A Study on Acid-Catalyzed Transesterification of Crude Rice Bran Oil for Biodiesel Production

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ABSTRACT

Biodiesel is a renewable, biodegradable and nontoxic fuel for diesel engines. It is derived from oils and fats by transesterification with alcohols. As alternative fuel biodiesel has attracted considerable attention during the past decades. The main hurdle to the commercialization of biodiesel is the cost of raw materials. The high value of soybean oil or canola oil as a food product makes production of a cost-effective fuel very challenging. Use of edible oils as biodiesel feedstock cost about 60-70% of raw material cost. Nonedible, inexpensive, low-grade oils with value added byproducts is utmost important to make the biodiesel production economical. Rice bran oil ranks first among the non-conventional, inexpensive, low-grade vegetable oils. Furthermore, crude rice bran oil is a rich source of high value-added byproduct. Therefore, use of rice bran oil as raw material for the production of biodiesel not only makes the process economical but also generates value added bio-active compounds. Isolation and purification of these byproducts make the process attractive and remunerative.In the present investigation a systematic studies of transesterification of high free fatty acid rice bran oil was carried out to establish optimal reaction condition. It was found that acid-catalyzed methanolysis of fatty acids are faster than pure triglycerides or pure triglycerides plus 5% water. More than 99% of FA were converted to their corresponding FAME with 20 min of reaction times at temperature of boiling point of methanol otherwise almost for 6 hours reaction none of TG were converted. Effect of chain length and unsaturation of fatty acid on rate of esterification of fatty acid with methanol are equally reactive irrespective of difference in their chemical structures. Fatty acids from different sources shows similar conversions and change in the fatty acids composition has no effect on rate of methanolysis.

KEYWORDS: Acid catalyzed, bioactive compounds, biodiesel, crude rice bran oil, rice bran, transesterification.

INTRODUCTION: More than 90% world's rice production coming from Asia. Rice production first among agricultural commodity of Indonesia. Rice bran is a brown layer present between rice and the outer husk of the paddy. Rice bran oil is an important derivative of rice. Depending on variety of rice and degree of milling, the bran contains 16-32 wt% of oil [1]. About 60-70% of the oil produced from this bran is non-edible oil, due to the problems attributed to the stability and storage of the rice bran and the dispersed nature of rice milling [2, 3]. Rice bran oil (RBO) is considered to be one of the most nutritious oils due its favorable fatty acid composition and unique combination of naturally occurring biologically active and antioxidant compounds [2, 4, 5]. RBO has been difficult to refine because of the its high content of free fatty acid (FFA), unsaponifiable matter and dark color [6]. Due to the presence of an active lipase in the bran, FFA content in CRBO is higher compared to the other edible oil. Because of this reason, RBO is not considered as edible oil, particularly in Indonesia, Philippines, Taiwan etc. Almost all the bran produced is used as boiler fuel and as poultry and cattle feed. Thus, this valuable by-product of rice is underutilized.

In Indonesia, rice bran and rice polishing are used as feed for poultry, pigs, and some cattle because they are cheap and do not require processing. Biodiesel, from vegetable oil and animal fats, is a promising alternative diesel fuel obtained from transesterification. Approximately 60-70% of biodiesel cost is attributed to raw material cost. However, use of cheap and non-edible oil as substrate and utilization of by product may result in substantial reduction in the BD production cost [7, 8]. Among the non-conventional oils, rice bran oil ranks first in terms of availability and low cost. Using RBO as biodiesel with transesterification process followed by removal of esters (BD) gives an unsaponifiable lipid fraction which contains highly concentrated nutraceutical and biologically active antioxidant compound (γ-oryzanol, tocopherols, tocotrienols, phytosterols, polyphenols and squalene) in the residual unsaponifiable lipid fraction. Utilization of these compounds further, reduces the cost of BD and makes it competitive or even less costly than conventional fuel. In the present investigation a systematic studies of transesterification of high free fatty acid rice bran oil was carried out to establish optimal reaction condition. This studies were pointed on the influence of free fatty acid in transesterification process.

EXPERIMENTAL PROCEDURES

Materials. Samples of rice bran of different free fatty acid content were obtained from the rice millings located around Taipei City. Thin-layer chromatography (TLC) aluminum plates were 20 x 20 cm of thickness 250 μm were purchased from from Machery-Nagel (Schweiz, Germany). Free acid content of the oil was determined according to AOCS method #Ca 5a-40. Hexadecanoic acid (>99%) was obtained from Sigma Chemical Co., USA. Oleic acid extra pure was purchased from E-Merck, Germany. All solvents and chemical reagents were either HPLC-grade or AR-grade were obtained from commercial sources.

Extraction of Oil. 50 g of rice bran was taken in to an extraction thimble. The thimble was then placed in the butt tube and extracted with 250 mL of solvent in the extraction flask. Oil was extracted with hexane on a Soxhlet apparatus and then recovered by filtering and drying the extract over anhydrous sodium sulfate and evaporating the solvent under vacuum at 40°C on a rotary evaporator (oil yield 18-21%). The

oil was stored under N_2 at 0°C until further use.

Transesterification: The acid-catalyzed transesterification of rice bran oil was carried out using 20:1 molar ratio of methanol (MeOH) to crude rice bran oil (CRBO) and 10%HCl as a catalyst (weight percent of oil). In a typical reaction a mixture of 1g of crude rice bran oil, 0.75g of methanol and 0.1 of HCl (wt 37%) were taken in to a 50 mL two necked round-bottomed flask equipped with refluxing condenser and thermometer. The contents were refluxed under constant magnetic stirring at 70°C in an oil bath. Aliquots of reaction mixture (100 µL) were withdrawn with 1mL of disposable pipette at definite interval for analysis.

Sampling and analysis. Samples were withdrawn at pre-specified time intervals. Approximately 10 samples were collected during the course of each reaction (1 hour). The frequency of sample collection varied and was dictated by the reaction condition. Reaction with pure fatty acid and high FFA CRBO (60%FFA) as a substrate required more frequent sampling at the beginning of the reaction. Samples were taken at 1- and 2-min time intervals, early in the reaction, and at 5 to 15 min intervals, later in the reaction. Aliquots of reaction mixture (100 µL) were collected into a 10-mL test tubes containing 2 mL water and 2 mL hexane. The contents were vortexed and centrifuged. The top organic phase, which contain fatty acid methyl ester (FAME), unreacted triglyceride, diglyceride and monoglyceride pipette out while the aqueous phase containing residual MeOH, glycerol and catalyst is discarded. The extent of reaction was monitored by analytical TLC. The samples of reaction product (hexane phase) was spotted on TLC plate and developed in eluting solvent system of n- hexane/ ethylacetate/ acetic acid (90:10:1, v/v/v). The spots were visualized by exposing the plates to Iodine vapour and identified by comparison with the R_f values of authentic standards.

Preparation of FFA from oil. Oil (crude rice bran oil or soybean oil) (25 g) was added to solution of NaOH (5.75 g) in water (11 mL) and 99.5% ethanol (66 mL). The mixture was refluxed at 65°C at atmospheric pressure. The saponification was completed in less than 2 h, and the reaction mixture was allowed to cool to room temperature. Distilled water was added to the saponified mixture, and the unsaponifiable matter was separated by extraction with hexane and discarded. The aqueous phase containing saponifiable matter was acidified to pH 2 with HCl/H_2O=1:1 (v/v). The mixture was transferred to a separatory funnel, and the hexane layer containing FFA-CRBO. Hexane in the organic phases was recovered by vacuum filtration using an Ace Bünchner funnel (25-50 mm).

TLC/FID analysis of Esterification of FA. The quantitative analysis of the degree of methanolysis of pure fatty acids was analyzed by a TLC/flame-ionization detector (FID) analyzer (Iatroscan MK-5; Iatron Co., Tokyo, Japan). Analyses were performed using an IATROSCAN TH-10 Analyzer MKIII. One hundred microliters of sample was dissolved in 1 mL of chloroform and filtered through cotton plug. The volume of the solution was reduced to 200 µL by passing N_2 gas. One micro liter of this lipid solution was spotted on silica gel Chromarods-SIII and developed in n-hexane/ethyl acetate/acetic acid = 97:3:0.3, v/v/v. solvent systems. The rods were developed for 40 min and air dried for 5 min, and then analyzed by

scanning the rods with a speed of 30s/rod. The Iatroscan was operated with hydrogen and airflow rates of 160 mL/min and 2000 mL/min, respectively.

GC analysis of FA composition. The composition of FFA were analyzed by gas chromatography after converted into their corresponding methyl esters (FAME). Fatty acids were converted to FAME by heating with 20% BF_3/methanol at 60^{o}C. The FFA composition was analyzed by a China Chromatography model 8700F (Taipei, Taiwan) gas liquid chromatograph equipped with a FID. The column used was SP-2330 (30 x 0.25 mm i.d; Supelco, Bellefonte, PA). The temperature of the injector and detector were set at 250 and 260^{o}C, respectively. The column was held at 160^{o}C for 2 min and then increased to 235 ^{o}C at a constant rate of 15^{o}C /min, then kept for 8 min. The split ratio was 1:50. One microlitre of sample was injected. The peaks were identified using authentic standard samples.

RESULTS AND DISCUSSION

The most commonly used alkalis in base catalyzed alcoholysis are NaOH, KOH, carbonates and corresponding sodium and potassium alcoxides. Alkali-catalyzed transeterification is much faster than acid-catalyzed transesterification and is used in the commercial production of biodiesel. Even at ambient temperature, the alkali-catalyzed reaction proceeds rapidly usually reaching 95% conversion in 1~2 h. On the other hand, the acid-catalyzed reaction commonly requires temperatures above 100°C [9], and reaction times of 3-48 h have been reported, except when reactions were conducted under high temperature and pressure [10,11]. However, for alkali-catalyzed transesterification, the starting materials (oil or fats) must be dry and free of FFA. Ma et al. [12] suggested that the FFA content of the refined oil should be as low as possible (below 0.5%), and Fuege and Grose [13] also stressed the importance of oils being dry (< 0.06%) and free of FFA. Freedman et al. [14] reported that ester yields were significantly reduced if the reactants did not meet these requirements. The presence of minor amount of FFA and moisture in the reaction mixture produces soap, which lowers the yield of esters and renders the separation of ester and glycerol as well as the water washing difficult. Moreover, FFA consumes the catalyst and reduced catalyst efficiency. Therefore base-catalyzed transesterification require highly refined oils in order to get efficient transesterification and not suitable for oils and fats with high FFA content, like rice bran oil.

Acids used for transesterification include sulphuric acid, phosphoric acid, hydrochloric acid, and organic sulfonic acids. Although transeterification by acid catalysis is much slower than that by alkali catalysis, acid-catalyzed transerification is more suitable for oils and fats that have relatively high FFA contents and more water [7, 14]. It has been reported that acid-catalyzed transesterification can be used when the starting materials are low-grade fats or have a high FFA content [15, 16]. Thus, acid-catalyzed transerification is more suitable to produce biodiesel from crude rice bran oil.

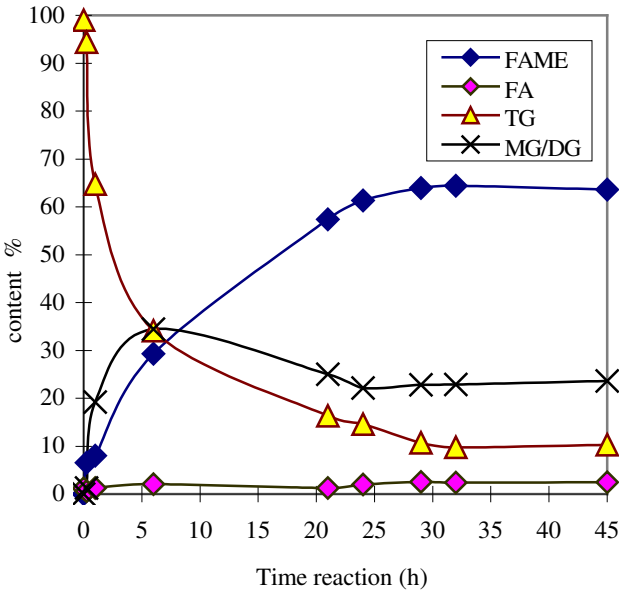

FIG. 1. The product composition of acid-catalyzed methanolysis of refined soybean oil. Reaction conditions: Molar ratio of methanol/oil : 20:1, Catalyst: 10% Methanolic HCl, Temperature: 70±2°C.

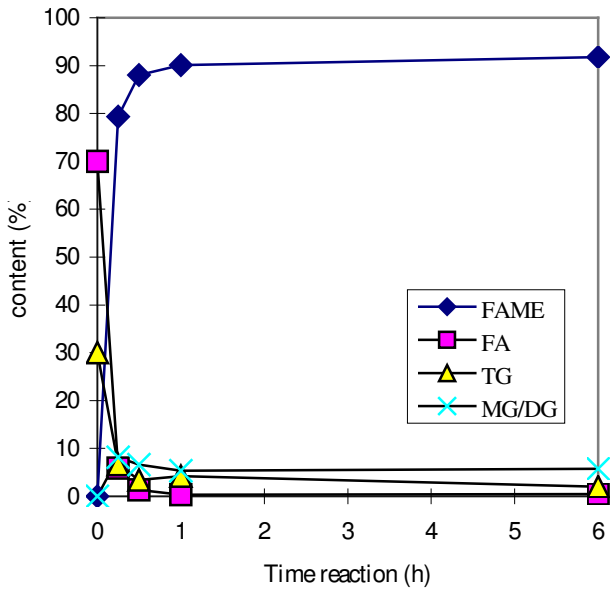

FIG. 2. The product composition of acid-catalyzed methanolysis of high FFA (60%) crude rice bran oil. Reaction conditions: Molar ratio of methanol/oil : 20:1, Catalyst: 10% Methanolic HCl, Temperature: 70±2°C.

Figure 1 shows the acid-catalyze transesterification of refined (>99%TG) soybean oil using molar ratio of methanol to oil 20:1 with 10%HCl (weight percent of oil). The results show, FAME content could not reached more than 65% after 45 h reaction time. On the other hand, experiments carried out with low grade high FFA (~60%) rice bran oil (Fig. 2) shows high conversion with FAME content of more than 90% with 6 h of reaction time. These results suggest that the acid-catalyzed methanolysis is more suitable for low grade high FFA oils like rice bran oil. In the initial stage, the reaction is fast and FAME content reaches to more than 85% with in 1 h reaction time. Further increase in reaction had not significant increase in the FAME content even after 6h of reaction. A number of researchers have worked with feedstocks that have elevated FFA to FAME using basic catalyst after removal of FFA. However, these methods are not economically feasible and generate large amount of soap stock.

To confirm that FFA is more susceptible to acid-catalyzed methanolysis than TG, we performed the methanolysis reaction with pure substrates such as refined TG and Free fatty acids from saponified soybean oil. During this study the influence o f water on degree of methanolysis of TG is also studied simultaneously by incorporating 5% water to the soybean oil. Figure 3 shows a qualitative picture of thin-layered chromatogram of methanolysis reaction product of 0, 1 and 6h reaction. The results show that metahnolysis of TG is slow and the product FAME is not observed even after 6 h reaction. However, minor partialglycerides were observed due to hydrolysis of TG after 6h of reaction (Fig. 3A). Addition of 5% water enhances both hydrolysis and methanolysis of TG to a small extent (Fig. 3B). Increase in reaction

time up to 23 h has no significant change in the product formation as shown in Fig. 4B. In contrast, the reaction with pure FA is fast and a complete conversion of (>99%) FA to their corresponding FAME is observed within 1h of reaction time. The results suggest that acid-catalyzed methanolysis of FA are faster than pure TG. The difference in the reaction rate between acid-catalyzed methanolysis of pure TG and FA is attributed to difference in their chemical structure. Fatty acids are simple in structure compared to bulky acylglycerides, which probably may hinder the methanolysis reaction.

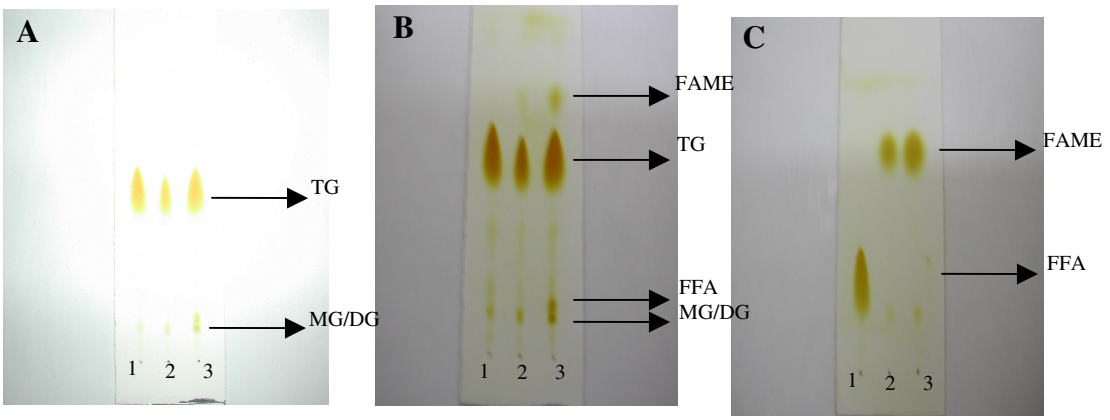

FIG. 3. Typical thin-layered chromatogram methanolysis of refined soybean TG (A), TG + 5%H_20 (B), and pure FFA from SBO (C), lane 1, 2 and 3 represents the reaction product of 0, 1 and 6h respectively.

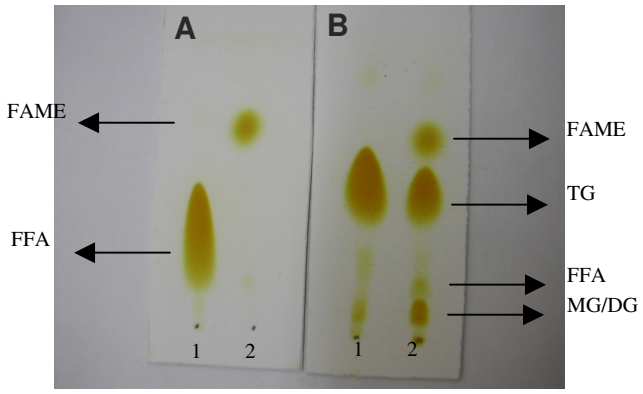

FIG. 4. Typical thin-layered chromatogram methanolysis of Pure FFA from SBO (A), TG + 5%H_20 (B). Lane 1 and 2 represents the reaction product of 0 and 23 h respectively.

The quantitative analysis carried out by TLC-FID of acid-catalyzed methanolysis of pure substrates such as TG form rice ban oil (Isolated by column chromatography), TG plus 5% water and pure fatty acids of soybean oil are shown in Figures 5,6 and 7 respectively.

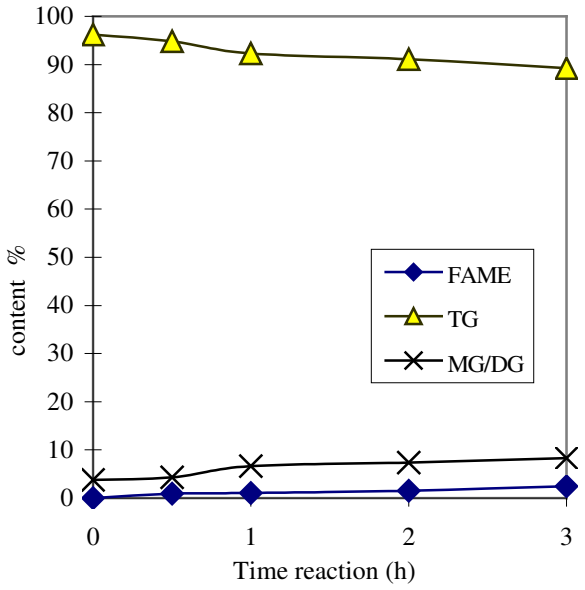

FIG. 5. Time-course of product composition of methanolysis of pure TG (from CRBO) +5% water Reaction conditions: Molar ratio of TG to methanol 1: 20, Catalyst: 10%methanolic HCl, Temperature 70±2°C.

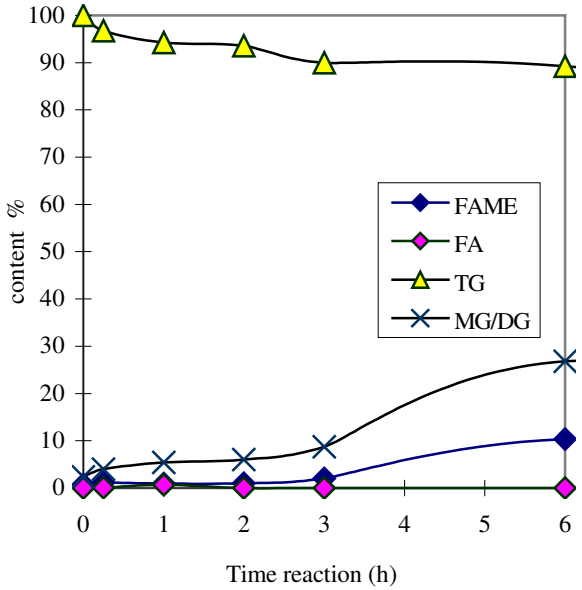

FIG. 6. Time-course of product composition of methanolysis of TG +5% water Reaction conditions: Molar ratio of TG to methanol 1: 20, Catalyst: 10%methanolic HCl, Temperature 70±2°C.

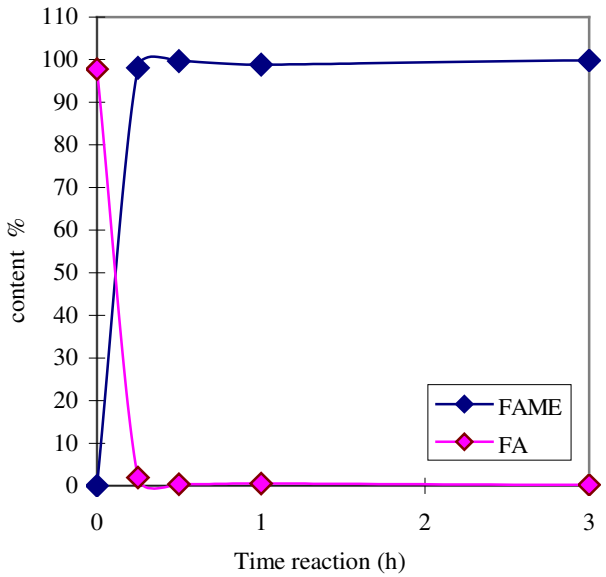

FIG. 7. Time-course of methanolysis of pure FFA from saponified SBO. Reaction conditions: Molar ratio of FFA to methanol 1: 20, Catalyst: 10%methanolic HCl, Temperature 70±2°C.

The results shows that reaction with pure FA is faster and about 99% of FA were converted to their corresponding FAME in less than 1h. On the other hand, the reaction with pure TG is quite slow and FAME content reached to 2% after 3 h of reaction. However, addition of 5% water increases the hydrolysis

of TG resulting the formation of FA, DG and MG. The formation of FA increases the FAME content moderately 16% after 3 h. Increase in the reaction time had no effect in FAME content. These results indicate that acid-catalyzed methanolysis of FA are faster than pure TG and these observations were in accordance as reported by Yucel and Türkay.

Rate of methanolysis of pure FA from SBO is faster than methanolysis of pure TG (from CRBO) or pure TG+5%H_2O. This is most probably due to different in the mechanism of esterification and transesterification. Methanolysis of free fatty acids proceed via simple esterification while TG procees via transesterification which consists a number of consecutive, reversible reaction [12, 14]. The triglyceride is converted stepwise to diglyceride, monoglyceride and finally glycerol. A mole FA ester is liberated at each step. The reactions are reversible, although the equilibrium lies towards the production of fatty acid esters and glycerol.

Esterification of Pure Fatty Acid from different Oil

In order to study the effect of variation of fatty acid composition on degree of methanolysis, two fatty acid mixtures form obtained from saponifined soybean oil and rice bran oil were investigated. Figure 8 shows the gas-chromatogram of composition of purified fatty acid from soybean oil and crude rice bran oil. The chromatograms show that oleic and palmitic acids are two major FA in both soybean oil and rice bran oil. Fatty acids with identical chain lengths were found in both oils samples with small variation in their percentage composition.

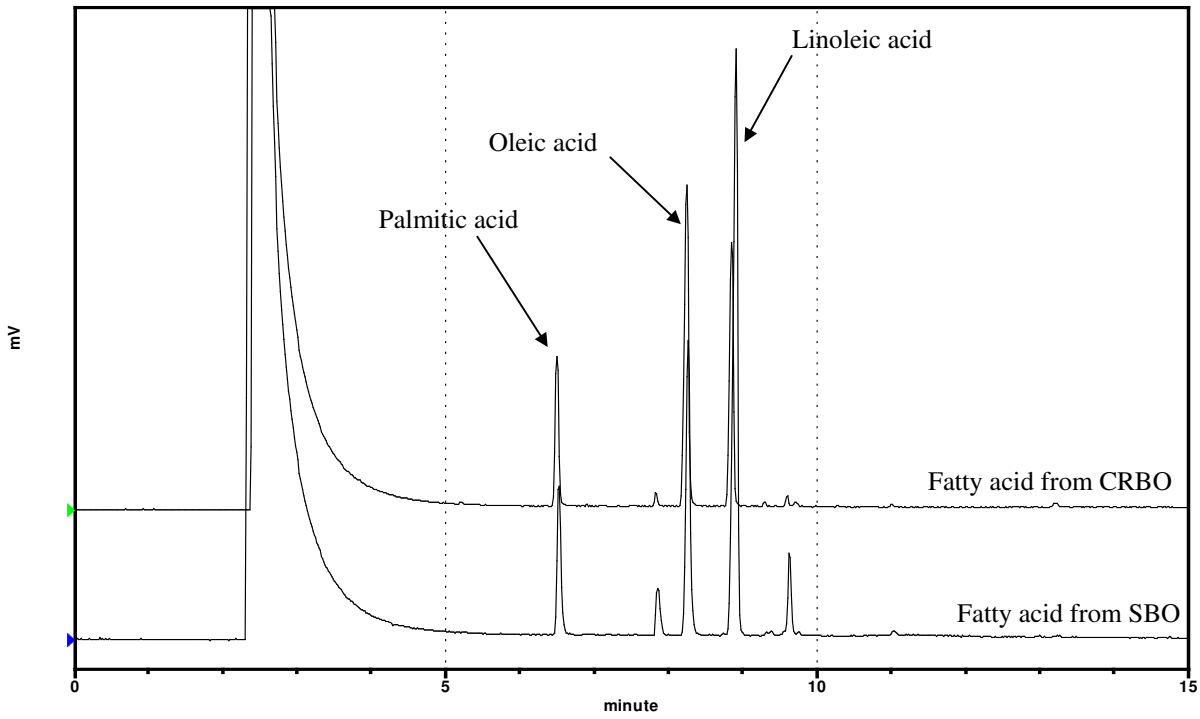

FIG. 8. Gas chromatogram of fatty acid composition of crude rice bran oil and soybean oil.

TABLE 1 Fatty acid composition (area %) of crude rice bran oil and soybean oil.

Oils	FAA composition (area %)						
	C14:0 Myristic acid	C16:0 Palmitic acid	C18:0 Stearic acid	C18:1 Oleic acid	C18:2 Linoleic acid	C18:3 Linolenic acid	C20:0 Arachidic acid
Rice bran oil	0.3366	17.2096	1.7112	45.7510	33.4208	0.3645	1.2063
Soybean oil	-	4.3401	11.3665	23.9698	53.8682	-	6.4554

Figure 9. Shows the time-course of formation of FAME during acid-catalyzed methanolysis of fatty acids obtained from saponified soybean oil and rice bran oil. The results shows that fatty acids from different sources show similar conversions and change in the FA composition has no effect on rate of methanolysis. More than 99% of FA were converted to their corresponding FAME with 20 min of reaction at temperature of boiling point of methanol.

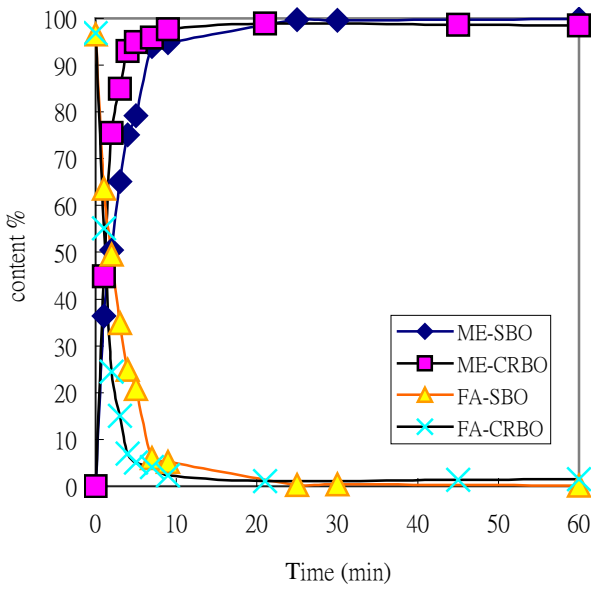

Fig. 9. Time-course of formation of FAME during acid-catalyzed methanolysis of fatty acids from SBO and RBO. Reaction conditions: Molar ratio of FA to methanol : 1: 20, catalyst =10%methanolic HCl; Temperature 70±2°C

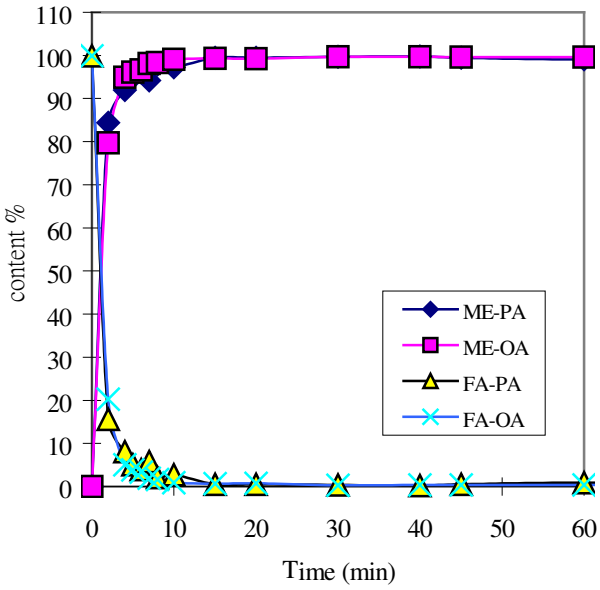

FIG. 10. Time-Course of formation of FAME during esterification of PA and OA with methanol. Reaction conditions: Molar ratio of FA to methanol: 1: 20, Catalyst 10%methanolic HCl, Temperature: 70±2°C.

Effect of chain length and unsaturation on esterification of FA with methanol

FA such as palmitic acid and oleic acid were selected to study the effect of chain length and unsaturation on rate of esterification of FA with methanol. Palmitic acid ($C_{16:0}$) is a long chain saturated fatty acid containing 16 carbon atoms with no double while oleic acid ($C_{18:1}$) is a long chain monounsaturated FA containing 18 carbon atoms and one double bond between carbon atoms 8 and 9. Another difference

between these two FA is that PA is a solid at room temperature while OA is a liquid. Acid-catalyzed esterification of these FA with methanol were conducted at 70^{o}C after melting the PA. About 99.1% OA and 97 % of PA were transformed to their corresponding FAME with in 10 min of reaction. The results suggest that both these FA are equally reactive irrespective of difference in their chemical structures. The time course of formation of FAME is shown in Fig. 10.

CONCLUSION

The objective of this study was to conduct a systematic studies of transesterification of low grade high FFA rice bran oil to establish optimal reaction condition. The variables of substrate that affecting ester formation were investigated to determine the best strategy for producing biodiesel. The following conclusions can be drawn from the acid-catalyzed biodiesel production study.

1. The amount of free fatty acid in oil can have a significant effect on the transesterification reaction.
2. The rate reaction of fatty acid to methyl ester faster than rate reaction of triglycerides.
3. Effect of chain length and unsaturation of fatty acid on rate of esterification of fatty acid with methanol are equally reactive irrespective of difference in their chemical structures.
4. Fatty acids from different sources given similar conversions and change in the fatty acids composition has no effect on rate of methanolysis.
5. Acid-catalyzed transesterification is suitable for low grade high FFA oils like rice bran oil than alkaline-catalyzed.

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