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Production of Biodiesel Fuel and Reduction of Phorbol Esters by Transesterification of *Jatropha curcas* Oil with Methyl Acetate

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Edible oil such has been used as raw material for biodiesel fuel (BDF). However, if the production of biofuel increases, it may compete with food and livestock feed and the price of oil may rise. Therefore, non-edible *Jatropha curcas* oil containing toxic phorbol esters has attracted attention. Recently a new BDF process which coproduct is not glycerin but triacetin has been developed. For triacetin can also be used as an anti-knock agent for biodiesel fuel, it is not necessary to remove triacetin from biodiesel fuel. The purpose of this study was to investigate the production of biodiesel fuel and reduction of toxic phorbol esters by transesterification of *Jatropha curcas* oil with methyl acetate. The formation of monoacetin, diacetin and triacetin was also confirmed by the spectrum estimated as the peaks of those substances. The peak of glycerin was not found among the major peaks of the chromatogram. As the molar ratio of methyl acetate to material oil was increased from 5 to 50, the yield of FAME was increased. The maximum FAME yield was 90 %. and the maximum concentration of FAME in the final product was 0.71 g/g. The concentration of phorbol esters in crude *Jatropha curcas* oil used in this study was 1.7 mg/g. The phorbol ester concentration of degummed oil decreased by a small amount from the crude oil. Phorbol esters decreased after deacidification and the concentration in phorbol esters in deacidified oil was 1.2 mg/g. The concentration in phorbol esters decreased to 0.22 mg/g after mixing step of oil with potassium methoxide. No phorbol esters were detected in any reaction time in the final product after the transesterification reaction in this study. The process used in this study is superior in that the phorbol ester contained in *Jatropha curcas* oil can be completely decomposed, compared with the conventional method using methanol as a raw material.

1. Introduction

In recent years, due to mass consumption of fossil fuels by automobiles, industries and home use, greenhouse gases emitted during the depletion of fossil fuels and combustion are becoming a problem (Casallas et al., 2018). Biodiesel fuel (BDF) using vegetable oil has attracted much attention in the world because biofuel is carbon neutral and renewable. Edible oil such as rapeseed oil has been used as raw material for BDF. However, if the production of BDF increases, it may compete with food and livestock feed and the price of edible vegetable oil may rise (Popp et al., 2014). Therefore, *Jatropha curcas* oil containing toxic phorbol esters has attracted attention (Kato et al., 2014). The seed of *Jatropha curcas* contains 50 % of oil. *Jatropha curcas* can grow almost anywhere, even on gravelly, sandy and saline soils where food crops cannot grow. The production of BDF from *Jatropha curcas* oil may not compete with food and livestock feed when *Jatropha curcas* is cultivated in the area not suitable for edible vegetable oil. Phorbol esters in *Jatropha curcas* have an inflammatory action and are skin tumor promoters (Hirota et al., 1988). For that toxicity of phorbol esters, it is necessary to reduce them as much as possible from BDF produced from *Jatropha curcas* oil (Kato and Takechi, 2015).

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In a conventional BDF process, fatty acid methyl esters and glycerin are generally produced by the transesterification reaction of fats and oil with methanol. The produced glycerin needs to be removed as it damages the engine or coagulates under the cloud point (Yamane et al., 2015). The glycerin removed from biodiesel fuel contains catalysis and various other substances originated from material oil and thus the recycling use of the glycerin from BDF process is a difficult problem (Sadano et al., 2010).
A new BDF process not producing glycerin was proposed recently (Casas et al., 2011). The proposed process produces fatty acid methyl esters (FAME) and triacetin by transesterification reaction of fats and oils with methyl acetate. For triacetin can also be used as an anti-knock agent for biodiesel fuel, it is not necessary to remove triacetin from biodiesel fuel (Casas et al., 2010).
The purpose of this study was to investigate the formation of FAME and the reduction of phorbol esters by transesterification of *Jatropha curcas* oil with methyl acetate. Additionally, degumming and deacidification processes were studied for the degradation of toxic phorbol esters.

2. Methodologies

2.1 Degumming and deacidification of *Jatropha curcas* oil

Jatropha curcas oil was obtained from Okinawa Biodiesel Ltd. The oil had been stored in a deep freezer kept at under -70 °C until the start of the experiment. Thawed crude *Jatropha curcas* oil was degummed by mixing with hot water. 17 mL of water was added to 100 mL of crude oil and the mixture was shaken for 10 min at 150 rpm in a hot water bath at 80 °C. Then 13 mL of hot water was added to the mixture and the mixture was centrifuged at 3000 rpm for 10 min. After centrifugation, the water phase was removed. These operations were repeated until the removed water phase became clear and transparent.
Degummed oil was deacidified by adding sodium hydroxide solution. Sodium hydroxide was dried at 120 °C for 2 h and made 0.1 % sodium hydroxide solution. The sodium hydroxide solution was added to the degummed oil and shaken for 10 min at 150 rpm in a water bath at 70 °C. Then the shaken mixture was centrifuged at 3000 rpm for 10 min. The upper oil phase of the centrifugate was recovered. The recovered oil was washed by citric acid solution by mixing and shaking at 150 rpm for 1 min. After washing, the mixture was separated by centrifugation and the upper oil phase was taken. The washed oil was dried in a rotary vacuum evaporator for 1 h. The saponification value of the dried oil was determined by neutralization titration method (JIS K 0070:1992) and the average molecular weight of oil was calculated.

2.2 Synthesis of BDF

Methyl acetate was desiccated before use by adding molecular sieve 4A. BDF was produced by transesterification of *Jatropha curcas* oil and methyl acetate using potassium methoxide for catalysis (Casas et al., 2011). The reaction was performed in a 300 mL Erlenmeyer flask placed on a hot stirrer with a polytetrafluoroethylene magnet. Deacidified and dried *Jatropha curcas* oil was heated to 50 °C. After heating, potassium methoxide catalyst was mixed with the oil for 30 minutes at 50 °C. Potassium methoxide catalyst to deacidified oil molar ratio was 0.4. The rotation speed of the magnet during the mixing was 700 rpm. After mixing was completed, methyl acetate having a molar ratio of methyl acetate to deacidified oil of 5 or 50 was added to initiate the reaction. The reaction temperature was set to 50 °C and the rotation speed was set to 700 rpm. After the reaction, phosphoric acid was added to terminate the reaction. Thereafter, the catalysis was removed as a potassium phosphate salt by centrifugation at 3000 rpm for 10 min. Methyl acetate was vaporized by a rotary vacuum evaporator at 60 °C for 1h. BDF yield was calculated by dividing actual yield obtained from the experiments by the theoretical yield obtained from the stoichiometric equation.

2.3 Analysis of FAME and phorbol ester

The concentration in phorbol esters in *Jatropha* oil and products was analyzed by High-Performance Liquid Chromatography (HPLC) (Prominence UFLC, Shimadzu) (Yu et al., 2016). Phorbol esters in oil samples before transesterification were extracted with the same volume of methanol as the samples for 3 times. Then 20 μL of the sample was injected into a LiChrospher 100 RP-18 (5 mm) LiChroCART 250-4 column (Merck-Millipore) and eluted from the column by acetonitrile gradient in the water at constant flow rate of 1.3 mL/min. Phorbol esters were detected with a UV detector at the wavelength of 280. The column oven temperature was 45 °C. Analytic gradients of HPLC were shown in Table 1. The UV absorbance spectrum was scanned by HPLC detector to confirm the suspected peaks of phorbol esters. Using phorbol-12-myristate 13-acetate as an external standard, results were converted to phorbol-12-myristate 13-acetate equivalent (Tosa and Ishizuka, 2017). The concentration of FAME in *Jatropha* oil and products was also analyzed by HPLC (Carvalhol et al., 2012). Each sample of the final product was diluted 50-fold with methanol for the determination of FAME.

FAME was detected with a UV detector at the wavelength of 205 nm. Other analytic conditions were the same as the analysis of phorbol esters. The calibration curves of phorbol ester and FAME were shown in Figure 1.

Table 1: HPLC analytical gradient conditions

Time (min)	Acetonitrile (%)	Water (%)
0	40	60
15	75	25
35	100	0

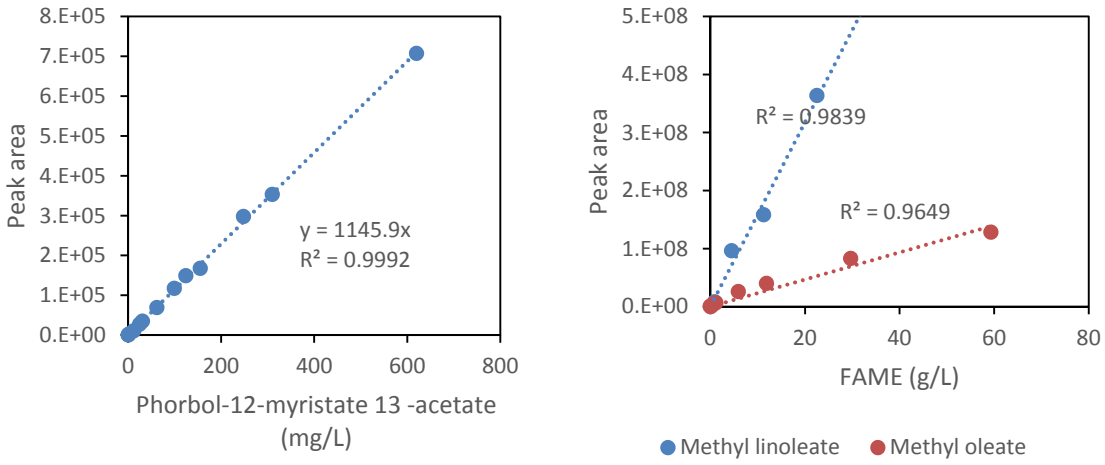


Figure 1: Calibration curve of phorbol ester and FAMEs

2.4 Qualitative analysis of the final product by GC-MS

The final product was diluted 50-fold with methanol and then analyzed by GC-MS (GCMS-QP2010, Shimadzu) with a column (RTX-5ms, 30 m, 0.25 mm ID, 0.25 μm). The conditions used for GC-MS analysis is shown in Table. 2. Qualitative analysis was performed from the retention time and mass spectrum of each peak on the chromatogram.

Table 2: GC-MS analytical conditions

Oven	40 °C (hold 1 min) to 300 °C at 10 °C/min
Carrier Gas	He, constant flow
Flow rate	3.0 mL/min
Linear Velocity	62.5 cm/s
Detector	MS Scan mode
Ionization Mode	EI

3. Results and Discussion

3.1 Component of the final product

The formation of monoacetin, diacetin and triacetin was confirmed by the spectrum estimated as the peaks of those substances by GC-MS. In this study, triacetin was one of the final products expected from formula (1) but diacetin and monoacetin are not the ones. Dicaetin and monoacetin may be produced from triacetin or reaction intermediate products by hydrolysis. The peak of glycerin was not found among the major peaks of the chromatogram. Glycerin has high water solubility and might be removed to phosphoric acid solution from the final product.

The peaks of FAME were also found in the GC-MS chromatogram of the analyzed final product. The major FAME detected by GC-MS qualitative analysis were methyl linoleate and methyl oleate. Some other FAME were also detected but the peaks in the chromatogram were very small. Then methyl linoleate and methyl oleate were used for the calculation of BDF yield.

3.2 Synthesis of FAME

The mixture of oil, methyl acetate and catalyst formed a single phase during the transesterification. The mixture of vegetable oil and methanol usually forms two phases and the intense mixing is necessary for the transesterification of vegetable oil with methanol. The reaction used in this study may need less energy for mixing than the reaction of vegetable oil and methanol. Figure 1 shows the relationship of reaction time and BDF yield in the final products at methyl acetate to oil molar ratio of 50 and catalyst to oil molar ratio of 0.4. It was found that FAME had the maximum yield at the reaction time of 45 min and gradually decreased thereafter. The maximum FAME yield was 90 % and the maximum concentration in FAME in the final product was 0.71 g/g. Methyl linoleate was rapidly synthesized and the concentration in the final product reached the plateau within15 min. The synthesis rate of methyl oleate was slower than that of methyl linoleate and the concentration reached the plateau at 45 min of reaction time. Figure 2 shows the relationship of reaction time and FAME concentration in the final products at methyl acetate to oil molar ratio of 5 and catalyst to oil molar ratio of 0.4 of reaction time. In contrast to the results shown in Figure 1, the FAME yield in the final product increased even after 30 minutes and reached the plateau at 3 h of reaction time. The maximum FAME yield was 59 % and the maximum concentration in FAME in the final product was 47 g/g. The synthesis of methyl linoleate at was also slower at methyl acetate to oil molar ratio of 5 than that at methyl acetate to oil molar ratio of 50. The concentration in methyl linoleate reached the plateau at 3 h of reaction time. The synthesis rate of methyl oleate was also slower than that of methyl linoleate and the concentration in methyl oleate reached the plateau at 4 h of reaction time.

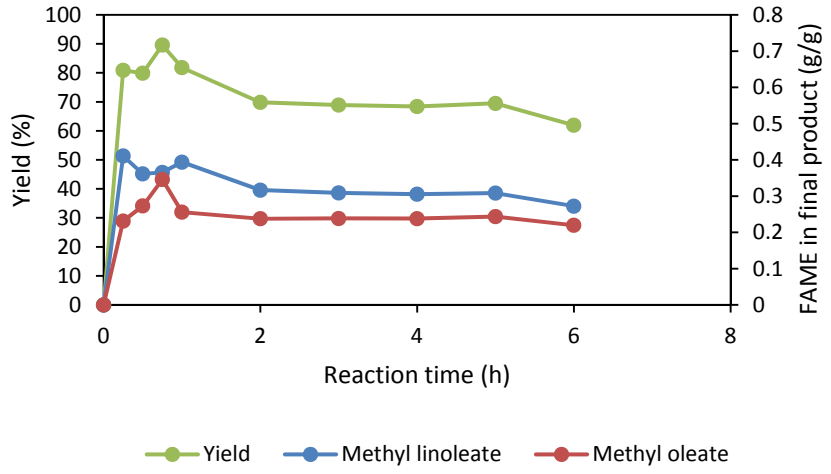


Figure 1: Time course of FAME in the final product at methyl acetate to oil molar ratio of 50

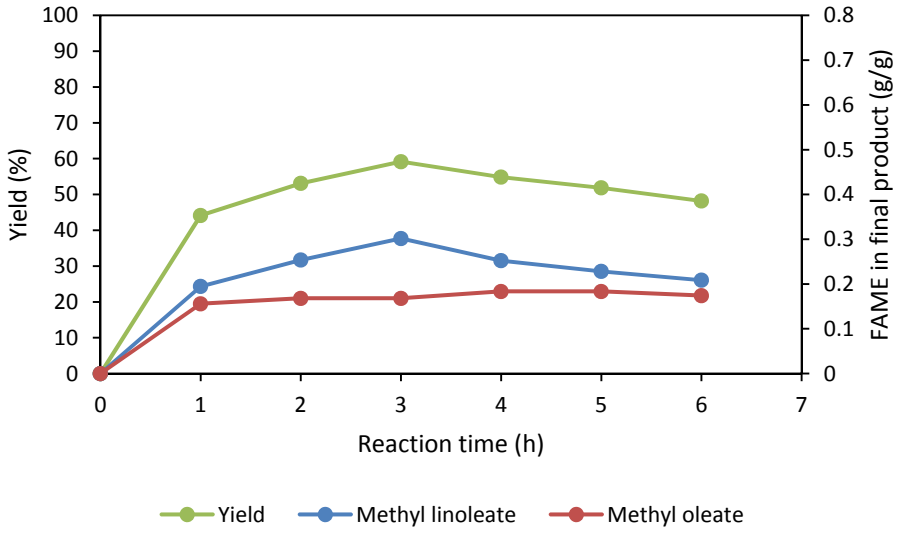


Figure 2: Time course of FAME in the final products at methyl acetate to oil molar ratio of 5

A trace amount of moisture contained in raw materials might react with the catalyst to produce potassium hydroxide and methanol so that potassium hydroxide and fatty acid methyl ester react with each other and the produced FAME might change to soap. When methanol is produced, glycerol might be formed by the transesterification reaction of fat and oil with methanol using potassium methoxide as a catalyst. To examine the effect of moisture, methyl acetate not desiccated was also used for the synthesis of FAME. The FAME yield with not desiccated methyl acetate was almost the same as that with desiccated methyl acetate.

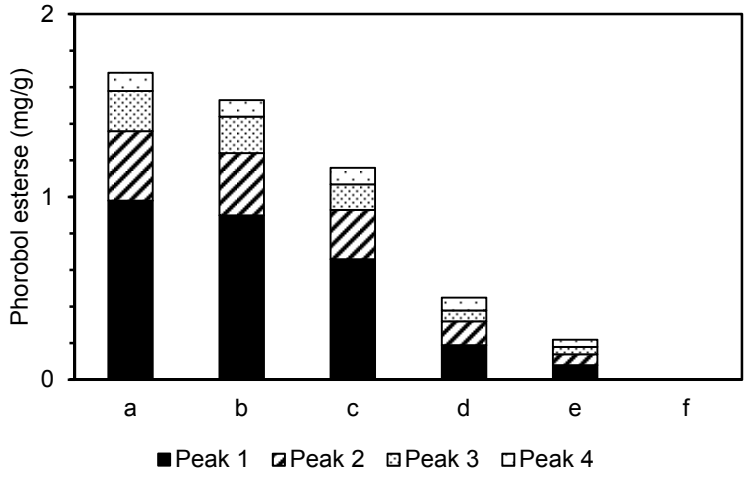
Casas *et al*. synthesized BDF from sunflower oil and methyl acetate, and the mass fraction of FAME in the final reaction product was 76.7% (Casas et al., 2011). In that research, chemical equilibrium was achieved within 10 minutes. The mass fraction of FAME in the final reaction product of this study was 71.1% at the maximum, which was 76.7% of the yield in the study by Casas *et al*. Moreover, the time required for chemical equilibrium in this research was 45 min even at methyl acetate to oil molar ratio of 50. The oil used in this study was *Jatropha curcas* oil, which property and component are different from the sunflower oil used by Casas *et al*. (Casas et al., 2011). The difference in these results may be partly due to the differences in fatty acid composition and other ingredients in the material oil. Kusumaningtyas *et al*. produced BDF from *Jatropha* oil and ethyl acetate and the maximum yield was about 14% (Kusumaningtyas et al., 2016). That yield is lower than the yield of this study. In this study, the ester used in the transesterification reaction is methyl acetate, whereas the ester used by Kusumaningtyas *et al*. was ethyl acetate. The differences in the esters used for raw material may be the cause of the difference in yield. Kusumaningtyas *et al*. also found an increase in molar ratio of ethyl acetate to *Jatropha* oil decreased yield of biodiesel. That result is not the same as the results obtained in this study.

3.3 Changes in phorbol esters in BDF process

Clear intense four peaks suspected as phorbol esters were detected in HPLC chromatogram of the methanol extract from deacidified *Jatropha curcas* oil. Phorbol esters exist in the four peaks (Makkar et al., 2009). The UV absorbance spectrum was scanned by HPLC detector and compared with the spectrums reported before (Makkar et al., 2009). Then those four peaks were confirmed as phorbol esters.

Figure 3 shows the change in the concentration of phorbol esters after each process. The concentration of phorbol esters in crude *Jatropha curcas* oil used in this study was 1.7 mg/g. The phorbol ester concentration of degummed oil decreased by a small amount from the crude oil. There was a possibility that hydrolysis of phorbol ester occurred because hot water was used for degumming. Phorbol esters decreased also after the deacidification process. The concentration of phorbol esters decreased to 1.2 mg/g after deacidification. Free fatty acids react with sodium hydroxide, forming soap and are removed from oil in the deacidification process. It is highly probable that sodium hydroxide also reacted with phorbol esters and thus formed soap. The concentration of phorbol esters decreased to 0.22 mg/g after mixing step of oil with potassium methoxide.

No phorbol esters were detected in any reaction time in the final product after the transesterification reaction. Although some peaks were seen in the chromatograms, the retention time of the peaks is not the same as the peaks of crude oil extract, meaning that the peaks in the chromatograms of the final product are not the peak of phorbol esters. Therefore, it was found that the transesterification reaction products of *Jatropha curcas* oil with methyl acetate did not contain phorbol esters.



a) Crude oil, b) Degummed oil, c) Deacidified oil, d) Dried and deacidified oil, e) Dried and deacidified oil after mixing step of oil with potassium methoxide and f) The final product.

Figure 3: Phorbol esters in crude Jatropha curcas oil and BDF process products

In *the Jatropha* BDF process with methanol as raw material, phorbol esters concentration in crude oil, degummed oil, deacidified oil, and BDF are reported as 0.429, 0.382, 0.141 and 0.016 mg/g, respectively (Matsukawa et al., 2010). The reduction of phorbol esters in these degumming and deacidification processes is about the same as this study. On the contrary, phorbol esters were not detected in BDF in this study, whereas in the conventional production method using methanol it was 0.016 mg/g. The process used in this study is superior in that the phorbol ester contained in Jatropha oil can be completely decomposed, compared with the conventional method using methanol as a raw material.

4. Conclusions

Crude *Jatropha curcas* oil was degummed, deacidified and then transesterified with methyl acetate using potassium methoxide as a catalyst. FAME is certainly synthesized by the process used in this study. Phorbol esters are reduced by degumming and deacidification processes. No phorbol esters were detected in any reaction time in the final product after the transesterification reaction. The process used in this study is superior in that the phorbol ester contained in *Jatropha curcas* oil can be completely decomposed, compared with the conventional method using methanol as a raw material.

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