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# Synthesis of Fatty Acid Methyl Ester from Palm Oil using Crude Lipase from Papaya Latex and Rice Bran

**Pudji-Hastuti** [a], Tranggono, Retno-Indrati [b] and Tyas-Utami
[a] hastutipudji@yahoo.com [b] rindrati@yahoo.com
Dept. of Agriculrutal Product Processing, Faculty of Agricultural Technology,
Gadjah Mada University, Yogyakarta 55281, Indonesia

## Abstract

The ability of crude lipase from papaya latex (CPL) and from rice bran (RBL) to catalyze trans-esterification of palm oil with methanol to produce fatty acid methyl Esters (FAMEs) were investigated. The work had two objectives: (a) to study the effect of temperature, reaction time and ratio of freeze dried *Carica papaya* latex (CPL) and defatted rice bran (RBL) to oil on the formation of FAME from palm oil through lipase-catalyzed methanolysis using response surface methodology (RSM) approach to determine the optimum process condition, (b) to evaluate of scaling up of batch process up to 4500 g of palm oil used and continuous process using packed bed reactor. The optimum condition for enzymatic synthesis of FAME by CPL was found to be at 55.13 $^0$C, with the ratio of CPL to oil 15.95:100. Methanol was added into oil by stepwise, three times every 4 hours, the reaction was continued until 24 hours. The FAME formed in this optimum condition found to be 21,73 %. Using RBL, the optimum condition for synthesis of FAME found to be at 61.7 $^0$C with the ratio of RBL to oil 16.9:100. Methanol was added into oil by stepwise, three times every 4 hours, and the reaction was continued until 54 hours. The FAME formed at this optimum condition found was 21.74%. Scaling up process using palm oil from 45 up to 4500 g giving FAME formed around 80% and 20 % for CPL and RBL, respectively. However, further investigation on continuous process need to be established.

**Key word**: palm oil, methanol, *Carica papaya* lipase, rice bran lipase, fatty acid methyl ester

## Introduction

Methanolysis of vegetable oil is an important reaction to produce fatty acid methyl esters (FAMEs), which is a valuable intermediate product in oleochemistry and an excellent substitute for diesel fuel. Lipase-catalyzed in a solvent free medium is important in industrial application, due to the advantages by avoiding problem of flammability, toxicity and separation of the organic solvents, which is lowering risk and cost of production. The use of lipase as biocatalyst for oil modification in food and other industries is becoming more and more important. Microbial lipase such as from *Candida antartica, C.rugosa,* and *Mucor miehei* have been widely used and provided commercially. However, plant origin lipase has not or less been explored yet, although some plant extracts have been studied and showed lipolytic activities (Caro et al., 2000). *Carica papaya* latex is well known as a source of protease, some previous studies shown it has lipase activity (Giordani *et al*., 1991; Villeneuve *et al*., 1995; Mukherjee and Kiewitt, 1996; Caro *et al*., 2000; Gandi and Mukherjee, 2000, Suhardi *et al*., 2004; Indrati *et al.* 2004). Several studies on rice bran have also found lipase activity (Funatsu *et al.,* 1971; Herawan, 2004, Hutomo *et al.,* 2002). Some reaction parameters need to be studied in order to find the optimal condition for enzymatic FAME production using lipase from plant origin. Surface response methodology (RSM) approach was taken to determine the optimal reaction parameters. This paper

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reports: (a) the effect ratio of the freeze dried *Carica papaya* latex (CPL) and defatted rice bran (RBL) to oil, temperature and reaction time on the formation of FAME from palm oil through lipase-catalyzed methanolysis using RSM approach, (b) evaluation of scaling up batch reaction up to 4500 g of palm oil, and continuous process using packed bed column

**Experimental**

*Materials*. *Carica papaya* latex was obtained from papaya plantation in Surakarta countryside, Central Java, Indonesia. It was collected early in the morning by tapping the green fruits through 5 (five) longitudinal incisions depth of 2-3 mm on the green fruit epidermis using a stainless steel blade. Plate-like devices were attached on the tree for collecting the exudates latex. It was then frozen prior to freeze drying at -50$^0$C for 48 hours. The tapping procedure was repeated 3 times at 10 days interval for the same fruit. The moisture, protein content and the lipolytic activity were were determined. Rice bran was also collected from the village rice mill in Surakarta countryside Indonesia. The rice bran was sieved using 30 mesh sieving device before it was defatted using diethyl ether as the solvent. The solvent containing fat was decanted, and the defatted rice bran was then air dried at room temperature. Palm oil was purchased from Yogyakarta local market, and was analyzed for moisture content, free fatty acid and peroxide values. Methanol analytical grade was purchased from Sigma. Other chemicals used were also analytical grade from Sigma, otherwise stated.

*Lipolytic activity* (Marseno *et al.,* 1998). Freeze dried *Carica papaya* latex ,CPL, (0.02 g) was added into screw cap reaction tube containing 2 ml 60% olive oil in isooctane. It was incubated at 50, 55 and 60$^0$C for 20 minutes in a shaker waterbath with shaking rate at 100 strokes per minute. The tube was then cooled in an icebath for 5 minutes. Sample (400 μl) was taken and added into a mixture of 3600 μl of isooctane and 800 μl of 5% Cu-acetate pH 6. The mixture was shaken for 90 seconds followed by centrifugation at 2000 rpm for 2 minutes. The absorbance of isooctane fraction was measured at λ 715 nm using isooctane as a blank. The fatty acid liberated from the lipolysis was calculated with fatty acid standard curve as the reference(Marseno, et al., 1998).
1 Unit lipase activity = the amount of enzyme which produce 1 μ mol fatty acid per minute

*Methanolysis* . A mixture of palm oil / methanol (1:1 mol/mol) was used as the substrate of this reaction. In the methanolysis using CPL, methanol was added into palm oil in three stepwise with interval time as stated in Table 1. An aliquot of oil was accurately weighed in the glass stoppered erlenmeyer flask . An appropriate amount of methanol and CPL were added into the flask immediately before incubation in the shaker waterbath with shaking rate at 100 strokes per minute. All reactions conditions were as stated in Table 1, and were continued until 24 hours.

Table 1. Experimental design of methanolysis using papaya latex powder (CPL)

| Variable | Level code | | |
|---|---|---|---|
| | -1 | 0 | 1 |
| Temperature, °C | 50 | 55 | 60 |
| Time, hours * | 3 | 4 | 5 |
| Ratio of CPL to palm oil | 10:100 | 15:100 | 20:100 |

* Interval time of methanol addition into reaction system, reaction was continued until 24 hours

In the methanolysis using RBL, methanol was added into palm oil in three stepwise with interval time 4 hours, and the reaction was continued as stated in Table 2.

Table 2. Design of methanolysis experiment using defatted rice bran as a source of lipase (RBL)

| Variable | Level code | | |
|---|---|---|---|
| | -1 | 0 | 1 |
| Temperature, °C | 55 | 60 | 65 |
| Time, hours * | 24 | 48 | 72 |
| Ratio of RBL to oil | 15:100 | 20:100 | 25:100 |

* Methanol was added stepwise (3 steps) every 4 hours, reaction was continued until the stated time

*Scaling up*. Scaling up of the reaction was conducted two times of tenfold, from initial scale 45 g, followed by 450 g and 4500 g of palm oil. The methanolysis of 45 and 450 g of palm oil were carried out in a glass reactor equipped with stirrer and was incubatied in a waterbath at 55$^0$C. The reactor for 4500 g palm oil was a water-jacketed stainless steel and equipped with stirrer. The CPL and RBL used were obtained from fresh preparation.

*Continuous process.* A continuous process was studied in a packed bed reactor, which is designed based on residence time of 24 hours. Either CPL or RBL was packed in the jacketed column. The temperature was set close to the optimum temperature obtained in the batch process, 55$^0$C. The substrate (a mixture of palm oil and methanol) was continuous stirred in the feed stock tank before it was pumped into the column. Sample was drawn every hour from the eluate for FAME analysis.

*FAME analysis by GC* (Christopherson and Glass,1997. with modification) The methyl ester was determined using Shimadzu chromatograph GC 9 AM equipped with FID and packed column (3 mm x 1m, supelcoport 80-100 mesh DEGS). The injector and detector temperatures were 250 and 195$^0$C, respectively. Sample (150 μl) was diluted in hexane (2850 μl) in the screw capped reaction tube and followed by methylation. Into the tube, 150 μl of 2N methanolic KOH was added and shaked for 5 minutes prior to centrifugation at 2000 rpm for 15 minutes. Subsampel (before and after methylation) was taken from the supernatant and was injected into GC. The FAME formed was calculated as the ratio of total methyl ester before and after methylation.

**Result and Discussion**

The Palm oil used in this experiment had moisture content and free fatty acid of 0.1 % and 12.47 %, respectively. The peroxide value (PV) was 7.46 m eq kg$^{-1}$ oil, so that it can be expected that this value will not affect the activity of lipase since lipase activity in oil will be affected by PV more than 20 meq kg$^{-1}$ oil (Bimberg, 1993). The lipolytic activity of dried papaya latex powder (CPL) at different temperature is shown in Table 3. It is likely the CPL has the maximum lipolytic activity at around 55$^0$C. This values were measured immediately before it was used. This activity is much lower compared to the lipolytic activity of fresh latex and immediately after it was freeze dried 330.9 and 262.3 U/g, respectively. The lipolytic activity is likely lowered by storage time and freeze drying.

Table 3 . Dried papaya latex properties

| | | Values |
|---|---|---|
| Moisture content, % | | 7,9 |
| Protein content, % | | 67,7 |
| Lipolytic activity at different temperature, U/g dried latex | $50^0$C | 90,6 |
| | $55^0$C | 181,0 |
| | $60^0$C | 156,9 |

*Methanolysis using CPL in batch process*. The FAME formed (%) after 24 hours of reaction under different condition of methanolysis using CPL is displayed in Tabel 4. The quadratic response surface model is in the form of the canonis equation I :

$$Y = 21{,}7346 - 9{,}9565X_1^2 - 7{,}9400X_2^2 - 5{,}8356X_3^2 \qquad (I)$$

where Y is the response variable ( FAME formed, %), $X_1$ is the temperature ($^0$C), $X_2$ is the interval time (hours) of methanol addition into reaction system before reaction was continued until 24 hours, and $X_3$ is the ratio of CPL to palm oil. Thus, the optimum condition was calculated and found to be at temperature of 55,1°C; interval time of methanol addition into reaction system of 3,9 hours and ratio of CPL to palm oil 15,95 %. This optimum condition gave FAME formed 21,73 %. Figure 1 shows contour and surface plots of the effect of (A) temperature and interval time of methanol addition into reaction system, (B) temperature and ratio of CPL:Oil, and (C) interval time of methanol addition into reaction system and ratio of CPL:Oil, on the formation of FAME at 24 hours of reaction time.

*Methanolysis using RBL in batch process.* The FAME formed (%) under different condition of methanolysis using RBL is shown in Tabel 5. The quadratic response surface model is in the form of the canonis equation II :

$$Y = 16.9354 - 6.016X_1^2 - 4.5429X_2^2 - 3.714X_3^2 \qquad (II)$$

Where Y is the response variable ( FAME formed, %), $X_1$ is the temperature ($^0$C), $X_2$ is the reaction time (hours), and $X_3$ is the ratio of RBL to palm oil. The optimum condition was found to be at temperature of 61.7°C; reaction time of 54.0 hours and ratio of RBL to palm oil 16.7 %. This optimum condition gives FAME formed of 16.9 %. Figure 2 depicts the contour and surface plots of the effect of (A)temperature and reaction time (B) ratio of RBL to oil and temperature, (C) ratio of RBL to oil and reaction time on the FAME formed by enzymatic methanolysis of palm oil.

*Scaling up* . There is no significant different of FAME formed (%) for different level of palm oil weight up to 4500g either using CPL or RBL, separately. However, FAME formed by CPL were higher than that by RBL in all level of palm oil weight used (Table 6). It is likely due to the lower RBL activity than the CPL activity. The FAME formed either using CPL or RBL in this experiment were higher than that in previous experiment (optimization experiment). It is due to different batch of lipase preparation resulted in different lipase

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activity and differences in mixing system used in the reactors. During optimization experiment, the mixing process of the reactants was carried out by shaking in the water bath shaker, while in the scaling up experiment the reactor was equipped with a stirrer . This stirring process keep the methanol was completely dispersed in the mixture of the reactant-enzyme resulted in higher FAME formed. While using water bath shaker the methanol may remained as droplets dispersion in the oil, may resulted in a lower FAME formed.

Table 4 . FAME formed (%) after 24 hours of reaction under different condition of methanolysis using CPL

| Process Conditions | | | FAME formed (%) Y |
|---|---|---|---|
| Temperature(°C) $X_1$ | Time (hrs) $X_2$ | Ratio CPL:Oil (%) $X_3$ | |
| 50 | 3 | 15 | 3.55 |
| 50 | 4 | 10 | 4.47 |
| 50 | 4 | 20 | 4.46 |
| 50 | 5 | 15 | 4.22 |
| 55 | 3 | 10 | 2.34 |
| 55 | 3 | 20 | 14.95 |
| 55 | 5 | 10 | 1.90 |
| 55 | 5 | 20 | 11.26 |
| 55 | 4 | 15 | 20.69 |
| 55 | 4 | 15 | 22.2 |
| 55 | 4 | 15 | 21.45 |
| 60 | 3 | 15 | 3.96 |
| 60 | 4 | 10 | 1.93 |
| 60 | 4 | 20 | 3.73 |
| 60 | 5 | 15 | 21.88 |

Table 5. FAME formed (%) under different condition of methanolysis using RBL

| Process Conditions | | | FAME formed (%) Y |
|---|---|---|---|
| Suhu (°C) $X_1$ | time (hrs) $X_2$ | Ratio RBL:Oil (%) $X_3$ | |
| 55 | 24 | 15 | 1,07 |
| 55 | 48 | 10 | 0,93 |
| 55 | 48 | 20 | 4,90 |
| 55 | 72 | 15 | 3,71 |
| 60 | 24 | 10 | 4,93 |
| 60 | 24 | 20 | 7,93 |
| 60 | 72 | 10 | 5,49 |
| 60 | 72 | 20 | 10,75 |
| 60 | 48 | 15 | 15,82 |
| 60 | 48 | 15 | 15,82 |
| 60 | 48 | 15 | 15,82 |
| 65 | 24 | 15 | 4,83 |
| 65 | 48 | 10 | 6,12 |
| 65 | 4 8 | 20 | 12,52 |
| 65 | 72 | 15 | 12,48 |

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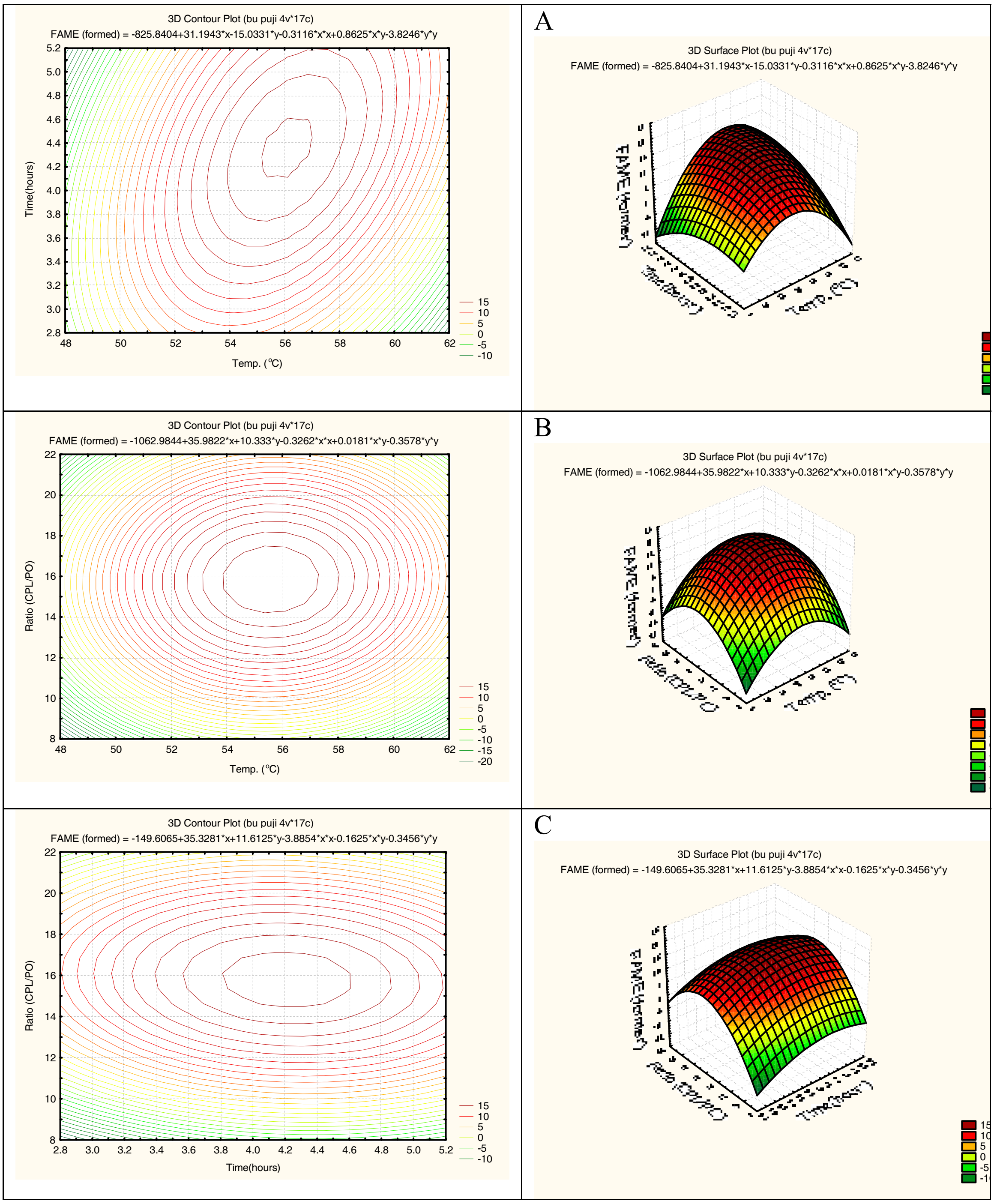


Figure 1. The contour and surface plots of the effect of (A) temperature and interval time of methanol addition into reaction system, (B) temperature and ratio of CPL:Oil, and (C) interval time of methanol addition into reaction system and ratio of CPL:Oil, on the formation of FAME at 24 hours of reaction time.

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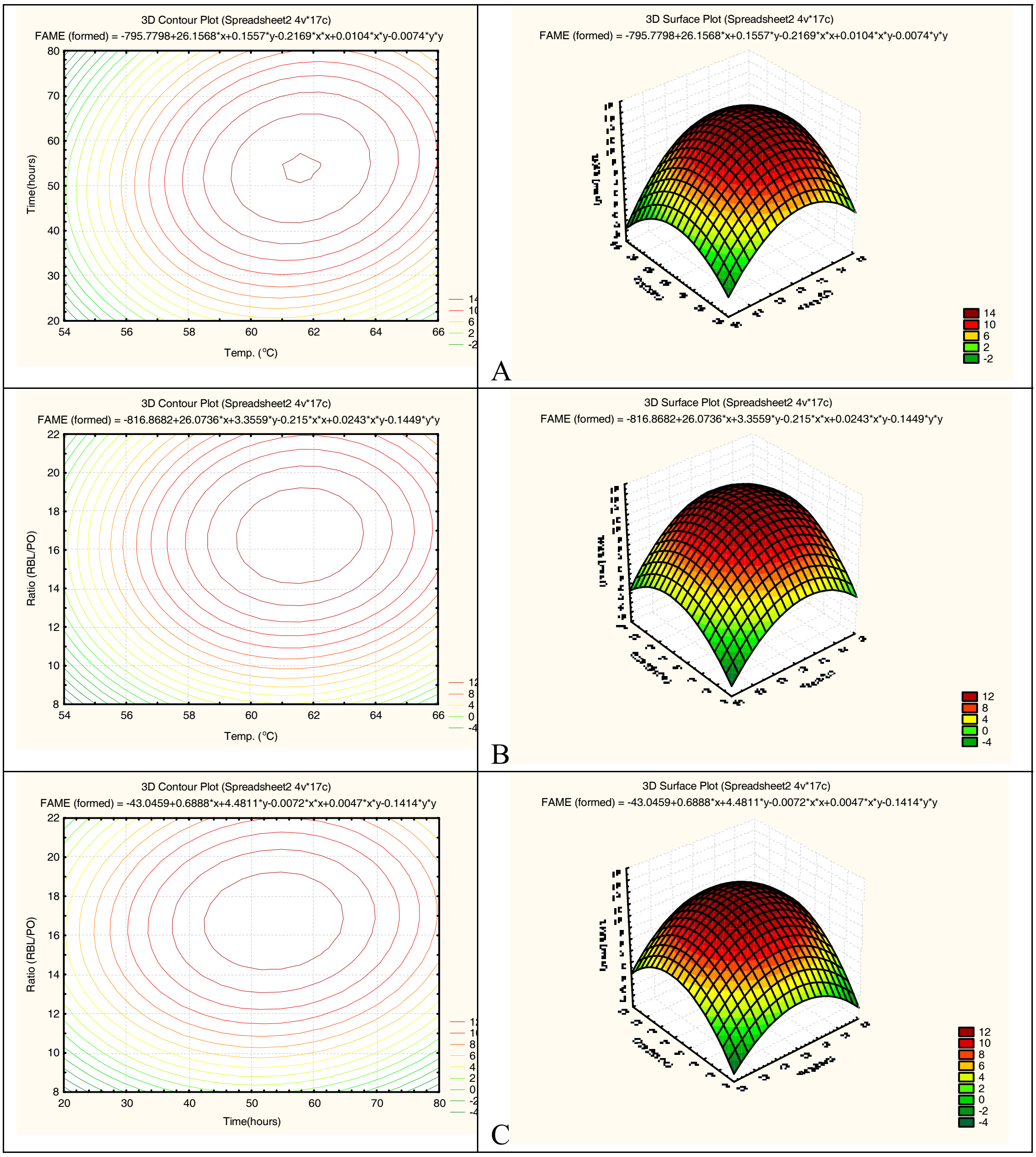


Figure 2. The contour and surface plots of the effect of (A)temperature and reaction time (B) ratio of RBL to oil and temperature, (C) ratio of RBL to oil and reaction time on the FAME formed by enzymatic methanolysis of palm oil.

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Table 6. FAME formed (%) by enzymatic methanolysis using CBL and RBL at different weight of palm oil used (45 g, 450 g, and 4500 g), reaction time of 24 hours

| Catalysts | FAME formed, % | | |
|---|---|---|---|
| | 45 g | 450 g | 4500 g |
| CPL | 76.6 | 81.4 | 80.8 |
| RBL | 21.5 | 20.2 | 23.5 |

When the time course of the methanolysis using 4500 g palm oil was continued until 48 and 72 hours, the FAME formed found to be 76.0 and 76.9% (for CPL) and 23.5 and 23.2% (for RBL), respectively. These values mean that the time course of reaction does not need to be continued until 72 hours.

*Continuous process.* From the stability test packed bed reactor with running time 72 hours. (Table 7) RBL packed column seem to be more stable than that of CPL column in term of giving FAME formed. It is likely due to stability of the position of CPL in the column, which is less stable than that of the RBL. Some CPL was found to be leached out from the matrix and was found in the eluate, means packing process for CPL in the column need to be improved. The FAME formed by continuous process through RBL column (Table 7) was close to the result in batch process (Table 6). It is therefore, the continuous process using CPL still need to be further studied.

Table 7. Stability test in a new packed column for enzymatic methanolysis using CBL and RBL. Reaction condition: residence time 24 hours, temperature 55$^0$C,

| Running time, hours | FAME formed (%) | |
|---|---|---|
| | CPL | RBL |
| 24 | 15.7 | 27.3 |
| 48 | 8.5 | 22.4 |
| 72 | 11.9 | 15.4 |

In conclusion, lipase-catalyzed methanolysis of palm oil using either freeze dried *Carica papaya* latex and defatted rice bran is a viable method for the production of fatty acid methyl ester. The FAME formed found to be much higher using CPL than that using RBL.

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