Chiang Mai J. Sci. 2018; 45(6) : 2438-2453
http://epg.science.cmu.ac.th/ejournal/
Contributed Paper

Synthesis and Characterization of Biolubricant from POME Oil and Hepatopancreas Lipase from Pacific White Shrimp (*Litopenaeus vannamei*)

Thanaphorn Rakkan [a], Nisa Paichid [a], Tewan Yunu [a], Sappasith Klomklao [b] and Kanokphorn Sangkharak* [a]

[a] Department of Chemistry, Faculty of Science, Thaksin University, Phatthalung 93210, Thailand.

[b] Department of Food Science and Technology, Faculty of Technology and Community Development, Thaksin University, Phatthalung 93210, Thailand.

* Author for correspondence; e-mail: skanokphorn@yahoo.com

Received: 21 August 2017
Accepted: 17 November 2017

ABSTRACT

POME polyol ester, an alternative biolubricant, was produced through transesterification using POME oil methyl ester and thrimethylolpropane. In addition, hepatopancreas lipase from Pacific white shrimp was utilized as an enzymatic catalyst. The highest percentage of biolubricant conversion (85%) was obtained at 70 kUnit/10 g substrate, 3.15:1 substrate to TMP molar ratio, 3% water content, 30°C reaction temperature, 250 rpm agitation speed and a reaction time of 18 and 21 h for the lab- and large- scale reactors, respectively. Gas chromatography (GC) and attenuated total reflection-Fourier transform infrared spectroscopy (ATR-FTIR) were used to ensure the conversion of substrate into biolubricant. The POME polyol ester exhibited high viscosity index (152), high flash point (164°C) and moderate oxidative stability. The biolubricant properties were within the recommended standard as specified by ISO VG32. Therefore, residual oil from POME as substrate of biodiesel and hepatopancreas lipase is a potential substrate and biocatalyst for the biolubricant industry.

Keywords: biodiesel, biolubricant, hepatopancreas, lipase, POME, transesterification

1. INTRODUCTION

With a growing concern about environmental problems and a decreasing of petroleum reserves, the production of biologically based products that are eco-friendly, biodegradable and use natural substances as substrates is an interesting topic for the scientific and industrial communities [1]. It is estimated that 20% of the 5.2 million tons of lubricant consumed every year in Europe is released into the environment. In Thailand, the consumption of lubricant is 4-times lower than Europe; however, a kilogram of said mineral oil is capable of polluting a million liters of water [2]. Therefore, pollution caused by lubricants is far from insignificant. The petroleum-based lubricants can also contaminate soil directly and pollute the air due to their volatility [3]. This pollution is hazardous to not only plants and animals inhabiting the contaminated areas

but potentially also to human residents as well [4].

Therefore, the use of vegetable oil-based lubricants in place of the commonly used petroleum-based lubricants could reduce both eco- and energy-concern. These products, known as biolubricants, carry several environmental, health and performance benefits over current petroleum-based lubricants [5]. The advantages of biolubricants include rapid biodegradability, nontoxic nature, no risk to the environment or operators, and better lubricity. In addition, their flash point, viscosity indices, shear stability, and resistance to humidity dispersancy are high with a low content of volatility and compressibility [4-6]. However, biolubricants are still not widely used due to several major challenges and difficulties regarding their performance and production. Aside from issues regarding feedstock reliability and consistency as well as industrial acceptance, biolubricants also have two main negative physical properties including poor low temperature performance and low thermal oxidative stability [3, 5-7]. However, through appropriate enzymatic processes as well as modifying the chemical structures of biolubricants using suitable substrate and alcohol, these two properties can be improved [8]. Enzymatic synthesis has a lot of advantages over conventional (chemical) synthesis methods such as reduced energy demand, less expensive waste treatment, mild conditions, no need for harmful reagents, and an ability to use cheap bio-based raw materials. The cost of enzymes was reduced by using lipase from Pacific white shrimp hapatopancreas. A low cost catalyst, lipase from the hepatapancreas of Pacific white shrimp (*Litopenaeus vannamei*), was determined for biodiesel production. The best conditions for biodiesel contain 70 kUnit lipase, 4:1 methanol to oil molar ratio, 3% water, 45°C reaction temperature and a 16 h reaction time. The maximum biodiesel yield reached 97.01% under these optimum conditions [9].

The investigation of biolubricant preparation has been conducted using a number of vegetable oils such as coconut, soybean, jojoba, rapeseed, palm and sunflower oil. However, the aforementioned sources are consumable feedstocks which are not suitable for large scale application. Many researchers have evaluated the production of biolubricants from palm oil and palm oil methyl ester. Little information has been reported on the production of biolubricants from palm oil mill effluent (POME). Syaima et al. [10] synthesized biolubricants from POME using enzymatic hydrolysis and non-catalytic esterification. The highest production was achieved from hydrolysis reaction with a rate of 0.1639 mg/sec.L. The optimum conditions for enzymatic hydrolysis contained 20 U/mL of enzyme loading, 40°C, pH 7.0, 650 rpm and 50% (v/v) of POME. However, it had no information on the production of biolubricants from POME biodiesel. In our previous study, >80% of residual oil was extracted from POME. The residual oil has 45% palmitic acid (C16:0), 38% oleic acid (C18:1Δ9) and 10% linoleic acid (C18:2Δ9,12) [11]. Residual oil from POME was suitable to be utilized as biodiesel substrate because of its availability, reliability and consistency as our previous study reported [11]. Therefore, the residual oil from POME was used as substrate for biodiesel throughout this study. Moreover, the use of biodiesel to develop a higher added-value product is a very attractive solution for biodiesel production. 2-ethyl-1-hexyl oleate (biolubricant) was synthesized from biodiesel and 2-ethyl-1-hexanol using immobilized lipase (Lipozyme TL IM). The transesterification reaction was performed in a solvent-free and water-free system.

2-Ethyl-1-hexyl oleate was successfully synthesized in 50 L scale with 100% conversion in 10 h of reaction time at 60°C [1]. Enzymatic synthesis and characterization of the biolubricant was obtained via the transesterification of different methyl fatty acids esters (biodiesel produced from castor, soybean and jatropha oils) and 2-(hydroxymethyl)-2-ehtylpropane-1,3-diol or trimethylolpropane (TMP). The performance of different lipases to catalyze these reactions has been compared. The best lipase depended on the substrate but a lipase from *Candida rugosa* and castor oil were selected due to the promising properties of their final product. After optimizing the experimental parameters of the processes, the optimal values were: an enzyme content of 4%, a water content of 1%, a molar ratio of castor oil biodiesel/TMP of 3.915:1, a temperature of 40°C and a residence time of 24 h. Under these conditions, a conversion yield higher than 95% was obtained [12]. The utilization of TMP as alcohol has high interest due to its branching structure without a beta-hydrogen. The presence of such beta-hydrogen decreases the thermal stability of the compound (e.g. natural vegetable oils) [8, 13]. Other alcohols such as pentaerythritol and neopentyl glycol do not have a beta-hydrogen structure [8].

The aim of the present study was to enzymatically synthesize trimethylopropane using biodiesel as a substrate. A novel biolubricant with good properties was expected. Lipase from Pacific white shrimp hepatopancreas was utilized as a low cost catalyst. In addition, the effect of commercial lipase from *Rhizomucor miehei* (Sigma-Aldrich) and *C. rugosa* (Type VII, Sigma-Aldrich) were also compared. The properties of the biolubricant including viscosity, viscosity index, flash point, fire point, pour point, thermal stability and oxidative stability were determined. The characteristics of the biolubricant (this study) were compared with a commercially available lubricant.

2. MATERIALS AND METHODS

2.1 Materials

POME biodiesel was obtained from laboratory scale as previously described [24]. The obtained POME methyl ester was already analyzed using GC. POME biodiesel shows the significant presence of methyl ester. The fatty acid methyl ester content from POME oil contains mainly methyl palmitate (46.5%), followed by methyl oleate (36.4%). In addition, few amounts of methyl linoleate (9.7%) and methyl stearate (4.2%) were also observed. The POME biodiesel was washed and treated with silica gel for 30 min to remove impurities. Then the sample was filtered and dried overnight in an oven at 105°C before used.

Trimethylolpropane (TMP) (98%) and commercial lipases from *R. miehei and C. rugosa* (type VII) were purchased from Sigma-Aldrich. The free lipase powder from Pacific white shrimp hepatopancreas was kindly provided by Assoc. Prof. Dr. Sappasith Klomklao (Thaksin University, Thailand). The hepatopancreas lipase was prepared following the Kuepethkaew method [9] and purification of hepatopancreas lipase was already reported in Kuepethkaew et al. [26]. Analytical-grade reagents were utilized throughout this study.

2.2 Enzyme Activity Assay

An enzyme activity test for lipase was performed as described by Kuepethkaew et al. [9]. The substrate solution was prepared by dissolving 30 mg *p*-NPP in 10 mL of propan-2-ol. The lipase assay was carried out at 37°C by direct mixing in a cuvette of 100 µL of an appropriately diluted enzyme with 810 µL of preincubated 50 mM phosphate buffer (pH 8.0) and 90 µL of substrate

solution. The amount of liberated *p*-nitrophenol was determined at 410 nm during the first 5 min of reaction. One unit of enzyme activity was defined as the amount of enzyme that liberated 1 µmol of *p*-nitrophenol per minute under the assay conditions.

2.3 Transesterification Reaction for Biolubricant Production

In this study, POME biodiesel and trimethylolpropane (TMP) was utilized as substrate via a transesterification reaction using lipase from Pacific white shrimp hepatopancreas. POME polyol ester (PomePE), products of transesterification, was performed. Polyol ester (PE), a transesterification product of fatty acid and fatty acid ester with a polyol such as TMP, was developed as base oil for various types of lubricating oil and prepared in various ways. The formation of PomePE is illustrated in Figure 1. The process for biolubricant production was modified from da Silva et al. [12], Heikal et al. [15] and Sripada et al. [25]. For the reaction, 11.25 mmol (1.51 g) TMP and water (1-12% if the total biodiesel and TMP mass) were added to the reactor. Once TMP was solubilized, POME biodiesel (11.8-47.22 mmol, corresponding to 10-40 g) was added. The reaction was carried out at 30-50°C. After the system had reached the desired temperature, lipase was added (10-100 kUnit/10 g substrate, corresponding to 2.83-28.31 g enzyme/10 g substrate). Then, the mixture was stirred (150-300 rpm) and the vacuum pump was turned on (whenever necessary to remove the produced methanol; shifting the reaction equilibrium and therefore increasing biodiesel conversion) (Figure 2a). The system was operated in a temperature-controlled 500 mL volume 3-neck round-bottomed flask and incubated for 10-15 min to stabilize the chosen reaction temperature. The mixing was performed by using a mechanical stirrer. The reaction was followed for 60 h and the sample was withdrawn every 12 h. Finally the mixture was filtered in filter paper and centrifuged (5000 rpm, 20 min). The formation of POME polyol ester (PomePE, biolubricant) was followed on time by Gas Chromatography (GC). The result was compared with *R. meihei* and *C. rugosa* lipases as the commercial catalyst under the same condition in the transesterification reactions.

2.4 Up-scale of Transesterification Reaction

The reaction was performed in a temperature-controlled glass reactor (1.5 L) with mechanical stirring. A reactor (Biostat, Satorius Germany) was surrounded by a heating jacket connected to a temperature controlled water bath and equipped with an overhead stirrer (Figure 2b). Initial reaction mixture consisted of 3% water content with a molar ratio of POME biodiesel/TMP at 3.15:1. The mixture was incubated for 1 h to reach a constant temperature of 30°C. Afterwards, hepatopancreas lipase (70 kUnit/10 g substrate, corresponding to 19.81 g enzyme/10 g substrate) was added. The stirring rate was kept at 250 rpm. The reactor outlet tube positioned in the reactor was covered by a metal filter (0.5 mm mesh size) to keep the substrate and enzyme in the reactor. The methanol forming during the reaction was controlled by alcohol probe and the excess methanol was removed by turn on the vacuum pump (Vacuum pump V-300, Buchi Switzerland). The vacuum was kept at 50-100 mm Hg for removal of the formed methanol. The reaction was operated for 24 h and the sample was withdrawn every 3 h. Thereafter, the sample was filtered from the enzyme and the reaction product was evaporated from the formed side products and characterized [1].

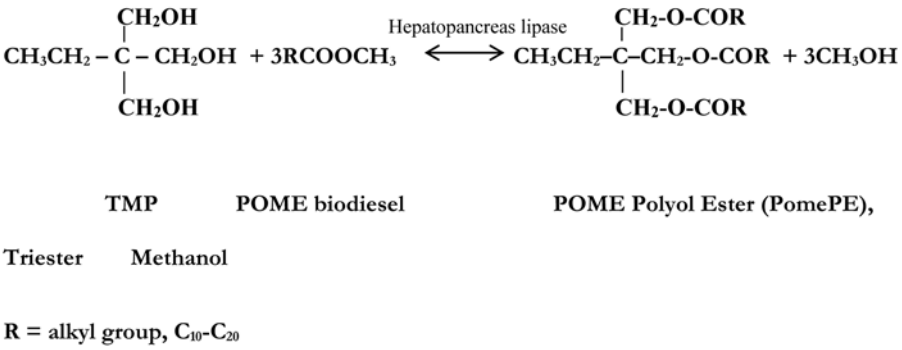


Figure 1. The formation of POME polyol ester via transesterification of POME biodiesel and trimethylolpropane.

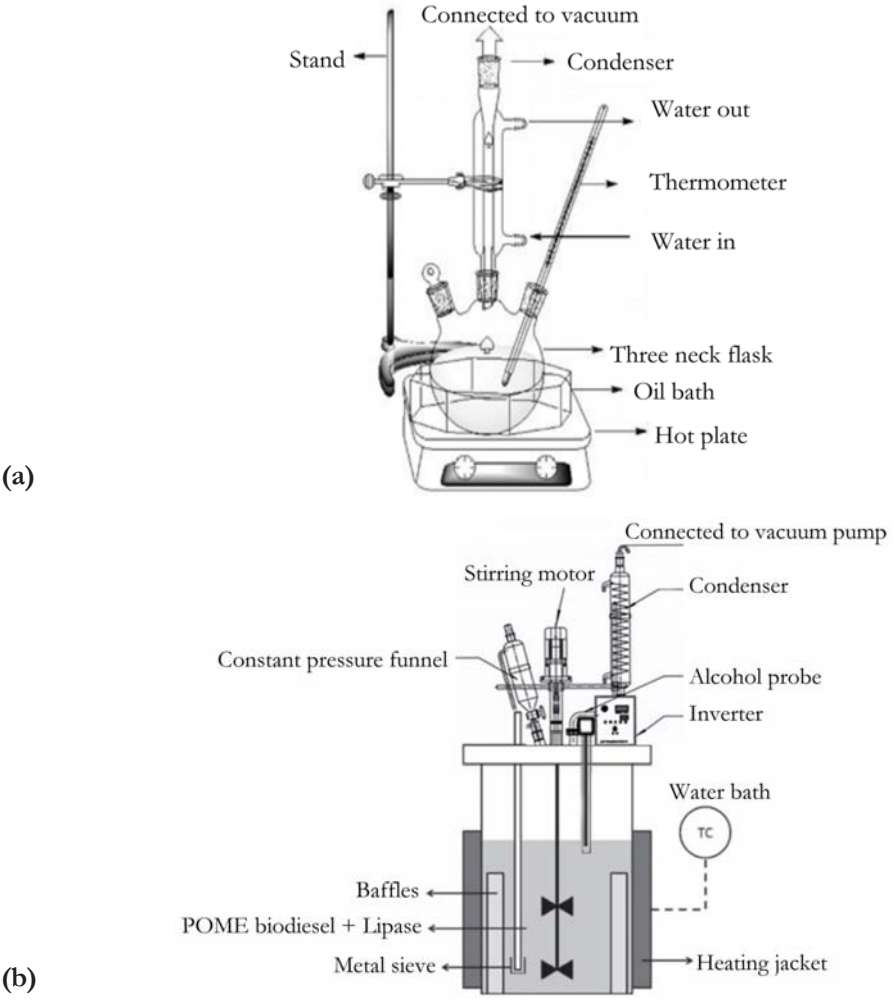


Figure 2. Schematic of reaction setup for transesterification reactions of POME biodiesel with trimethylolpropane under lab-scale (a) and up-scale reactor (b).

2.5 Analytical Method

The progress of the reaction, fatty acid, mono-esters (ME), di-esters (DE) and tri-esters (TE) content was analyzed by Gas Chromatography (GC). GC spectra were recorded using GC equipped with a flame ionization detector and capillary column SGE 12 m × 0.53 mm IDHT5 with a film thickness (0.15 µm) of stationary phase (polyethylene glycol). Hydrogen at 26.7 mL/min was used as a carrier gas. The oven temperature was programed from 80 to 340°C and the conditions were as follows: an initial temperature of 80°C, then hold for 3 min, increase 6°C/min and hold for another 6 min. The other chromatographic conditions were as follows: an injector temperature of 300°C and a detector temperature 360°C, and a sample injection volume of 1 µL [16]. The yield of each product was determined from the GC chromatogram calibrated against the known samples according to the procedure described by Yunus et al. [14]. The formulated biolubricant from POME biodiesl conversion (% of conversion) is calculated by the following formula:

$$\text{Percentage of conversion (\%)} = \left[1- \frac{C_{TMP,P}}{C_{TMP,I}}\right] \times 100\%$$

where $C_{TMP,P}$ is the amount of TMP in product, $C_{TMP,I}$ is the initial amount of TMP added to the reaction system.

The viscosity of samples was evaluated by a Redwood Viscometer as per ASTMD2270 [4]. In addition, the viscosity index (VI) value was determined through calculation of ASTMD2270 which takes in account the product viscosities at 40°C and 100°C. The flash point was estimated by Cleveland open cup equipment as per ASTMD92 [4]. The pour point was estimated according to the ASTMD97 method. After pre-heating, the sample was cooled at a specified rate and observed in 3°C intervals to evaluate the flow characteristics. The oxidative stability was done using the Rotating Pressure Vessel method (RPVOT-ASTMD2272). A steel vessel containing an oil sample and a standard metal catalyst (copper) was pressurized with pure oxygen (90 psi). Afterward, the vessel was kept at 150°C and the time required for a 25 psi decrease in the pressure due to the oxygen consumption was measured. This time is the oxidation resistance of the sample and is accepted as a measure of oxidative degradation [12].

Attenuated total reflection-Fourier transform infrared spectroscopy (ATR-FTIR) was used to determine the product conversion by the chemical group shift since it has a rapid and easy detection method. The ATR-FTIR spectra were taken using Agilent Cary 630 FTIR with the specified range of 800-4000 cm^{-1} [9].

3. RESULTS AND DISCUSSION

3.1 Biolubricant Optimization Parameters

3.1.1 Effect of enzyme loading

The effect of lipase from Pacific white shrimp hepatopancreas was firstly evaluated. Enzyme amount is a key factor that influences biolubricant production. The amount of enzyme on transesterification was investigated in the range of 10-100 kUnit/10 g substrate with constant conditions of molar ratio biodiesel:TMP (3.15:1), temperature (35°C), water content (3%), 150 rpm and 16 h of reaction time. The reaction was vacuumed to increase the conversion by methanol removal and to avoid this alcohol from competing with TMP. In addition, the process was operated at atmospheric pressure following the da Silva report [12]. The production of biolubricant was evaluated by a percentage of conversion.

Therefore, the conversion increased with the increasing amount of lipase (Figure 3). The highest conversion at 80% was achieved with 70 kUnit of hepatopancreas lipase/10 g substrate. Afterward, the conversion remained constant. It was presumed that increasing the enzyme may increase the viscosity and reduce the reaction rate [9]. The optimum enzyme loading (70 kUnit/10 g substrate) was applied to the subsequent reaction.

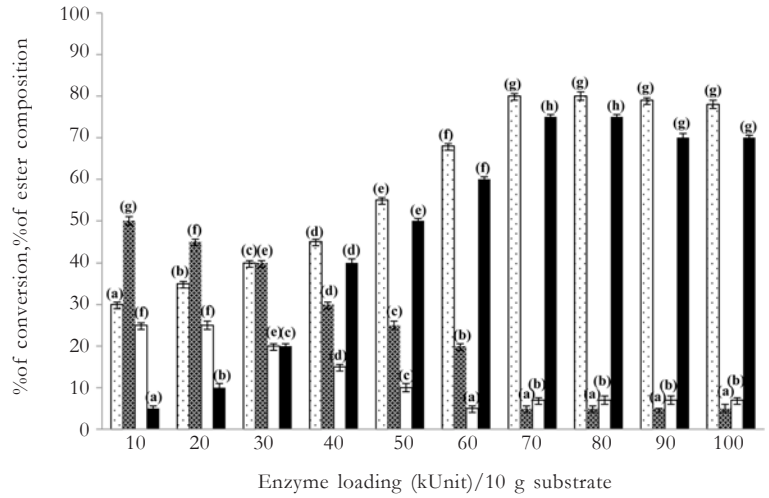


Figure 3. Effect of enzyme loading on % of conversion (▒) and % composition of monoester (▓), diester (□) and triester (■) of POME biodiesel with TMP. The condition was operated of molar ratio biodiesel:TMP (3.15:1), temperature (35°C), water content (3%) with 16 h of reaction time. Different letters (a, b or c) above the columns indicate significant difference based on statistical test (P<0.05). Data are means ± standard error of mean (s.e.m).

3.1.2 Effect of water content

The biolubricant catalyzed by hepatopancreas lipase in the presence of water content at 1-11% was determined. The result indicated that 3% of water content yielded the highest biolubricant production (82%). In addition, the production was decreased when the water content was over 3% (Figure 4). The appropriate water content is an important parameter in a transesterification reaction that could affect the reaction rate and final yield greatly. The addition of the appropriate water content is necessary to maintain lipase activity and to increase the available interfacial area between water and sample, thereby enhancing the reaction. However, since lipase usually catalyzed hydrolysis in aqueous media, excess water might also stimulate the competing hydrolysis reaction. In addition, high water content generates a thicker water layer around the enzyme surface and causes a diffusion problem from the substrate and product from the enzyme active site [9, 17]. Therefore, 3% water content was selected for biolubricant preparation.

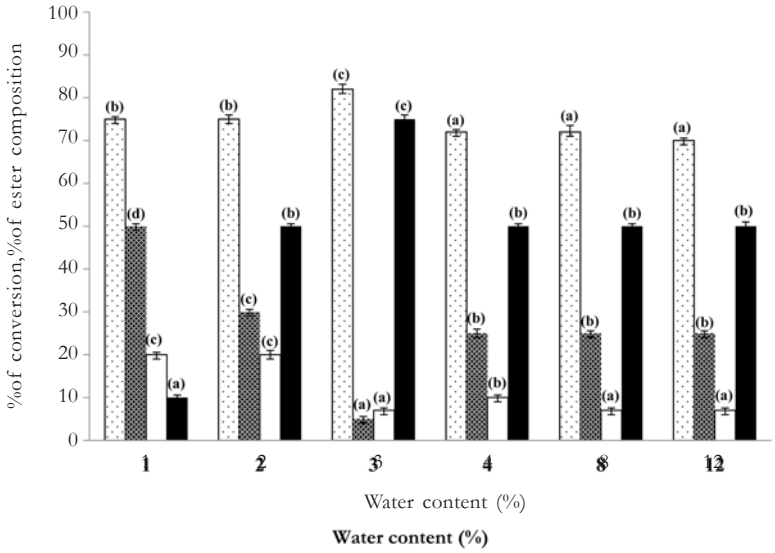


Figure 4. Effect of water content on % of conversion (▒) and % composition of monoester (▓), diester (□) and triester (■) of POME biodiesel with TMP. The condition was operated of molar ratio biodiesel:TMP (3.15:1), temperature (35°C), enzyme loading (70 kUnit/10 g substrate), 150 rpm with 16 h of reaction time. Different letters (a, b or c) above the columns indicate significant difference based on statistical test (P<0.05). Data are means ± s.e.m.

3.1.3 Effect of molar ratio of POME biodiesel to TMP

The effects of the molar ratio were performed in the range of POME biodiesel : TMP at 1.05-4.20:1. There was negligible effect of the molar ratio of POME biodiesel to TMP on %conversion when the ratio was varied between 3.15:1 and 4.2:1 (Figure 5). However, it has been reported that the suitable molar ratio for palm oil substrate was in the range of 3.8:1-4.0:1. With a high molar ratio (>4:1), the amount of partial ester in the product, especially triester, was reduced [12, 16]. Sulaiman et al. [16] indicated that the molar ratio of 3.8:1 is the optimum ratio for triester produce from palm oil methyl ester and TMP. When the molar ratio was high, the amount of monoester was reduced which is important for the lubricant behavior [18, 19]. A similar ratio of substrate to TMP (3.92:1) was also observed in the production of biolubricant from castor oil biodiesel [12]. In view of this result, a POME biodiesel to TMP ratio of 3.15:1 was used in the further experiment.

3.1.4 Effect of reaction temperature

The effect of temperature on the transesterification reaction has been investigated due to the fact that a higher temperature can help the substrate molecules to obtain adequate energy to overcome the energy barrier and enhance the reaction rate, but a significant increase in the temperature can lead to the denaturation of the enzyme [9]. An increase of reaction temperature from the lowest level (30°C) to the highest (50°C) caused a decrease of biolubricant production (Figure 6). In lipase catalyzed reactions, temperature significantly influences both the initial rate of the reaction and stability of the enzyme. Hepatopancreas lipase from Pacific white shrimp was found to be thermostable and did not deactivate up to

60°C [20]. However, the best enzyme activity was obtained at 30°C which yielded the highest biolubricant (83%). Therefore, the further experiments were carried out at this temperature. This property of the enzyme is advantageous for us as it enables us to carry out the reaction at low temperature and thus makes the process more energy efficient [21]. Thus, the other experiments were carried out under this optimum temperature (30°C).

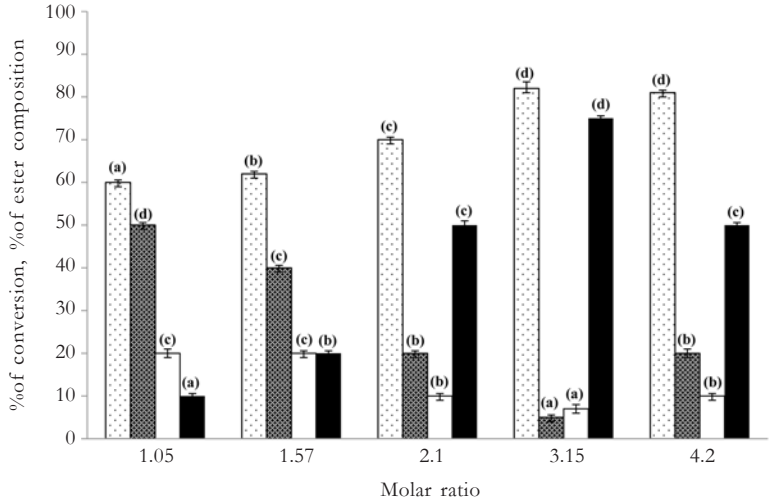


Figure 5. Effect of molar ratio on % of conversion (▒) and % composition of monoester (▓), diester (□) and triester (■) of POME biodiesel with TMP. The condition was operated at water content (3%), temperature (35°C), enzyme loading (70 kUnit/10 g substrate), 150 rpm with 16 h of reaction time. Different letters (a, b or c) above the columns indicate significant difference based on statistical test (P<0.05). Data are means ± s.e.m.

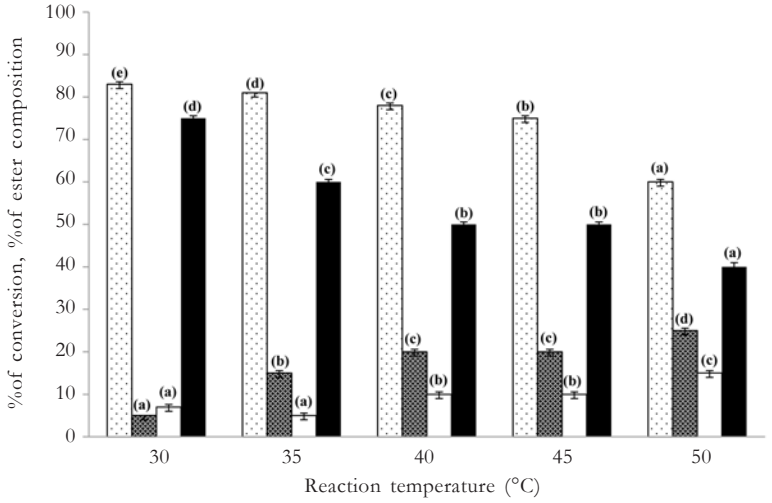


Figure 6. Effect of reaction temperature on % of conversion (▒) and % composition of monoester (▓), diester (□) and triester (■) of POME biodiesel with TMP. The condition was operated of molar ratio biodiesel:TMP (3.15:1), water content (3%), enzyme loading (70 kUnit/10 g substrate), 150 rpm with 16 h of reaction time. Different letters (a, b or c) above the columns indicate significant difference based on statistical test (P<0.05). Data are means ± s.e.m.

3.1.5 Effect of agitation speed

Agitation is required to disperse the reaction mixture in the form of emulsion. Agitation is an energy consuming process which has a considerable effect on enzymatic reaction [21]. The effect of agitation speed on the transesterification process for biolubricant production was varied from 150-300 rpm. The highest percentage of conversion (85%) was attained when the agitation speed reached 250 rpm (Figure 7). The production was dropped when the speed was increased beyond 250 rpm. The increase of agitation speed reduces the droplet size and consequently increases the interfacial area between the substrate and enzyme in the aqueous phase. However, the stronger the speed, the contact between these two surfaces is decreased due to the increase in the shear on the enzyme [10]. In addition, an adequate agitation is required to create circulatory flow to dissolve the particle of TMP. If the TMP particle could not dissolve completely, it may reduce the production yield after the catalyst was added [16]. When the agitation speed was increased, the substrate was drawn down into the charge and dissolved the TMP which settled down at the bottom layer. Therefore, the formation of biolubricant (triester) was increased proportionally by agitation speed especially from 150-250 rpm. Therefore, an agitation speed at 250 rpm was applied to the subsequent experiment.

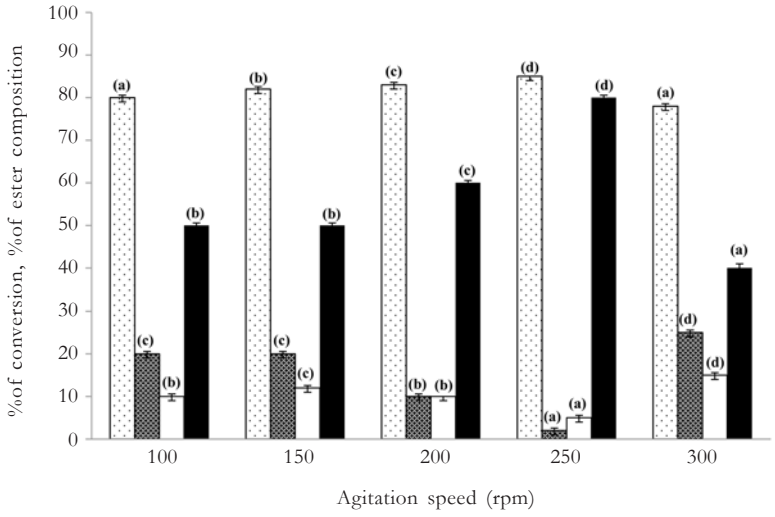


Figure 7. Effect of agitation speed on % of conversion (▒) and % composition of monoester (▓), diester (□) and triester (■) of POME biodiesel with TMP. The condition was operated of molar ratio biodiesel:TMP (3.15:1), water content (3%), enzyme loading (70 kUnit/10 g substrate) temperature (30°C), with 16 h of reaction time. Different letters (a, b or c) above the columns indicate significant difference based on statistical test (P<0.05). Data are means ± s.e.m.

3.1.6 Effect of reaction time

Effect of reaction time on the transesterification process of POME biodiesel with TMP using partially purified lipase from Pacific white shrimp hepatopancreas at a level of 70 kUnit was investigated up to 60 h. The biolubricant samples were withdrawn at an interval of 6 h and analyzed by GC. The results indicated that the product gradually increased with increasing the reaction time ($p < 0.05$). A constant conversion rate was obtained after 18 h of reaction time (Figure 8).

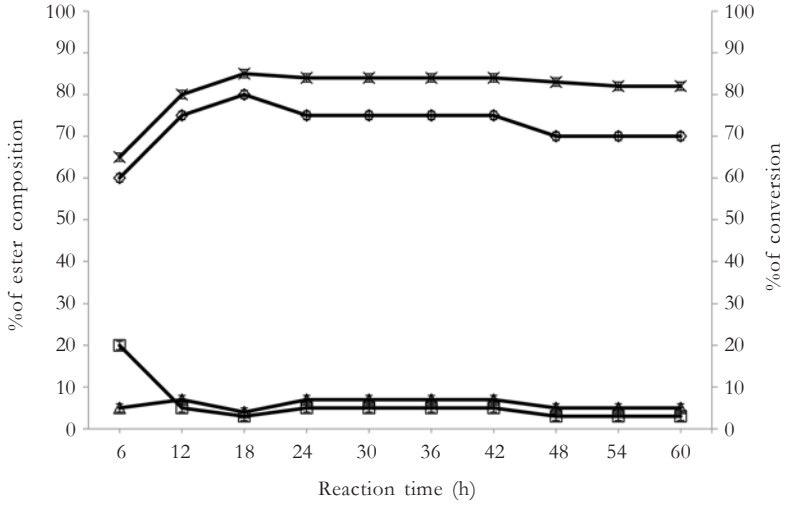


Figure 8. Effect of reaction time on % of conversion (X) and % composition of monoester (□), diester (Δ) and triester (◊) of POME biodiesel with TMP. The condition was operated of molar ratio biodiesel:TMP (3.15:1), water content (3%), enzyme loading (70 kUnit/10 g substrate) temperature (30°C), within 60 h of reaction time. Data are means ± s.e.m.

3.2 Up-scale of Transesterification Reaction

Large-scale reaction was also determined after lab-scale experiment for transesterification reaction of POME biodiesel with TMP using hepatopancreas lipase. The synthesis of POME polyol ester was carried out in a 3 L reactor with 1.5 L of working volume. The optimum conditions included molar ratio biodiesel:TMP (3.15:1), water content (3%), enzyme loading (70 kUnit) temperature (30°C), with 24 h of reaction time. The conversion of 83% was reached in 21 h of reaction time and remained constant afterward. Interestingly, the production of biolubricant in the large-scale reactor reached the maximum content slower than in the small-scale experiment (Figure 9). Linko et al. [22] and Kleinaite et al. [1] reported that an increase in the quantity of enzymes resulted in a decrease of the apparent enzyme activity, owing to an increase of diffusion limitation, a problem that in large-scale experiments may be minimized. In order to compare the best conversion result, the same activity of commercial lipase from *R. miehei* and *C. rugosa* (70 kUnit/10 g substrate) were also tested under the same conditions. The similar yield of biolubricant (85%) was obtained from *R. miehei* lipase. However, low biolubricant conversion was obtained from commercial lipase (80% conversion). This result could be explained by their high content of oleic and linolenic acid

in substrate. *C. rugos*a lipase is not specific for several chain lengths of fatty acids with low activity in the presence of long chain polyunsaturated fatty acids [12]. Hou and Shimada [23] concluded that the specificity of *C. rugosa* lipase is affected by the length of the fatty acid linear chain as well as by the amount and position of the double bonds.

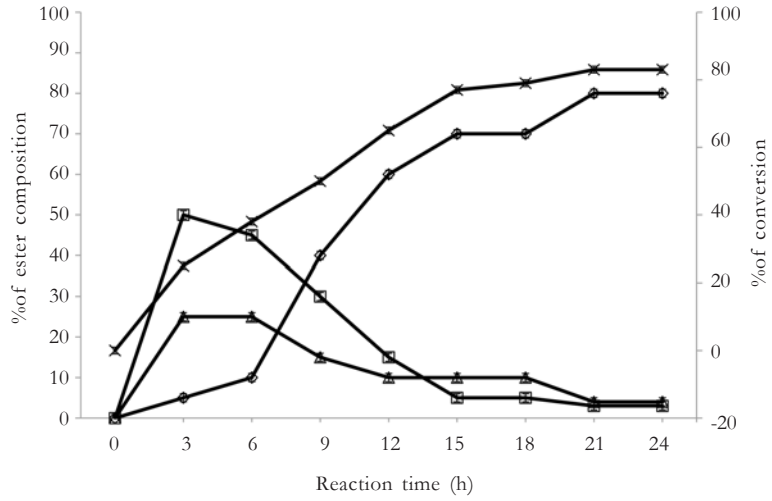


Figure 9. Time course of transesterification reaction of POME biodiesel to TMP and % of conversion (X) and % composition of monoester (□), diester (Δ) and triester (◊) of POME biodiesel with TMP. The condition was operated of molar ratio biodiesel:TMP (3.15:1), water content (3%), enzyme loading (70 kUnit/10 g substrate) temperature (30°C), within 24 h of reaction time. Data are means ± s.e.m.

3.3 Characterization of Biolubricant

The ATR-FTIR of both POME biodiesel and biolubricant is illustrated in Figure 9. The spectra clearly show the absorption band at 1742 cm^{-1}. These absorption bands due to the C=O and C-O stretching vibration in ester which led to prove the presence of oxygen in POME methyl ester and POME polyol ester. In addition, the peak of hydroxide group (OH) at 3326 cm^{-1} was not detected in spectra, indicating that the biolubricant reaction was completed. GC chromatogram of esters is shown in Figure 11. The peaks appeared was identified and labeled based on the number of alkyl carbon groups that attached to TMP backbone. The esters formed are identified by using the standard of triglyceride (TG), diglyceride (DG) and monoglyceride (MG). The composition of products contained monoester 3.3%, diester 8.0% and triester 84.0%.

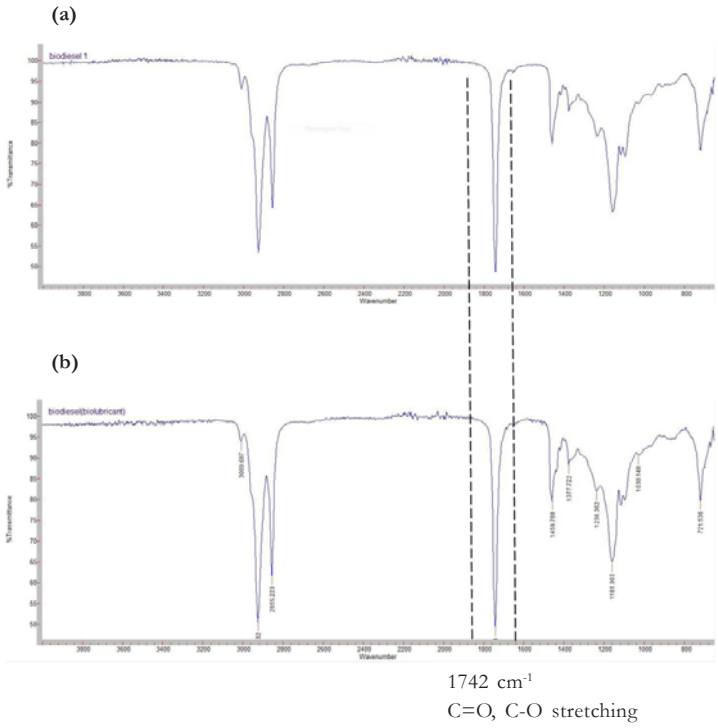
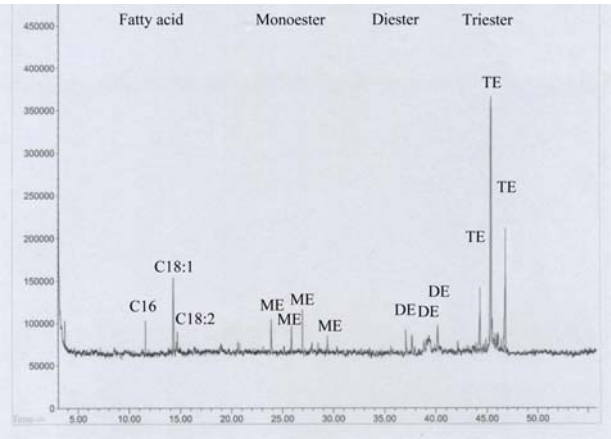


Figure 10. IR spectra of biodiesel from POME oil (a) and biolubricant (b) produced from POME biodiesel and TMP using hepatopancreas lipase from Pacific white shrimp. The condition was operated of molar ratio biodiesel:TMP (3.15:1), water content (3%), enzyme loading (70 kUnit/10 g oil) temperature (30°C), within 21 h of reaction time.



Composition	Percentage (%)
Fatty acid	0.8
Monoester	3.3
Diester	8.0
Triester	84.0

Figure 11. GC chromatogram of polyol ester via transesterification of POME biodiesel and trimethylolpropane.

The sample was analyzed in order to determine the physical parameters and to compare with POME biodiesel as well as commercial lubricant analogues. This product (POME polyol ester) from the optimized transesterification reaction had a viscosity of 48.0 and 8.4 cSt at 40°C and 100°C, respectively and correspond to the viscosity index (VI) of 152. The VI of oil is a number that indicates the effect of temperature change on its viscosity. A high VI signifies relatively little change in viscosity over a wide range of temperatures. The ideal oil for most purposes is one that maintains a constant viscosity throughout different temperature changes [15]. The critical concern for the lubricant industry is a low pour point. Table 1 shows that pour point of biolubricant from methyl oleate, canola biodiesel and castor biodiesel were -51, -66 and -39°C, respectively. However, a high pour point (5°C) was observed from the biolubricant in this study. It had been reported that *Cis*-unsaturation levels in the oleic fraction favor a decreased pour point in vegetable oils [24]. Therefore, high palmitic content in oil POME biodiesel (45%) may increase chain length and saturation of fatty acids and appear to rescind the effect of *Cis*-unsaturation resulting in the high pour point in final product (Table 1). In addition, the characteristics of biolubricant from oil POME biodiesel and TMP using hepatopancreas lipase as a catalyst were compared to commercial industrial oil ISO VG32. The test of oxidative stability was also performed. High oxidative stability (45 min) was observed. This superior oxidative stability of enzyme catalysis products is due to less byproducts formation. These byproducts affect the oxidative stability because they are molecules that do not present the whole TMP structure replaced [12]. Oxidation properties are often used to predict actual lubricant service that lie in high temperature and other extreme applications. The more resistant a lubricant is to oxidation, the less the tendency it has to form deposits, sludge and corrosive byproducts in grease, engine oil and industrial oil applications. It is also more resistant to undesirable viscosity increases during use [15]. The biolubricant (this study) was 3-times higher than the products obtained through the methoxide catalysis of the same substrate.

Table 1. Characterization of the product from transesterification of oil POME biodiesel with TMP using hepatopancreas lipase as catalyst. The condition was operated of molar ratio biodiesel:TMP (3.15:1), water content (3%), enzyme loading (70 kUnit/10 g substrate) temperature (30°C), within 21 h of reaction time.

Specification	Pome Polyol ester (This study)	Biolubricant from			ISO VG32
		Methyl oleate[a]	Canola biodiesel[a]	Castor oil biodiesel[b]	
Yield (%)	83.5	91.2	90.9	95	Not specified
Kinematic viscosity at 40°C (Cst)	48.0	48.9	40.5	290.2	>28.8
Kinematic viscosity at 100°C (Cst)	8.4	9.3	7.8	28.5	>4.1
Viscosity index	152	191	204	132	>90
Pour point (°C)	5.0	-51	-66	-39	-6
Flash point (°C)	164	-[c]	-	-	204
Oxidative stability (min)	45.0	124.8	44.4	40.0	Not specified

[a] Data obtained from Sripada et al. [25], [b] Data obtained from da Silva et al. [12], -[c] Data not reported.

4. CONCLUSION

Biolubricant, an environmentally friendly product, is an attractive product to study in several countries. The aim of this work was to utilize waste oil from palm oil mill effluent and TMP using partial purified hepatopancreas lipase from Pacific white shrimp for biolubricant production through transesterification. The results indicated that POME oil biodiesel and TMP using hepatopancreas lipase showed good potential as a substrate and catalyst in biolubricant production. The highest percentage of biolubricant conversion (83.5%) was obtained at 70 kUnit/10 g substrate, 3.15:1 substrate to TMP molar ratio, 3% water content, 30°C reaction temperature, 250 rpm agitation speed and a reaction time of 18 and 21 h for the lab- and large- scale reactors, respectively. Despite their high pour point, POME polyol ester had good frictional properties compared to ISO VG32 specification. This work has indicated the feasibility of an alternative process for bilubricant using waste from the agriculture industry. However, from the economic point of view, the process can be improved by immobilized enzyme and repeatability of immobilized enzymes as well as the preparation cost of free and immobilized enzyme has been under development.

ACKNOWLEDGEMENTS

The authors would like to thank the Energy Conservation Promotion Fund (AE-PSU-59-03), Thailand Research Fund (RTA6080010), and the Research and Development Institute at Thaksin University for financial support. Finally, we would like to thank Mr. Christopher Joseph Forti (English adviser and English proof-reader, Thaksin University) for useful comments and suggestions on the language and structure of our manuscript.

REFERENCES

[1] Kleinaite E., Jaska V., Tvaska B. and Matijosyte I., *J. Clean. Prod.*, 2014; **75**: 40-44. DOI 10.1016/j.jclepro.2014.03.077.

[2] Garces R., Martinez-Force E. and Salas J.J., *Grasas. Aceities*, 2011; **62(1)**: 21-28. DOI 10.3989/gya.045210.

[3] Nagendramma P. and Kaul S., *Renew. Sust. Energ. Rev.*, 2012; **16(1)**: 764-774. DOI 10.1016/j.rser.2011.09.002.

[4] Rani S., Joy M.L. and Nair K.P., *Ind. Crop. Prod.*, 2015; **65**: 328-333. DOI 10.1016/j.indcrop.2014.12.020.

[5] McNutt J. and He Q., *Ind. Eng. Chem.*, 2016; **36**: 1-12. DOI 10.1016/j.jiec.2016.02.008.

[6] Salimon J., Salih N. and Yousif E., *Eur. J. Lipid Sci. Technol.*, 2010; **112(5)**: 519-530. DOI 10.1002/ejlt.200900205.

[7] Luna F.M.T., Rocha B.S., Rola Jr. E.M., Albuquerque M.C.G., Azevedo D.C.S. and Cavalcante Jr. C.L., *Ind. Crop. Prod.*, 2011; **33(3)**: 579-583. DOI 10.1016/j.indcrop.2010.12.012.

[8] Linko Y.Y., Tervakangas T., Lamsa M. and Linko P., *Biotechnol. Technol.*, 1995; **11**: 889-892. DOI 10.1023/A:1018410316231.

[9] Kuepethkaew S., Sangkharak K., Benjakul S. and Klomklao S., *Renew. Energ.*, 2017; **104**: 139-147. DOI 10.1016/j.renene.2016.12.014.

[10] Syaima M.T.S., Ong K.H., Noor I.M., Zamratul M.I.M., Brahim S.A. and Hafizul M.M., *Renew. Sust. Energ. Rev.*, 2015; **44**: 669-675. DOI 10.1016/j.rser.2015.01.005.

[11] Suwanno S., Rakkan T., Yunu T., Paichid N., Kimtun P., Prasertsan P. and Sangkharak K., *Fuel*, 2017; **195**: 82-87. DOI 10.1016/j.fuel.2017.01.049.

[12] da Silva J.A.C., Soares V.F., Fernandez-Lafuente R., Habert A.C. and Freire D.M.G., *J. Mol. Catal. B*, 2015; **122**: 323-329. DOI 10.1016/j.molcatb.2015.09.017.

[13] Mouloungui Z. and Pelet S., *Eur. J. Lipid Sci. Technol.*, 2001; **103**: 216-222. DOI 10.1002/1438-9312(200104)103:4<216::AID-EJLT216>3.0.CO;2-J.

[14] Yunus R., Lye O.T., Fakhru'l-Razi A. and Basri S., *J. Am. Oil Chem. Soc.*, 2002; **79**: 1075-1080. DOI 10.1007/s11746-002-0606-3.

[15] Heikal E.K., Elmelawy M.S., Khalil S.A. and Elbasuny N.M., *Egypt. J. Pet.*, 2017; **26(1)**: 53-59. DOI 10.1007/j.ejpe.2016.03.003.

[16] Sulaiman S.Z., Chuah A.L. and Fakhru'l-Razi A., *Appl. Sci.*, 2007; **7(15)**: 2002-2005. DOI 10.3923/jas.2007.2002.2005.

[17] Abdulla R. and Ravindra P., *Biomass Bioenerg.*, 2013; **56**: 8-13. DOI 10.1016/j.biombioe.2013.04.010.

[18] Yunus R., Fakhru'l-Razi A., Ooi T.L., Iyuke S.E. and Idris A., *J. Oil Palm Res.*, 2003; **15**: 35-41.

[19] Zhang Y., Dube M.A., McLean D.D. and Kates M., *Bioresour. Technol.*, 2003; **89**: 1-16. DOI 10.1016/S0960-8524(03)00040-3.

[20] Kuepethkaew S., Sangkharak K., Benjakul S. and Klomklao S., *Int. J. Food Prop.*, 2017; **20(4)**: 769-781. DOI 10.1080/10942912.2016.1180534.

[21] Trivedi J., Aila M., Sharma C.D., Gupta P. and Kaul S., *SpringerPlus*, 2015; **4**: 165-173. DOI 10.1186/s40064-015-0937-3.

[22] Linko Y.Y., Lamsa M., Huhtala A. and Rantanen O., *J. Am. Oil Chem. Soc.*, 1995; **72(11)**: 1293-1299. DOI 10.1007/BF02546202.

[23] Hou C.T. and Shimada Y., *Encyclopedia of Microbiology*, Academic Press, Netherland, 2009.

[24] Rakkan T., Suwanno S., Paichid N., Yunu T., Klomklao S. and Sangkharak K., *Fuel*, 2017; **209**: 309-314. DOI 10.1016/j.fuel.2017.07.115.

[25] Sripada P.K., Sharma R.V. and Dalai A.K., *Ind. Crop. Prod.*, 2013; **50**: 95-103. DOI 10.1016/j.indcrop.2013.07.018.

[26] Kuepethkaew S., Sangkharak K., Benjakul S. and Klomklao S., *J. Food Sci. Technol.*, 2017; **54(12)**: 3880-3891. DOI 10.1007/s13197-017-2844-9.