KOH/g)	1.11	0.69
Viscosity at 40^{o}C (mm^{2}/s)	5.76	6.0
Flash point (^{o}C)	>120	141
Heating value (MJ/Kg)	94.04	40.11

ACKNOWLEDGMENT

We grateful acknowledge the financial supports from the National Research Council of Thailand.

REFERENCES

[1] K. Annapurna, M. Paramita, G. K. Vijay and B. Rintu, "Enzymatic transesterification of Jatropha oil," Biotech& Biofuels, vol. 2, no page, 2009.
[2] N. Kaili, X. Feng, W. Fang and T. Tianwei, "Lipase catalyzed methanolysis to produce biodiesel: optimization of biodiesel production," J. Mol. Catal. B: Enzym. Vol 43, 142-147, 2006.
[3] W. Hong, Z. Min-hua, L. Qian and W. Hua-chang, "Enzymatic conversion of waste oil to biodiesel in a solvent-free system," Prepr. Pap. Am. Chem. Soc., Div. Fuel Chem. Vol. 48, 533-534, 2003.
[4] R. Alcantara, J. Amores, L. Canoira, E. Fidalgo, M. J. Franco and A. Navarro, "Catalytic production of biodiesel from soy-bean oil, used frying oil and tallow," Biomass & Bioenergy. Vol 18, 515-527, 2000.
[5] A. Robles-Medina, P. A. Gonzalez-Moreno, L. Esteban-R Cerdan and E. Molina-Grima, "Biocatalysis: Towards ever greener biodiesel production," Biotechnology Advances., vol. 27, pp. 398-408, 2009.
[6] L. Lilin, D. Wei, L. Dehua, W. Li and L. Zebo, "Lipase-catalyzed transesterification of rapeseed oils for biodiesel production with a novel organic solvent as the reaction medium," J. Mol. Catal. B: Enzym., vol. 43, 58-62, 2006.
[7] G. Steinke, R. Kirchhoff and K.D. Mukherjee, "Lipase-catalyzed alcoholysis of crambe oil and camelina oil for the preparation of long-chain esters," J. Am. Oil Chem. Soc., vol. 69, 166-169, 2000.
[8] Y. Watanabe, Y. Shimada, A. Sugihara, H. Noda, H. Fukuda and Y. Tominaga, "Continuous production of biodiesel fuel from vegetable oil using immobilized Candida antarctica lipase," J. Am. Oil Chem. Soc., vol. 77, 355-360, 2000.
[9] W. Du, W. Li, T. Sun, X. Chen and D. Liu, "Perspectives for biotechnological production of biodiesel and impacts," Appl Microbiol Biotechnol, vol. 79, 331-337, 2008.
[10] Y. Shimada, Y. Watanabe, A. Sugihara and Y. Tominaga, "Enzymatic alcoholysis for biodiesel fuel production and application of the reaction to oil processing," J. Mol. Catal. B: Enzym., vol. 17, 133-142, 2002.
[11] O. Kose, M. Tuter and H. A. Aksoy, "Immobilized Candida Antarctica lipase-catalyzed alcoholysis of cotton seed oil in a solvent-free medium," Bioresour. Technol., vol. 83, 125-129, 2002.
[12] P. Villeneuve, A. Skarbek, M. Pina, J. Graille and T. A. Foglia, "Catalytic behavior of Carica papaya latex in transesterification reactions," Biotechnol. Tech., vol. 11, 637-639, 1997.
[13] M. Kaieda, T. Samukawa, T. Matsumoto, K. Ban, A. Kondo, Y. Shimada, H. Noda, F. Nomoto, K. Ohtsuka, E. Izumoto and H. Fukuda, "Biodiesel fuel production from plant oil catalyzed by Rhizopus oryzae lipase in a water containing system without an organic solvent," J. Biosci. Bioeng., vol. 88, 627-631, 1999.
[14] K. Ban, M. Kaieda, T. Matsumoto, A. Kondo and H. Fukuda, "Whole cell biocatalysts for biodiesel fuel production utilizing Rhizopus oryzae cells immobilized within biomass support particles," Biochem. Eng. J., vol. 8, 39-43, 2001.
[15] T. A. Foglia and P. Villeneuve, "Carica papaya latex-catalyzed synthesis of structured triacylglycerol," J. Am. Oil Chem. Soc., vol. 74, 1447-1450, 1997.
[16] [16] P. Angkanurukpun, P. Sriburi and P. Kanasawud, "Improvement of Carica papaya lipase for methanolysis of triolein," Chiang Mai J. Sci., vol. 33, 217-222, 2006.
[17] L. Cherng-Yuan and L. Rong-Ji, "Fuel properties of biodiesel produced from the crude fish oil from the soapstock of marine fish," Fuel Processing Technology., 130-136, 2008.
[18] D. Y. Kwon and J. S. Rhee, "A simple and rapid colorimetric method for determination of free fatty acids for lipase assay," J. Am. Oil Chem. Soc., vol. 63, 89-92, 1986.
[19] A. S. Ramadhas, S. Jayaraj and C. Muraleedharan, "Biodiesel production from high FFA rubber seed oil," Fuel., Vol. 84, 335-340, 2005.
[20] M. Canakci and J. G. Van, "Biodiesel production from oils and fats with high free fatty acids," Transactions of the ASAE., vol. 44, 1429-1436, 2001b.
[21] M. Canakci and J. G. Van, "A pilot plant to produce biodiesel from high free fatty acid feedstocks," ASAE, 2001a.
[22] K. Krisnangkura, T. Yimsuwan and R. Pairintra, "An empirical approach in predicting biodiesel viscosity at various temperature," Fuel., vol. 85, 107-113, 2006.
[23] L. A. Nelson, T. A. Foglia and W. N. Marmer, "Lipase catalyzed production of biodiesel," J. Am Oil Chem Soc., vol. 73, 1191-1195, 1996.
[24] V. Dossat, D. Combes and A. Marty, "Continuous enzymatic transesterification of high oleic sunflower oil in a packed bed reactor:

influence of the glycerol production," Enzyme Microb Technol., vol. 25, 194-200, 1999.
[25] L. Jike, N. Kaili, X. Feng, W. Fang and T. Tianwei, "Enzymatic synthesis of fatty acid methyl esters from lard with immobilized Candida sp. 99-125," Process Biochemistry., vol. 42, 1367-1370, 2007.

P. Pinyaphong was born at Uttaradit hospital, Thailand on 12 December, 1971. The degree of highest education was Doctor of Science (Biotechnology) from Chiang Mai University, Chiang Mai, Thailand and 5 year degree was earned.She is a lecturer and Head of Department of Chemistry, Faculty of Science and Technology, Uttaradit Rajabhat University, Uttaradit, Thailand. The previous research experience were (1) study of lipase production from Thermus TP441 and pancreatic lipase reaction, (2) purification and characterization of lipase from thermophile TLS63 (3) methanolysis of triolein by lipase from *Carica papaya* latex, (4) modification of palm oil structure to cocoa butter equivalent by *Carica papaya* lipase catalyzed interesterification and (5) synthesis of methyl ester from waste cooking oil by *Carica papaya* lipase for biodiesel production. Current researches are focused on synthesis of monoacylglycerol from glycerolysis of crude glycerol derived from biodiesel production and screening of bacteria from waste water containing fat for biodegradable plastic production.