Available online at
http://jifnp.tp.ugm.ac.id

INDONESIAN FOOD AND NUTRITION PROGRESS

*Indonesian Food and Nutrition Progress, 2014, Vol. 13, Issue 1*

# Synthesis of Fatty Acid Methyl Esters from *Jatropha curcas* Oil and Its Purification Using Solvent Fractionation

Avita Kusuma Wardhani and Chusnul Hidayat[*)]

Departement of Food and Agricultural Product Technology, Faculty of Agricultural Technology
Universitas Gadjah Mada, Jl. Flora 1, Bulaksumur, Yogyakarta 55281, Indonesia
[*)] Corresponding author. Tel/Fax.: +62274549650. E-mail: chusnulhi@ugm.ac.id

Received 8 June 2014; Accepted 22 August 2014; Published Online 10 September 2014

**Abstract**

Fatty acid methyl esters (FAME) are produced by transesterification. The problem in the product of transesterification is the presence of impurities such as mono-, di-, triglycerides, and free fatty acids. So that, the purification using solvent fractionation is needed to separate them from FAME. The objective of this research were to determine the effects of crude fatty acid methyl esters-to-acetone (CFAME/acetone) ratio on yield, purity, purification factor, and recovery of FAME after fractionation and to evaluate the impurities which were separated in each step of fractionation. FAME were produced from *Jatropha curcas* oil using Berchmans's and Tiwari's methods. The impurities were separated by solvent fractionation using acetone. CFAME/acetone ratios were 1, 2, 3, 4, and 5. Fractionation was done stepwise namely 21°C, 16°C, 12°C, and 5°C. The results showed that the conversion of FAME using Tiwari's method was 1.7-fold higher than Berchmans's method. Purification of FAME using solvent fractionation resulted that the best CFAME/acetone ratio was 1. Yield decreased 1.6-fold at CFAME/acetone ratio 4. Purity decreased 8.74% with an increase in CFAME/acetone ratio 1 to 5. Purification factor decreased 2-fold at CFAME/acetone 1 to 3. Recovery decreased 1.3-fold at CFAME/acetone ratio 1 to 4. The impurities which were separated from FAME were mono-, di-, triglycerides, and free fatty acids and the major component of impurities was triglycerides (>59%). The results indicated that solvent fractionation could be used as an alternative method for purifying FAME and further study to optimize this method was needed.

***Keywords***: fatty acid methyl esters (FAME), transesterification, *Jatropha curcas* oil, solvent fractionation

## Introduction

*Jatropha curcas* L. is one of such non-edible oil belong to the *Euphorbiaceae* family. It was found to be the most appropriate renewable alternative source of fatty acid methyl esters (FAME) (Kywe et al., 2009). Quantitative analysis of methyl ester components indicated that the FAME from *Jatropha curcas* oil contained mainly methyl linoleate (47.4%) and methyl oleate (32.4%), which were comparable to fatty acid composition in *Jatropha curcas* oil feedstock (Berchmans and Hirata, 2007).

Generally, FAME is produced by transesterification. Transesterification can be defined as the reaction between triglyceride (oil) and alcohol (methanol or ethanol) in the presence of a catalyst, such as sodium hydroxide or potassium hydroxide, to form methyl or ethyl esters. Glycerol, also known as glycerine, is the by-product of this reaction (Arumugam et al., 2007).

Berchmans et al., (2007) and Tiwari et al., (2007) produced FAME from *Jatropha curcas* oil with high content of free fatty acids (FFA) by two-step process. The first step was acid esterification as a pretreatment for removing FFA in the oil. Second step was alkali base catalyzed transesterification to convert triglyceride from *Jatropha curcas* oil to FAME. Transesterification consists of a number of consecutive and reversible reactions. The triglyceride is converted stepwise to diglyceride, monoglyceride, and finally glycerol in which a mole of fatty acid is released at each step. The reactions are reversible, although the

equilibrium lies towards the production of FAME and glycerol (Arumugam et al., 2007). However, it is very difficult to obtain full completion of the reaction. Mono- and diglycerides can also exist after transesterification of triglycerides. They are as intermediates product during the transesterification reaction. Other contaminant materials in FAME are residual alcohol, glycerol, and catalyst (Gerpen and Shanks, 2004). So that it is not easy to obtain pure FAME because the presence of impurities in final product can not be avoided.

An alternative to overcome this problem is that purification is needed to separate the impurities from FAME. For removing residual glycerol and catalyst, it is usually done by washing FAME with warm distilled water. Mono-, di-, triglycerides, and fatty acids are usually removed by several methods of purification, such as distillation (based on the difference of boiling points) and optimization of transesterification reaction (using resin as catalyst or increasing the concentration of alcohol and/or catalyst) which need more energy and cost. Because mono-, di-, triglycerides, and fatty acids have the difference in melting points, fractionation at low temperature can be done as an alternative method to separate impurities from FAME.

There are three types of fractionation at low temperature, namely dry, solvent and detergent fractionation (Mamat et al., 2005). Solvent fractionation is the most efficient of fractionation methods because it produces higher in yield and purity. The properties of a solvent, including chemical nature, polarity, solubility, viscosity, and structural organization of molecules, can influence the crystallization behavior of TAGs (Wellner et al., 1981; Yang et al., 1992; Hartel et al., 1992).

Most of the previous researchers on low-temperature crystallization of fats and fatty acids used acetone as the solvent. Acetone is usually used as a solvent in fractionation because of its solubility and polarity. In addition, by adding acetone, molecule of triglyceride in low temperature can form more stable crystal earlier (Yokochi et al., 1990).

In this study, the effects of fatty acid methyl esters-to-acetone (CFAME/acetone) ratio on yield, purity, purification factor, and recovery of methyl esters after fractionation were determined. The impurities which were separated in each step of fractionation were also evaluated.

## Materials and Methods

### Materials

*Jatropha curcas* oil used in this study was obtained from ENHIL Company, Grobogan, Central Java, Indonesia. It was crude unrefined oil without any purification process such as degumming, dewaxing, netralization, bleaching, or deodorization. It was stored in dark place to prevent oxidation. It was filtered by filter paper before it was used to produce FAME. Methanol, chloroform, petroleum ether, and isooctane were supplied by J.T. Baker (USA); glacial acetic acid, acetone, diethyl ether, sulphuric acid, oleic acid, methyl oleate, pyridine, copper (II) acetate, and sodium hydroxide were purchased from Merck KGaA (Germany); olive oil was supplied by Sigma-Aldrich (USA).

### Equipments

The equipments were: a crystallization vessel, cooling machine (EYELA Cool Ace CA-1111), thermocouple (Lutron TM-903 A), stirrer (Glas-Col), rotary vacuum evaporator (IKA-WERK), shaker waterbath (Julabo SW23), and stopwatch. The characteristic vessel dimension is shown in **Fig. 1**. Fractionation was conducted in crystallization vessel. It was connected with cooling machine. The temperature in cooling machine could be controlled. There was a glass sample in the vessel. The outside of the vessel was insulated to reduce heat loss through the vessel walls. The vessel was agitated by turbin impeller which was connected with overhead stirrer.

### Chemical Analysis of *Jatropha curcas* Oil

Water content, peroxide value, and saponification value were determined by AOAC method (1990). FFA content was determined by Marseno method (1998).

### Synthesis of FAME

A two step process, acid-catalyzed esterification process followed by base-catalyzed transesterification process was performed for converting *Jatropha curcas* oil to FAME. The first step was acid esterification for removing FFA in the oil which is mainly a pretreatment process, which could reduce the FFA. Second step was alkali base catalyzed transesterification to convert triglyceride from *Jatropha curcas* oil to FAME. Berchmans's and Tiwari's method were compared to choose the better method for FAME synthesis (**Table 1)**.

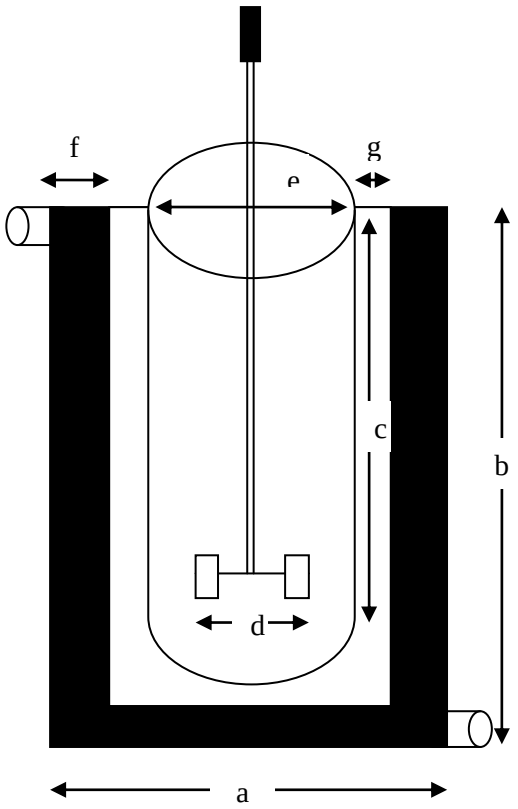


**Fig 1**. Crystallization vessel. a = vessel length = 24 cm, b = vessel depth = 20 cm, c = glass sample depth = 12 cm, d = impeller blade length =7.5 cm, e = diameter of glass sample = 9 cm, f = isolator thickness = 5 cm, g = medium circulation = 3 cm

**Table 1**. Differences between Berchmans's and Tiwari's methods

| Step | Differences | Berchmans's method | Tiwari's method |
|---|---|---|---|
| First step | Methanol-to-oil ratio (w/w) | 60% | 28% |
| | $H_2SO_4$-to-oil ratio (w/w) | 1 % | 1.43 % |
| | Reaction time | 1 h | 1,5 h |
| Second step | Methanol-to-oil after pretreatment ratio (w/w) | 24% | 16% |
| | NaOH-to-oil after pretreatment ratio (w/w) | 1.4 % | 0.5 % |
| | Reaction time | 2 h | 25 min |

In the first step, about 50 g *Jatropha curcas* oil was poured into the erlenmeyer and heated until 50°C. The solution of $H_2SO_4$ in methanol was prepared and added into the oil in erlenmeyer. Then, the mixture was incubated in shaker waterbath (70 strokes/min) at 60°C for esterification reaction. After the reaction, the mixture was allowed to settle for 30 minutes. After that, the methanol-water fraction was removed from oil. The FFA contents of oil were determined. The oil having FFA content less than 2% was used for the transesterification reaction.

In the second step, the oil product that has been pretreated from the first step was poured into the erlenmeyer and heated until 50°C. The solution of NaOH in methanol was prepared and added into the oil. Then, the mixture was incubated in shaker waterbath (70 strokes/min) at 60°C for transesterification reaction.

After the reaction, the mixture was allowed to settle for 30 minutes before separating the glycerol fraction at the bottom layer. The top layer including FAME fraction was removed in a separated erlenmeyer. Separated FAME layer was

purified by washing with warm distilled water (±60°C) until the washing distilled water was neutral to remove methanol, residual glycerol, residual catalyst, and soaps. Then, the FAME was weight and analyzed by Thin Layer Chromatography (TLC).

**Fractionation of FAME**

FAME after washing, namely crude fatty acid methyl esters (CFAME), were mixed with acetone. The variation of CFAME-to-acetone ratio was 1:1 (v/v), 2:1 (v/v), 3:1 (v/v), 4:1 (v/v), 5:1 (v/v). The volume of system was 100 mL. The mixture was poured into glass sample in crystallization vessel which was connected with cooling machine and strirrer. Fractionation was done step by step in 21°C, 16°C, 12°C, and 5°C with constant stirring rate of 100 rpm.

In each step of fractionation, if the temperature of sample reached the target temperature of fractionation, it would be maintained for two hours. Then the mixture was filtered using filter paper. Filtrate in early step would be an input for next step. Filtrate and residue in filter paper were weight and analyzed using TLC method. Acetone in filtrate from last step (5°C) was evaporated using rotary vacuum evaporator (556 mbar, 40°C).

During cooling process, the decrease in temperature of system was controlled every minute. For holding time, temperature of system and cooling medium were controlled every fifteen minutes. Temperature measurement was done using thermocople probe.

**Analysis of FAME Using TLC**

TLC plate G60 $F_{254}$ was dried in oven at 105°C for 2 h. 1 µL of sample, methyl oleat standard, olive oil, and oleic acid standard (concentration 20%) were spotted on TLC plate and developed in saturated chamber with petroleum ether : chloroform (3:1, by volume). The lipid bands were detected by exposure to iodine vapour after separation. Quantitative analysis was done using Camag Automatic TLC Scanner III.

**Analysis of Gliceride Profil Using TLC**

TLC plate G60 $F_{254}$ was dried in oven at 105°C for 2 hours. 1 µL of sample, methyl oleat standard, olive oil, and oleic acid standard (concentration 20%) were spotted on TLC plates and developed in saturated chamber with petroleum ether : diethyl ether : glacial acetic acid (60:40:1, by volume). The lipid bands were detected by exposure to iodine vapour after separation. Quantitative analysis was done using Camag Automatic TLC Scanner III.

**Experimental Designs and Statistical Analysis**

A completely randomized design was used as the experimental designs. Factor which was study was CFAME-to-acetone ratio with five levels. Fractionation process was duplicated at each fractionation temperature (21°C, 16°C, 12°C, and 5°C) and duplicate analysis were performed on each replicate. Data were analyzed by one-way analysis of variance (ANOVA) at $P > 0.05$.

**Results and Discussion**

**Chemical Properties of *Jatropha curcas* Oil**

In many cases, *Jatropha curcas* oil's quality reduces gradually because of hydrolysis of glycerides to fatty acids due to improper handling and inappropriate storage conditions. So that, the chemical properties such as FFA content, moisture content, peroxide value, and saponification value of *Jatropha curcas* oil must be determined before oil is used. They would be responsible for the quality of *Jatropha curcas* oil as the material for FAME synthesis. The results of the FFA content, water content, peroxide value, and saponification value in raw *Jatropha curcas* oil are shown in **Table 2**.

According to **Table 2**, FFA content of *Jatropha curcas* oil was high (6.25%). The FFA and water contents have significant effect on the transesterification of glycerides with alcohol using base catalyst (Goodrum, 2002). The high FFA content (>1% w/w) will cause soap formation and the separation of products will be difficult, and as a result, it has low yield of FAME product. The acid-catalyzed esterification of the oil was an alternative (Crabbe et al., 2001), but it is much slower than the base-catalyzed transesterification reaction. Therefore, an alternative process such as a two-step process was investigated for feedstock having the high FFA content (Ghadge and Raheman, 2005).

**Table 2**. Chemical properties of *Jatopha curcas* oil

| Properties | |
|---|---|
| FFA content | 6.25% |
| Water content | 0.16±0.03% |
| Peroxide value | 51.27±1.71 meq/kg oil |
| Saponification value | 128.77±0.45 mg KOH/g oil |

According to **Table 2**, the water content of *Jatropha curcas* oil was low (0.16%), so it didn't effect transesterification reaction. It is important that water is kept out of the FAME production process. While most processes can tolerate up to 1% water, even this low level will increase soap production and measurably affect the completeness of the transesterification reaction (Gerpen and Shanks, 2004). When water is present, particularly at high temperatures, it can hydrolyze the triglycerides to diglycerides and form FFA. This may disturb the transesterification by catalysts loss and unwanted soap production, which is contribute to the lower yield of FAME (Arumugam et al., 2007).

The usual method of assessment hydroperoxides (primary oxidation products) is by determination of peroxide value (Gunstone, 2004). According to **Table 2**, the peroxide value of *Jatropha curcas* oil showed a high value (51.27 meq/kg). Oxidation of FAME causes hydroperoxide formation, which can lead to FAME polimerization during fuel storage, which can lead to formation of insoluble compounds and thus cause filter blockage. Akbar et al., (2009) reported that peroxide value of *Jatropha curcas* oil which have oxidative stability was 1,93 meq/kg.

Saponification value of the *Jatropha curcas* oil was 128.77 mg KOH/g oil. Saponification value is affected by molecular weight and the composition of fatty acid of oil (Azam et al., 2005). High saponification value indicated that oils are normal triglycerides.

**Synthesis of FAME**

Tiwari's method have several advantages, namely (i) less in methanol-to-oil ratio both in first step or second step than Berchmans's method (**Table 1**), (ii) faster in reaction time than Berchman's method (**Table 1**), and (iii) the conversion of FAME was 1.7-fold higher than Berchmans's method (**Fig. 2)**. So that Tiwari's methods was selected for FAME synthesis.

The high FFA content (6.75%) of *Jatropha curcas* oil could be reduced to 0.75% in the first step. In the second step, transesterification reaction gave 95% FAME yield. Tiwari (2007) reported that first step could reduce the high FFA (14%) content of crude *Jatropha* oil to less than 1%. This process gave a yield of Jatropha's FAME above 99%. Based on analysis of FAME using TLC method, the average concentration of FAME was 2.23 µmol/µL (data not shown).

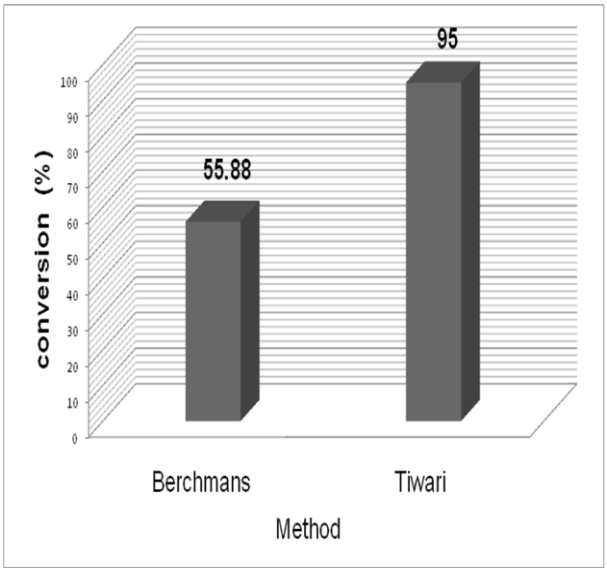


**Fig. 2**. Conversion of FAME in Berchmans's and Tiwari's method

**The Effects of CFAME-to-Acetone Ratio on Yield**

Yield was defined as the amount of fractionated material divided by the amount of CFAME before fractionation. Effects of CFAME/acetone ratio on yield are shown in **Fig. 3**. According to **Fig. 3**, yield of FAME decreased 1.6-fold at CFAME/acetone ratio 4. Increasing CFAME-to-acetone ratio to 5 didn't significantly effect to yield of FAME. In low temperature, the viscosity of fatty acids increased and would block glyceride crystal formation (Weiss et al., 1967). Decrease in yield of FAME with an increase in its concentration was caused by the addition of solvent which caused increasing in viscosity of mixture. So that formation of glyceride crystal would be faster. Solvent (typically acetone) crystallization is used for promoting TAG crystal formation, because TAG at low temperature generally form more stable crystals with solvent than without solvent (Lee et al., 2001). Talbot et al., (2006) reported that an increase in the concentration of solution would be lead to the formation of bigger crystal. It caused that FAME were trapped in the matrix. So that yield was decrease. Yield increased with an increase of solvent-to-oil ratio in fractionation of rapeseed lechitin with ethanol (Sosada et al., 1993).

Yield would be related with the amount of total mass residue in each ratio during filtration. The effects of CFAME/acetone ratio on total mass of residue are shown in **Fig. 4**. According to **Fig. 4**, total mass of residue increased with an increase in CFAME/acetone ratio. Total mass of residue had nonlinear relationship with yield of FAME. So, yield of FAME decreased with an increase of total

mass of residue. Total mass of residue increased significantly about 3.3-fold after CFAME/acetone ratio 3. It was correlated with an average decrease of yield of FAME about 0.67-fold at CFAME/acetone ratio 4 and 5 which was compared with CFAME/acetone ratio 1, 2, 3.

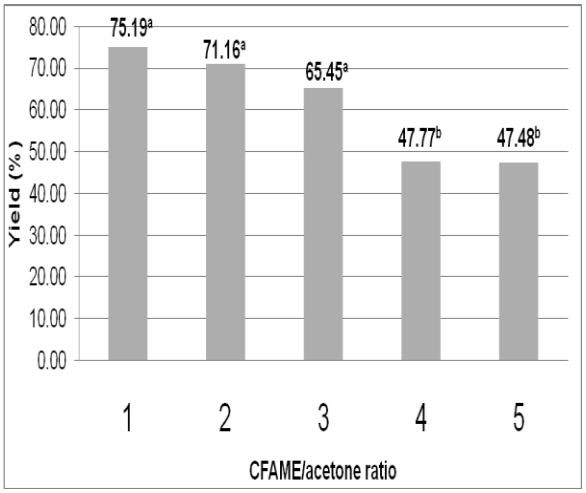


**Fig. 3**. The effects of CFAME/acetone ratio on yield

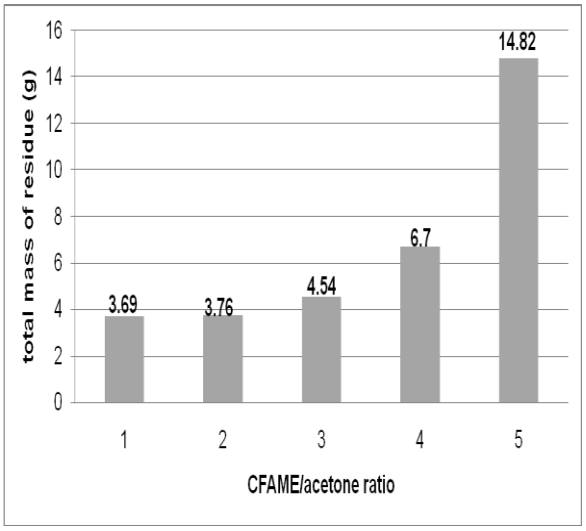


**Fig. 4**. The effects of CFAME/acetone ratio on total mass of residue

Based on colligative properties of solution, the decrease in the vapor pressure of the solvent which occurs when a solute is added to the solvent causes an increase in the boiling point and a decrease in the melting point of the solution. Therefore, the solution could reach the target of crystallization temperature. If acetone which was added to the system was fewer, it would cause less acetone in the next step of fractionation because acetone evaporated. It caused that the FAME was trapped before reaching its melting point and was measured as residue. So that increasing in total mass of residue was occurred.

**The Effects of CFAME-to-Acetone Ratio on Purity**

Purity was defined as the percentage (% area) of FAME as quintified by TLC scanner. The effects of CFAME-to-acetone ratio on purity are shown in **Fig. 5**. According to **Fig. 5**, purity decreased 8.74% with an increase in CFAME/acetone ratio 1 to 5. Residue, which was considered as impurities, contained FAME (FAME trapped in residue). It increased 3.7-fold with an increase in CFAME/acetone ratio 1 to 2 and increase 9-fold with an increase in CFAME/acetone ratio to 5 (**Fig. 6**). The yield decreased 1.6-fold at CFAME/acetone ratio 4 (**Fig. 3**). It also correlated with the results that an increase of total mass of residue about 3.3-fold in CFAME/acetone ratio 3 (**Fig. 4**).

In fractionation process, it was suggested that there is no FAME in residue with the assumption that residue only contained mono-, di-, and triglyceride as impurities because melting point of methyl oleat was -19.8°C. But in this study, residue also contained FAME. It was caused by the formation of crystal matrix, so the FAME was trapped in it.

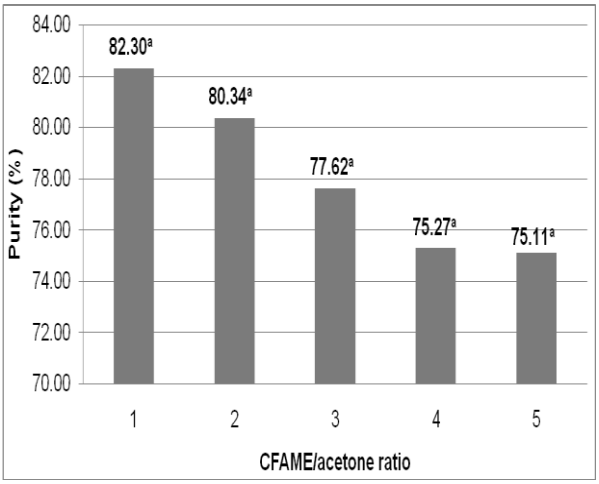


**Fig 5**. The effects of CFAME/acetone ratio on purity

**The Effects of CFAME-to-Acetone Ratio on Purification Factor**

Purification factor was defined as concentration of FAME in the product divided by concentration of CFAME. The effects of CFAME-to-acetone ratio on purification factor are shown in **Fig. 7**. According to **Fig. 7**, purification factor decreased 2-fold at CFAME/acetone ratio 1 to 3 and constant with an increase in CFAME/acetone ratio 3 to 5. It was occurred because in CFAME/acetone ratio 1 to 3, purity decreased about 2.89%, and in CFAME/acetone ratio 4 and 5, the purity showed constant trend with the average of 75.19% (data not shown). Purity had a

linear relationship with final concentration of FAME. If purity was constant, purification factor would be also constant.

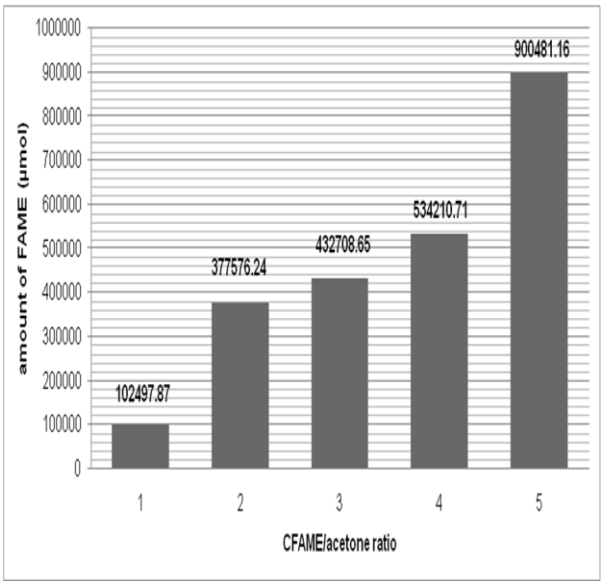


**Fig. 6**. FAME in residue

Purification factor had a linear relationship with purity. According to **Fig. 5**, purity decreased 8.74% with an increase in CFAME/acetone ratio 1 to 5. It was correlated with an increase in total mass of residue (**Fig. 4**). Based on residual analysis, amount of FAME increased 9-fold with an increase in CFAME/acetone ratio to 5 (**Fig. 6**). It would cause purity and purification factor decreased. Because purification factor indicated how purity of component, so purification factor would decrease with an increase of total mass of residue which obviously contained FAME.

**The Effects of CFAME-to-Acetone Ratio on Recovery**

Recovery was defined as mass of FAME in the product devided by total mass of CFAME. The effects of CFAME-to-acetone ratio on recovery can be shown in **Fig. 8**.

Recovery decreased 1.3-fold at increasing CFAME/acetone from ratio 1 to 4. Increasing CFAME-to-acetone ratio from 4 to 5 didn't significantly affect recovery. It was occurred because there was relationship between recovery with total mass of residue and the amount of FAME in residue. Total mass of residue increases with an increase in CFAME/acetone (**Fig. 4**). Based on residual analysis, amount of FAME increased 9-fold with an increase in CFAME/acetone ratio to 5 (**Fig. 6).**

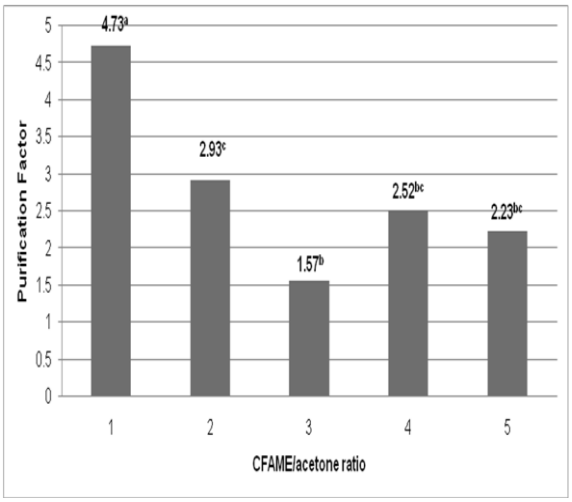


**Fig. 7**. The effects of CFAME/acetone ratio on purification factor

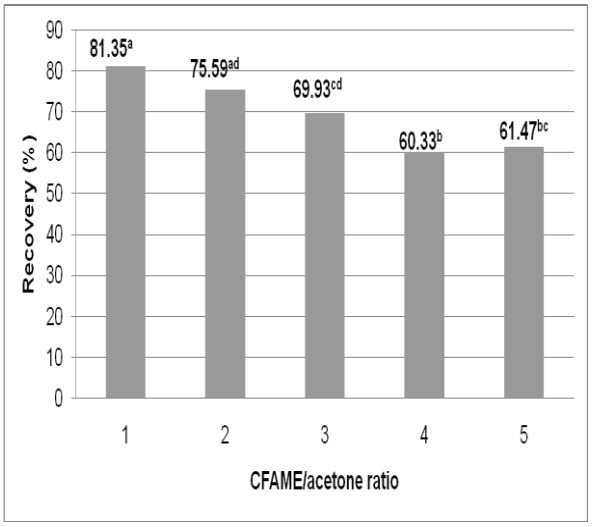


**Fig. 8.** The effects of CFAME/acetone ratio on recovery

Based on the definition of recovery, if the total mass of residue and the amount of FAME which was trapped in residue increased with an increase in CFAME/acetone ratio to 5, the amount of FAME in final product would decrease, so that recovery would also decrease.

**Analysis of Impurities**

The major component of impurities in 21°C, 16°C, 12°C, and 5°C was triglyceride (>59%) and the highest percentage of oleic acid was observed in 16°C (1.08%). There were always impurities (mono-, di-, and triglyceride) in product (filtrate in 21°C, 16°C, 12°C, and 5°C). It indicated that target of impurities, which were separated, was not appropriate with their melting point. Maa et al., (1999) reported that component which was separated in 21°C was diglyceride of oleate, which had melting point at 21.5°C; component which was separated at 16°C was oleic acid which had

melting point at 16.3°C; component which was separated at 12°C was monoglyceride of linoleate which had melting point at 12.3°C; and component which was separated at 5°C was triglyceride of oleate which had melting point at 5.5°C. It is occurred because *Jatropha curcas* oil contained the mixture of triglyceride. Triglyceride consists of the many fatty acids which had differences in melting points. The characteristic of glyceride was the totality of the characteristic of fatty acid. Glyceride, which was more saturated and had higher melting point, would be crystallized early (Gunstone, 1983). For example, at 21°C, the target of impurities was diglyceride of oleic which had melting point at 21.5°C. At this temperature, mono-, triglycerides, and fatty acids which had melting points higher than 21.5°C would be crystallized. In the other hand, mono-, triglyceride, and fatty acids which had melting points lower than 21.5°C wouldn't be crystallized. So that, at 21°C product still contained these three components.

**Conclusions**

The conversion of FAME using Tiwari's method was 1.7-fold higher than Berchmans's method. On solvent fractionation of FAME, yield decreased 1.6-fold at CFAME/acetone ratio 4; purity decreased 8.74% with an increase in CFAME/acetone ratio 1 to 5; purification factor decreased 2-fold at CFAME/acetone 1 to 3; and recovery decreased 1.3-fold at CFAME/acetone ratio 1 to 4. The impurities which were separated from FAME were mono-, di-, triglycerides, and FFA, and the major component of impurities was triglycerides (>59%). This study underscore that solvent fractionation can be done to purify FAME and further study to optimize this method is needed.

**References**

Akbar, E., Yaakob, Z., Kamarudin, S., Ismail, M., and Salimon, J. 2009. Characteristic and composition of *Jatropha curcas* oil seed from Malaysia and its potential as biodiesel feedstock. Eur. J. Sci. Res. 29 (3): 96-403.

AOAC. 1990. Official Methods of Analysis of the Association of Official Analytical Chemists. Association of Official Analytical Chemists, Washington DC.

Arumugam, S., Zinoviev, S., Foransiero, P., and Miertus, S. 2007. Bio-Fuels, Technology Status and Future Trends. ICS-UNIDO, Trieste, Italy.

Azam M.M., Waris, A., and Nahar, N.M. 2005. Prospects and potential of fatty acid methyl esters of some non-traditional seed oils for use as biodiesel in India. Biomass Bioenerg. 29: 293–302.

Berchmans, H.J. and Hirata, S. 2007. Biodiesel Production from crude *Jatropha curcas* L. seed oil with high content of free fatty acids. Bioresource Technol. 99: 1716-1721.

Crabbe, E., Nolasco-Hipolito, C.N., Kobayashi, G., Sonomoto, K., and Ishizaki, A. 2001. Biodiesel production from crude palm oil and evaluation of butanol extraction and fuel properties. Process Biochem. 37: 65–71.

Gerpen, J, Van. and Shanks, B. 2004. Biodiesel Production Technology. Midwest Research Institute, Colorado.

Ghadge, S.V. and Raheman, H. 2005. Biodiesel production from mahua (*Madhuca indica*) oil having high free fatty acids. Biomass Bioenerg. 28: 601–605.

Goodrum, J.W. 2002. Volatility and boiling points of biodiesel from vegetable oils sand tallow. Biomass Bioenerg. 22: 205–211.

Gunstone, F.D. 1983. Lipids in Food: Chemistry, Biochemistry, and Technology. Pergamon Press, New York.

Gunstone, F.D. 2004. Rapeseed and Canola Oil: Production, Processing, Properties and Uses. Blackwell Publishing Ltd, London.

Hartel, R.W. 1992. Solid-liquid equilibrium: crystallization in foods. In Physical Chemistry of Foods; Schwartzber, H. G., Hartel, R. W., Eds.; Marcel Dekker, New York, pp 47-81.

Kywe, Tint. and Oo, Mya. 2009. Production of Biodiesel from Jatropha Oil (*Jatropha curcas*) in Pilot Plant. Proceedings of World Academy of Science, Engineering, and Technology; 38: 481-487.

Lee, K.T. and Foglia, T.A. 2001. Fractionation of menhaden oil and partially hydrogenated menhaden oil: characterization of triacylglycerol fractions. J. Am. Oil. Chem. Soc. 78 (3): 297-304.

Maa, F. and Hanna, Milford. 1999. Biodiesel production: a review. Bioresource Technol. 70.

Mamat., A., I, Nor., and Said, M. 2005. Physicochemical characteristic of palm oil and sunflower oil blends fractionated at different temperatures. J. Food Chem. 91: 731-736.

Marseno, D.W., Indarti, R., and Ohta. 1998. A simplified method for determination of free fatty acids for soluble and immobilized lipase

assay. Indonesian Food and Nutrition Progress. 5: 79-83.

Sosada, M. 1993. Optimal conditions for fractionation of rapeseed lecithin with alcohols. J. Am. Oil. Chem. Soc. 70*:* 405–410.

Talbot, Geoff., Smith, K.W., and Cain, F.W. 2006. Solvent fractionation of palm oil. J. Lipid Sci. Technol. 17 (5): 324-326.

Tiwari., Kumar, A., and Raheman, H. 2007. Biodiesel production from *Jatropha curcas* with high free fatty acids: An optimized process. Biomass and Energy. 10: 10-16.

Weiss T.J. 1967. Salad Oil Manufacture and Control. J. Am. Oil. Chem. Soc. 44: 146A, 148A, 186A, 197A.

Wellner, E., Garti, N., and Sarig, S. 1981. Solution-mediated polymorphic transformation of stearic acid. Cryst. Res. Technol. 16: 1283-1288.

Yang., Chang S.C., and Chen, R.H. 1992. Effect of solvent polarity and fractionation temperature on the physicochemical properties of squid viscera stearin. J. Am. Oil. Chem. Soc. 69: 1192-1197.

Yokochi, T., M.T. Usita., Y, Kiamisaka., T, Nakahara., and O, Suzuki. 1990. Increase in the γ-linolenic acid content by solvent winterization of fungal oil extracted from *mortierella* genus. J. Am. Oil. Chem. Soc. 67: 846–851.