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in situ Transesterification of Ground *Pongamia pinnata* Kernel under Sub/Supercritical Methanol Conditions

S. PANPRANEECHAROEN[1], V. PUNSUVON[1,*] and Y.H. JU[2]

[1]Department of Chemistry, Faculty of Science, Kasetsart University, Bangkok, Thailand
[2]Department of Chemical Engineering, National Taiwan University of Science and Technology, 43 Keelung Road, Section 4, Taipei 106-07, Taiwan

*Corresponding author: E-mail: fscivit@ku.ac.th

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in situ Transesterification of ground *Pongamia pinnata* kernel under sub/supercritical conditions was studied. The effects of reaction temperature (200-250 °C), methanol-to-solid ratio (5:1-13:1 mL/g), reaction time (0-120 min) and stirring were investigated. A fatty acid methyl ester yield of 88.04 % was obtained under the following conditions:methanol-to-solid ratio 13:1 mL/g, reactant loading 80 %, with the reaction at 250 °C for 1 h with stirring. This process not only eliminated the oil extraction and esterification step for reducing the high free fatty acid content in *P. pinnata* kernel (up to 14 %) but also did not use the conventional acid/base catalyst for transesterification step to produce biodiesel.

Keywords: *Pongamia pinnata*, Subcritical methanol, Supercritical methanol, Biodiesel, Transesterification.

INTRODUCTION

P. pinnata oil seed is a potential low cost feedstock and trees of this species can be commonly found in many countries including India, Australia, Malaysia and Thailand. *P. pinnata* seeds, containing 20 to 40 % crude lipid, are found inside pods borne by the tree. The seeds have previously been investigated and utilized in biodiesel synthesis [1]. Most of these investigations focused on conventional biodiesel production methods, utilizing homogeneous catalysts in transesterification such as NaOH, KOH and $NaOCH_3$ [2,3]. Although homogeneous catalysts provide much faster reaction rates than heterogeneous catalysts, it is difficult to separate the catalyst from the reaction mixture resulting in large amounts of waste water being produced. Due to concerns of catalyst recovery, heterogeneous catalysts have gained renewed interest. Several heterogeneous catalysts for use in transesterification reactions of triglycerides have been developed and/or explored including alkali metal oxides, alkaline earth metal oxides, transition metal oxides, mixed metal oxides and derivatives, ion exchange resins, sulfated oxide, ash from waste materials and enzyme [4]. However, the main limitation of heterogeneous catalysts is their relatively slower rate in the transesterification reaction due to mass transfer limitations [5]. Another drawback is the availability and cost of the materials involved for the production and use of a heterogeneous catalyst.

One way to overcome the use of a catalyst is to produce biodiesel under subcritical or supercritical conditions of the oil/lipid and methanol mixture. Catalysts may be avoided in the supercritical methanol process due to the improved miscibility of the lipids in supercritical methanol. The reaction is also driven forward at a faster rate due to the high temperatures (270 to 350 °C) and pressures (20 to 42 MPa) involved, allowing the reaction to reach completion in 4 to 40 min [6]. As an alternative, reactions under subcritical methanol conditions have also been explored in the hope of lower process severity. The temperature and pressure of subcritical methanol are lower than the critical values of methanol (239 °C for temperature and 8.08 MPa for pressure) [7]. The use of subcritical methanol in the transesterification reaction can also achieve a high yield and conversion at less severe operating conditions but at the expense of a higher methanol requirement and a longer reaction time [8]. To take advantage of the high amount of solvent used, the *in situ* transesterification process under sub/supercritical solvent conditions may be adopted. The *in situ* transesterification process involves the simultaneous extraction and reaction of a lipid in seeds or kernels, which may result in a shorter overall processing time.

In present work, *in situ* transesterification was carried out under sub/supercritical conditions (below 239 °C and above 239 °C of temperature). The effects of the reaction temperature, methanol-to-solid ratio, reaction time and stirring on the fatty

acid methyl ester (FAME) yield were investigated. In addition, the obtained results from this process would be compared the advantage and disadvantage with the acid catalyst conventional process.

EXPERIMENTAL

P. pinnata pods were obtained from Ranong province (Thailand). Pods obtained were sun dried and kept at room temperature prior to use. Standards of fatty acid (FA), acylglyderides (AG) such as monoolein, diolein and triolein and fatty acid methyl ester (FAME) were obtained from Supelco (Bellefonte, PA). All the solvents and reagents used were either of high performance liquid chromatography or analytical reagent grade, obtained from commercial sources.

Sample preparation and characterization: *P. pinnata* seeds obtained from pods were sun dried and manually deshelled to obtain the kernels. Then, the *P. pinnata* kernels were ground using a food blender and sieved to a particle size of < 0.71 mm. The ground kernels were stored in a closed polyethylene bottle and kept at -20 °C for later use.

The moisture content of ground *P. pinnata* kernels was measured by placing 1 g of sample in a pre-dried glass tube (three replicates). The sample loaded tubes were placed into a freeze drier (Labconco Free Zone 2.5 L Model: 7670520, Kansas City, MO) operated at -44 °C and 0.110 bar for 24 h. The moisture content of each seed sample was calculated based on the difference in weight of the seed sample before and after freeze-drying.

To determine the amount of extractable crude lipid, ground kernel (5 g) was packed into a cellulose thimble and plugged with cotton. The thimble was then loaded into a Soxhlet extractor and extracted for 8 h using 150 mL of *n*-hexane as the solvent. At least two replicates were done for lipid extraction.

The free fatty acid content present in the crude lipid was determined using the titrimetric method following AOCS official methods (Method Ca 5a-40). In brief, 1 g of sample was accurately weighed in an Erlenmeyer flask and dissolved in hot and neutralized ethanol. Then, about 2-3 drops of phenolphthalein were added as indicator and the sample was titrated with 0.1 N KOH in ethanol solution.

The amount of unsaponifiable matter in the lipid sample was analyzed using AOCS official methods (Method 6b-53). The saponified lipids obtained after determination of the unsaponifiable matter were collected and acidified to pH 2 with concentrated sulfuric acid and continually stirred at 60 °C until the solution was clear. The solution was then allowed to settle until two phases formed. The upper layer consisting of fatty acids (FA) was extracted with hexane and recovered to determine the total available fatty acid convertible to FAME.

Dewaxing and degumming of the extracted lipids was carried out by using 1 g of crude lipid. The sample was dissolved in 6 mL acetone and heated in a 60 °C water bath until the solution was clear. The solution was allowed to cool to room temperature and then stored at 4 °C for 3 h. The solution was then filtered (0.22 mm pore size) to remove the precipitated wax and gum. The procedure was repeated twice.

***in situ* Transesterification:** Ground *P. pinnata*kernel (10.7-25.0 g) and methanol (125.0-139.3 mL) were loaded into a glass chamber (190 mL) and placed in a high-pressure reactor (290 mL) provided by HC Scientific and Instrument Co. (Taipei, Taiwan). The ground kernel was mixed and suspended in methanol to a total volume of 150 mL (about 80 % of the glass chamber). A detailed reactor description and configuration has been described elsewhere [9]. The reactor was equipped with an external electric heater and a magnetic stirrer. The temperature in the reactor was measured using a thermocouple and was controlled to within a range of ± 2 °C using a proportional-integral-differential controller. The reaction was carried out at the desired temperature (200-250 °C). The heating rate of the reactor was kept at 5 °C/min with a heating period of 40-45 min. The reaction time in this study was started as the desired reaction temperature was reached under the constant pressure (105-110 bar). After a predetermined reaction time (30-120 min), the reactor was rapidly cooled down to room temperature using an iced water bath. The pressure inside the reactor was released and the product in the reactor was collected at room temperature. The reaction product was vacuum filtered using a Buchner funnel with Advantec No. 2 filter paper (8 mm pore size) to separate the spent solid from the reaction product. The solid residues were each washed three times, using 30 mL methanol to recover the FAME produced. Methanol in the filtrate was removed and recovered using a rotary evaporator (BUCHI Labortechnik AG, Flawil, Switzerland) operated at 40 °C and 13.3 kPa. The concentrated crude product containing FAME was transferred to a separation funnel and was extracted four times,each using 25 mL *n*-hexane to recover the FAME produced. The recovered FAME was washed three times, each using 25 mL 5 % NaCl solution in a separate separation funnel to remove non-lipid products co-extracted by methanol. In between washings, the mixture was allowed to settle and clarify, resulting in a FAME-rich hexane layer. The upper hexane phase was recovered and hexane was removed using a rotary evaporator under vacuum. The final product was weighed and analyzed for its FAME content.

Gas chromatography analysis: A 20 mg aliquot of the lipid sample was dissolved in 1 mL of ethyl acetate and filtered through a 0.2 mm polytetrafluoroethylene hydrophobic membrane to remove moisture. From this prepared solution, a 1.5 µL sample was injected into high temperature gas chromatography for analysis. An external calibration curve was generated using 0.2-20 mg of a pure standard (methyl oleate) dissolved in ethyl acetate. Qualitative and quantitative analyses of FAME and unreacted free fatty acid in each sample were performed using a Shimadzu GC2010 chromatographer (Kyoto, Japan) equipped with a split-injector and a flame ionization detector. Separation was carried out on a ZB-5HT (5 % phenyl)-methylpolysiloxane non-polar column (15 m × 0.32 mm i.d., 0.1 mm film thickness) (Zebron, Phenomenex, Torrence, CA, USA). Detailed analysis conditions were adopted from the work of Go *et al.* [9]. Data analyses were carried out using the software "GC Solution version 2.3", Shimadzu (Kyoto, Japan).

RESULTS AND DISCUSSION

The moisture content of the ground *P. pinnata* kernels was 8.66 ± 0.12 %. The crude hexane extractable lipid content

of dry ground *P. pinnata* kernels was 32.08 ± 0.04 %.The lipid content in oil kernels and its quality largely depend on the origin of the kernels and storage. The lipid content of *P. pinnata* was reported to range from 9.5 to 46 % with an average lipid content of 31.40 % [10]. Most of the extracted lipids obtained 1.59 ± 0.01 of unsaponifiable. Further analysis showed that 85.41 ± 0.02 of the extracted lipid could be converted to fatty acid. The major fatty acid composition in *P. pinnata* is oleic acid that could be converted to FAME and obtained the theoretical maximum overall FAME yield is 89.68 ± 0.02 %. The crude lipid extracted from *P. pinnata* kernels contained 14.39 ± 0.14 % of free fatty acid and 2.16 ± 0.06 % of wax and gums. The acylglycerides were obtained from 100 % of crude lipid minus by free fatty acid, wax and gums and unsaponifiable values. The characteristics of the lipids extracted from *P. pinnata* kernels are summarized in Table-1.

TABLE-1
MASS FRACTION (%) OF LIPID COMPONENTS OF DRY GROUND *P. pinnata* KERNELS

Lipid component	Amount (%)
Free fatty acid (%)	14.39 ± 0.14
Wax and gum (%)	2.16 ± 0.06
Unsaponifiables (%)	1.59 ± 0.01
Acylglycerides	~ 81.86
Total fatty acid content (%)	85.41 ± 0.02
Theoretical FAME yield (%)	89.68 ± 0.02

Effect of reaction temperature: It was reported that an increase in temperature reduced the polarity of methanol due to breaking down of hydrogen bonding, which resulted in higher solubility of oil in methanol. Complete solubility occurred as the temperature approached the critical temperature of the mixture [11]. Zhou *et al.* [12] presented the density of methanol as a function of temperature. Methanol density decreases with increasing temperature, which may explain the change of phase holdup of methanol to oil from the top to the bottom in the reactor. Temperature not only affects the reaction rate but also the equilibrium of the reaction. In this study, the FAME yield was calculated base of extractable lipid from kernel. Fig. 1 shows the time courses of FAME yield at different temperatures under the same pressure (105-110 bar). A FAME yield of 83.07 ± 1.46 % was obtained at 250 °C.

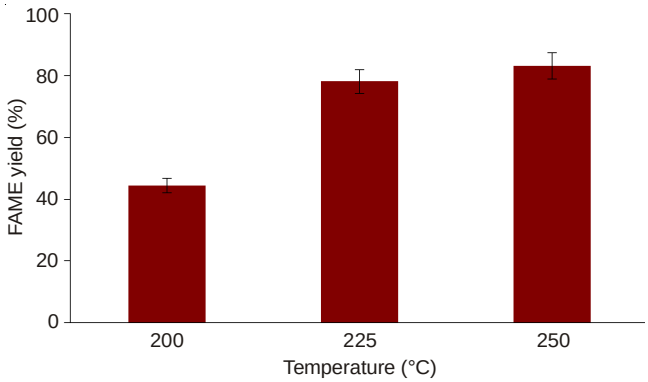

Fig. 1. Effects of reaction temperature on FAME yield. Reactions were carried out at a fixed reactor loading (~80 %) and 9:1 mL/g methanol-to-solid ratio for 120 min

Effect of methanol to solid ratio: Fig. 2 shows that the FAME yield increased from 72.12 ± 0.03 % to 83.07 ± 1.46 % when the methanol-to-solid ratio (mL/g) was increased from 5 to 9.Above that, the FAME yield increased only slightly from 83.07 ± 1.46 % to 86.30 ± 1.66 % when the methanol-to-solid ratio was increased from 9 to 13. Methanol not only was used as the reactant to produce biodiesel but also as the solvent for extracting lipid from the *P. pinnata* kernels. In this study, based on dry seed, a FAME yield of 29.77 ± 0.97 % can be obtained by a methanol-to-solid ratio of 13:1 mL/g for a reaction time of 2 h. However, although increasing the methanol amount results in increasing FAME yield, this adds additional cost to recover the excess methanol. In this study, the methanol-to-solid ratio used was limited to 13 mL/g in the *in situ* transesterification of *P. pinnata* kernels.

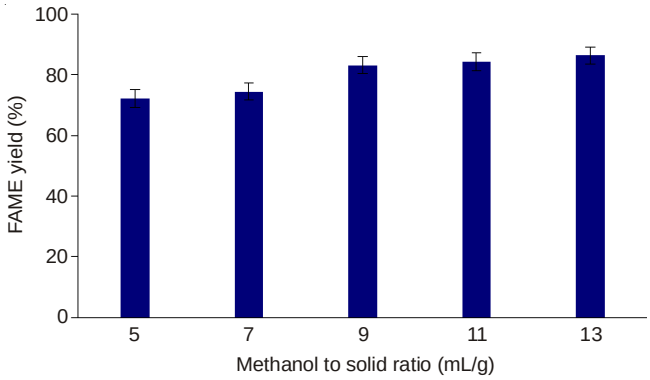

Fig. 2. Effects of methanol-to-solid ratio on FAME yield. Reactions were carried out at a fixed reactor loading (~80 %) for 120 min at 250 °C

Effect of reaction time: The effect of reaction time on the FAME yield was investigated and the results are shown in Fig. 3. Two methanol-to-solid ratios (9:1 and 13:1 mL/g) were employed at 250 °C and a fixed reactor loading of ~80 % was used. A FAME yield of about 10 % was observed at time zero due to the reaction that occurred during the 45 min of heating required to reach 250 °C. The FAME yield increased rapidly to ~75 % after 30 min and then increased slowly with time to ~85 % at 120 min.

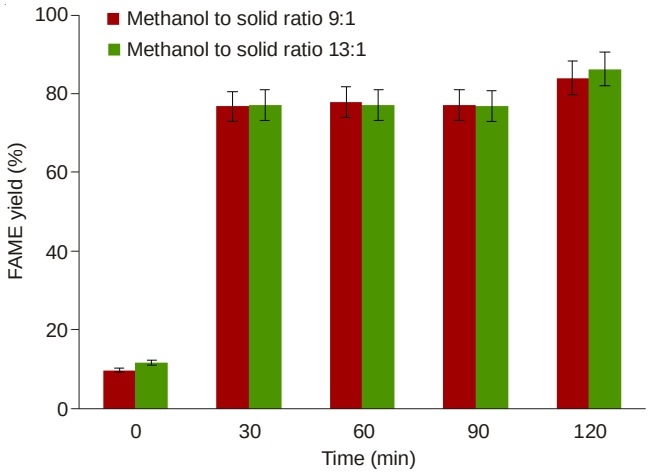

Fig. 3. Effects of reaction time on FAME yield at different methanol-to-solid ratios. Reactions were carried out at a fixed reactor loading (~80 %) at 250 °C

Effect of stirring: A magnetic stirrer was used to mix the solutions in this study. The stirrer reading was set at 600 rpm. Figs. 4 and 5 show the effect of stirring on the FAME yield when the methanol-to-solid ratio used was 9:1 and 13 mL/g, respectively. At a methanol-to-solid ratio of 9:1 mL/g, with the help of stirring, the FAME yield reached 82.10 % at 30 min which is almost the same as that obtained at 120 min without stirring (Fig. 4), while at a methanol-to-solid ratio of 13:1 mL/g, with the help of stirring, the FAME yield reached 88.04 % at 60 min which is higher than that obtained at 120 min without stirring (Fig. 5). As shown in Table 1, the theoretical maximum FAME yield that can be obtained is 89.68 %; so the FAME yield of 88.04 % that was obtained in this study means that the conversion achieved was 98.17 %. However, the addition of a high amount of excess methanol after optimum conditions are reached has a tendency to decrease the FAME yield because the amount of glycerol increased and remained in solution causing transesterification as a reversible reaction [13]. Thus, the effect of stirring can efficiently increase the contact between *P. pinnata* kernels and methanol during the reaction.

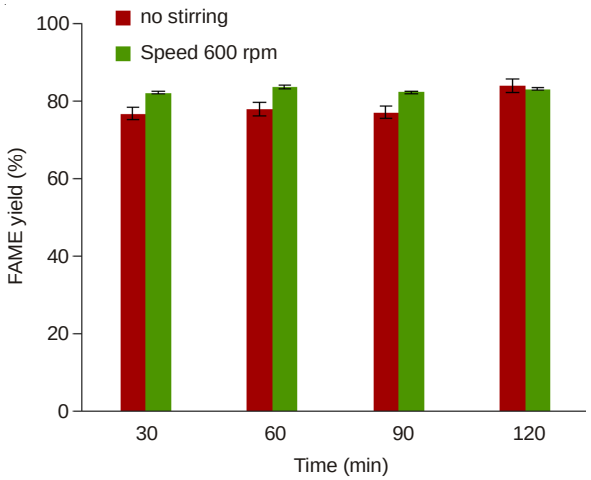

Fig. 4. Effects of stirring on FAME yield. Reactions were carried out at a fixed reactor loading (~80 %) and 9:1 mL/g methanol-to-solid ratio at 250 °C

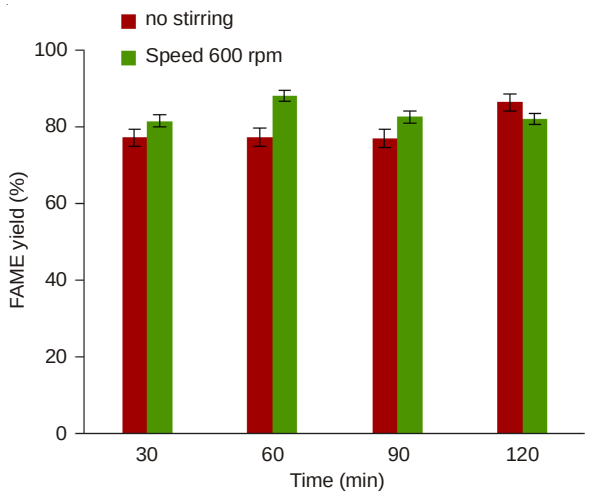

Fig. 5. Effects of stirring on FAME yield. Reactions were carried out at a fixed reactor loading (~80 %) and 13:1 mL/g methanol-to-solid ratio at 25 °C

Comparison with conventional conditions: The optimum conditions of *P. pinnata* oil in conventional transesterification reported in the literature were found to be comparable to those of the *in situ* sub/supercritical methanol conditions used in this study. Table-2 summarizes the results of studies on biodiesel production using *P. pinnata* kernel/oil. It can be observed that a single-step liquid acid catalyst could be used for biodiesel production from *P. pinnata* oil and the yield was as high as 89.8 % after being prolonged to 5 h.

TABLE-2
COMPARISON OF CONVENTIONAL CONDITION AND *in situ* SUB/SUPERCRITICAL METHANOL CONDITIONS FOR *P. pinnata* TRANSESTERIFICATION

Method	Catalyst	Conditions	FAME yield (%)	Ref.
Single-step liquid acid catalyst	2 wt. % H_2SO_4	6:1 methanol-to-oil molar ratio, 65 °C, 5 h	89.8	[14]
in situ Sub/ supercritical methanol	–	13:1 methanol-to-solid ratio (mL/g), 250 °C, 1 h	88.04	Present study

However, the biodiesel product using homogeneous catalysts and conventional transesterification conditions must be rinsed to remove the leftover catalyst using warm water that causes large amounts of waste water to be released. Moreover, all the conventional transesterification conditions had to be applied for a prolonged time to extract the lipid before use in the reported studies.

In this study, the *in situ* sub/supercritical methanol method was used to produce a high FAME yield under high temperature and pressure. Although the transesterification sub/supercritical method must be carried out under higher conditions than those needed in the conventional method, the sub/supercritical process not only eliminates the use of catalyst and skips the extraction lipid step but also involves a shorter reaction time to achieve a high FAME yield. The optimum conditions under sub/supercritical methanol conditions used 13:1 mL/g of methanol-to-solid ratio and fixed 80 % of the loading in the glass chamber at 250 °C for 1 h of reaction time with stirring and obtained a high FAME yield (88.04 %) of *P. pinnata* biodiesel product.

Conclusion

In this study, the *in situ* transesterification of *P. pinnata* kernels with high free fatty acid content was used for biodiesel production under sub/supercritical methanol conditions. The process used in this study eliminated not only the need for prior oil extraction from seeds but also no catalyst was required. The parameters affecting the FAME yield during the transesterification reaction were the reaction temperature, methanol-to-solid ratio, reaction time and stirring. A high FAME yield (88.04 %) and conversion (98.17 %) were obtained under the following conditions: methanol-to-solid ratio13:1 mL/g, reaction temperature 250 °C, reaction time 1 h, 80 % loading and constant stirring at 600 rpm. Lastly, this newly developed process provides an alternative route in *in situ* biodiesel production that can use feedstock with high moisture and free fatty acid content.

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