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APPLICATION OF RESPONSE SURFACE METHODOLOGY FOR OPTIMIZING TRANSESTERIFICATION OF COCONUT OIL

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ABSTRACT

It is considered in this work the use of coconut oil for the synthesis of renewable and environmentally friendly biodiesel as an alternative to conventional diesel fuel. Response surface methodology (RSM) with central composite design (CCD) was applied for the determination of optimum condition. The results showed that an optimum biodiesel yield of 93.03% could be obtained under the following reaction conditions: methanol content of 23.67% (by weight with respect to the oil), catalyst concentration of 0.5% (by weight with respect to the oil), and the reaction time of 120 minutes. These obtained results demonstrated the potential of coconut oil as good feedstock for biodiesel production in Mekong Delta.

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1 INTRODUCTION

Biodiesel is defined as the mono-alkyl esters of fatty acids derived from vegetable oils or animal fats (Demirbas, 2007). In simple terms, biodiesel is the product obtained when a vegetable oil or animal fat reacts with an alcohol to produce fatty acid alkyl esters. A catalyst such as sodium or potassium hydroxide is required. Glycerol is produced as a coproduct.

Biodiesel has many advantages compared to diesel fuels. It has a higher cetane number than diesel fuel, and contains no aromatics, almost no sulfur and 10–12% oxygen by weight. Biodiesel-fueled engines produce less carbon mono oxide (CO),

hydrocarbon (HC) and particulate matter (PM) than petroleum diesel-fueled engines (Lay, 2009). Biodiesel improves the lubricity, which results in longer engine component life. The flash point of biodiesel is higher than that of diesel fuel. Although the flash point does not directly affect the combustion, it makes biodiesel safer regarding the storage and transport.

However, there are some drawbacks of biodiesel. Biodiesel has a higher cost than diesel fuel, mainly due to the cost of virgin vegetable oils. The cold flow properties of biodiesel are poor and these properties may cause problems at the start of the engine and limit the use of biodiesel in cold climates. Another drawback of biodiesel is a tendency

to oxidize by air, especially at high temperatures. The heating value of biodiesel is approximately 8% lower than that of diesel fuel. When diesel engine is fueled with biodiesel, there is an increase in NO_x emissions compared to petroleum diesel-fueled engines due to the combustion and some fuel properties. However, in some studies, a reduction can be seen in NO_x emissions.

Biodiesel was produced by transesterification, which was affected by many factors, such as methanol content, reaction time and catalyst amount. Most of the studies on the transesterification (the conventional approach) changed one separate factor at a time (analysis of the effect of one particular reaction condition by keeping all the other ones constant). However, reaction system influenced simultaneously by more than one factor can be poorly understood with this approach (the optimum conditions obtained depend on the starting point). In recent years, the interest in use of the response surface methodology (RSM) for optimizing various processes has been increasing considerably including the transesterification reaction of vegetable oils. Yuan *et al.* (2008) optimized conversion of waste rapeseed oil with high free fatty acid (FFA) to biodiesel using RSM. In another study, Jeong and Park evaluated the application of RSM to optimize the process variables during transesterification of castor oil. Similarly, the optimization of transesterification variables for biodiesel production from *Moringa oleifera* oil using RSM has also been reported by Rashid *et al.* (2011)

Therefore, to understand the relationship between the factors and conversion to biodiesel, and to determine the optimum conditions for production of biodiesel from coconut oil, the experiments were performed according to central composite design (CCD) and RSM.

2 MATERIALS AND METHODS

2.1 Materials

Coconut oil was collected from Chemical Scientific Technological Joint Stock Company, Can Tho City Branch.

All used chemicals were analytical grade.

2.2 Methods

2.2.1 Base-catalyzed transesterification

Coconut oil was converted to methyl esters using a transesterification process in methanol by the use of an alkaline catalyst of KOH, the procedure details have been described as follows: A previously prepared solution of KOH in methanol was poured into the mixture of coconut oil and acetone (10 %

w/w with respect to oil phase) (Maeda *et al.*, 2011) while continually stirring. At first the mixture became cloudy, but soon separated into two layers. The glycerol layer felt down to the bottom, and the methyl ester (biodiesel) floated to the top. The system was set to stand for about an hour and the glycerol layer was then drained off. The methyl ester layer was washed with water until the pH became neutral. After washing, the final product was heated to 110°C for 10 minutes to remove moisture.

2.2.2 Fatty acid profile

Fatty acid methyl ester contents were analyzed by using a gas chromatography mass spectrometer (GC-MS), equipped with a TG-SQC GC column 15m × 0.25mm × 0.25µm (Thermo scientific). The carrier was helium gas with a flow rate of 1.2 mL/min. The following temperature program was employed: 60°C, heating at a rate of 10°C/min up to 260°C and holding at that temperature for 1.0 minute. The identification of fatty acid methyl esters and the corresponding relative amount were defined using the computer program installed in the GC-MS system.

2.2.3 Product yield

Product yield is defined as the weight percentage of the final product (transesterified and purified oil) relative to the weight of oil at the start (Eq. (1)). It, in fact, indicates the final results of the competition between the main reaction (transesterification) producing methyl esters and the side reactions (saponification) influencing the ester yield.

$$\text{Yield of methyl esters} = \frac{\text{weight of product}}{\text{weight of oil used in reaction}} \times 100 \quad (1)$$

2.2.4 Physicochemical properties

Physical properties of coconut oil and coconut oil biodiesel were analyzed such as viscosity measured with a Viscosity Measuring unit ViscoClock (Schott Instrument), according to the standard method of ASTM D44506. The acid value (AV), iodine value (IV), saponification value (SV) and peroxide value (PV) of coconut oil and coconut oil biodiesel were determined by volumetric titration.

2.2.5 NMR and Fourier Transform Infrared (FT-IR) Spectroscopy

The ¹H NMR data were recorded using a Bruker UltraShield 600 spectrometer operating at 600 MHz (Kenji Kanaori Lab. (Molecular Structural Chemistry) – Graduate School of Science and Technology, Kyoto Institute of Technology, Kyoto, Japan). FT-IR spectra were obtained on a Nicolet

6700 FT-IR spectrometer (Spectroscopy-Chromatography Lab., College of Natural Sciences, Can Tho University, Vietnam).

2.2.6 Experimental design

The range and level of the investigated variables are listed in Table 1.

Table 1: Factors and their levels of response surface design

Variable	Code	Unit	Level				
			-1,68 (-α)	-1	0	+1	+1,68 (+α)
Methanol content	X ₁	%	13.2	20	30	40	46.8
Catalyst loading	X ₂	%	0.16	0.5	1	1.5	1.84
Reaction time	X ₃	min	39.5	60	90	120	140.5

A CCD was applied with three design factors, namely, methanol content (X1), catalyst loading (X2) and reaction time (X3). The central values (zero level) selected for experimental design were: methanol content of 30%, catalyst loading of 1.0% (w/v) and reaction time of 120 minutes. This study was conducted in a total of 20 experiments (N = 2k + 2k + 6, where k = 3 is the number of independent variables) in accordance with a 23 complete factorial design plus six central points and six axial points (star points). The distance of the star points from the center point was provided by α = 2k/4, for three factors α=1.68. The experimental factors selected for optimization and their respective ranges were as follows: methanol content (13.2–46.8 wt.%), catalyst loading (0.16–1.84 wt.%) and reaction time (39.5–140.5 min). The Design Expert 6.0 software was used for regressive and graphical analyses of the data obtained. The maximum values of the yield were taken as the responses of the design experiments. Statistical analysis of the mod-

el was performed to evaluate the analysis of variance (ANOVA). Once the experiments were performed, the response variable (conversion to biodiesel) was fitted a second-order model in order to correlate the response variable to the independent variable. The general form of the second degree polynomial equation is as follows:

$$Y = b_0 + \sum_{i=1}^3 b_i X_i + \sum_{i=1}^3 b_{ii} X_i^2 + \sum_{i=1}^2 \sum_{j=i+1}^3 b_{ij} X_i X_j + \varepsilon \quad (2)$$

where i and j are the linear and quadratic coefficients, respectively; b is the regression coefficient; k is the number of optimized factors in the experiment and ε is the random error (Bezerra et al., 2008; Jeong et al., 2009)

3 RESULTS AND DISCUSSION

3.1 Key characteristics of biomass sources

The physical and chemical properties of coconut oil were analyzed and given in Table 2.

Table 2: The physicochemical properties and fatty acid composition of coconut oil

Items	Chemical structure	Value, %
8:0 (Caprylic)	CH ₃ (CH ₂) ₆ -COOH	7.25
10:0 (Capric)	CH ₃ (CH ₂) ₈ -COOH	6.38
12:0 (Lauric)	CH ₃ (CH ₂) ₁₀ -COOH	40.15
14:0 (Myristic)	CH ₃ (CH ₂) ₁₂ -COOH	20.4
16:0 (Palmitic)	CH ₃ (CH ₂) ₁₄ -COOH	11.01
18:0 (Stearic)	CH ₃ (CH ₂) ₁₆ -COOH	3.2
18:1 (Oleic)	CH ₃ (CH ₂) ₇ C=C(CH ₂) ₇ -COOH	9.99
Others		0.79
Saturated		89.22
Monounsaturated		9.99
APE		19.98
BAPE		0
Mean molecular weight		680.06
Kinematic viscosity at 40°C, mm ² /s		29.46
Acid value, mg KOH/g		0.82
Iodine value, g I ₂ /100g		11.02
Saponification value, mg KOH/g		246

APE = 2 × (C18:1 + C18:2 + C18:3): Allylic Position Equivalent. Calculated from Eq (4) in Ref. (Knothe et al., 2004)

BAPE = C18:2 + 2C18:3 : Bis-Allylic Position Equivalent. Calculated from Eq (6), according to Ref. (Knothe et al., 2002)

$$\Sigma\text{Sat} = \text{C8:0} + \text{C10:0} + \text{C11:0} + \text{C14:0} + \text{C16:0} + \text{C17:0} + \text{C18:0} + \text{C20:0}$$

$$\Sigma\text{Monounsaturat} = \text{C18:1} + \text{C20:1}$$

$$\Sigma\text{Polyunsaturat} = \text{C18:2} + \text{C18:3} + \text{C20:2} + \text{C20:3}$$

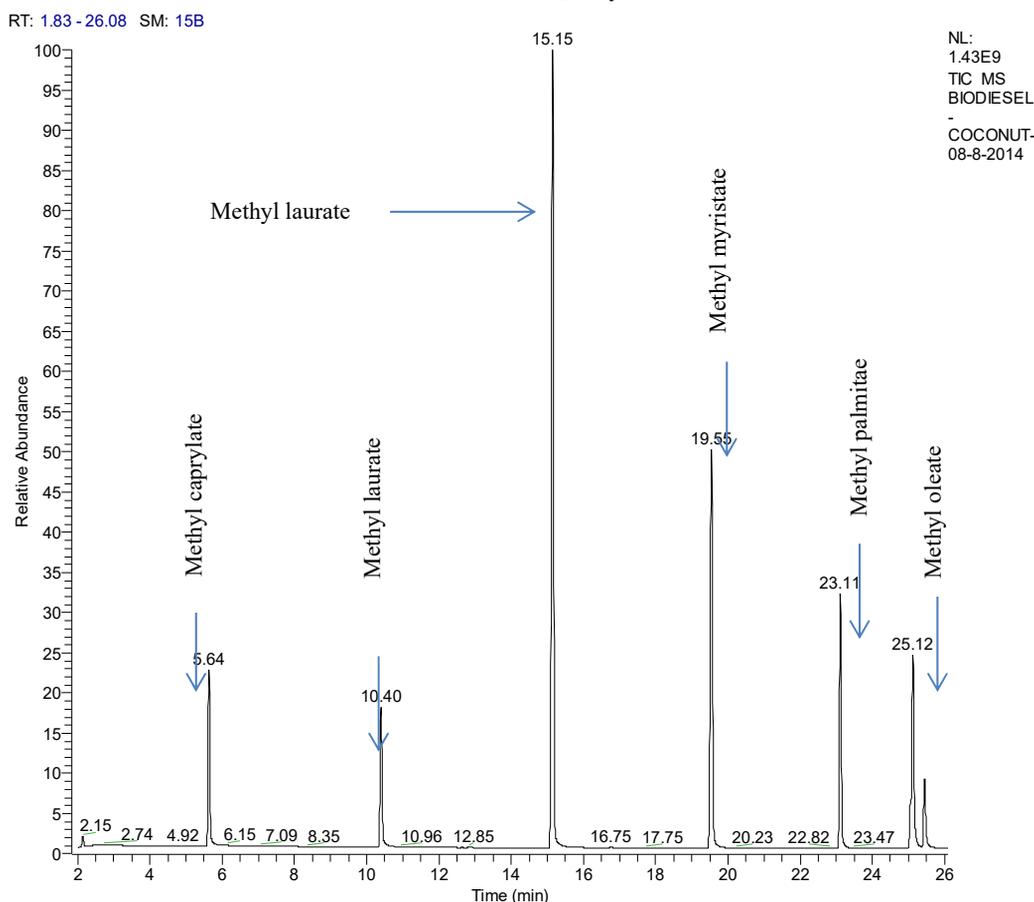


Fig. 1: Production of the reaction of coconut oil with methanol

Methyl laurate (C_{12:1}; 40.15 wt.%) and methyl myristate (C_{14:0}; 20.4 wt.%) were the major components of coconut oil according to the GC-MS analyses. Minor constituents included methyl caprylate (C_{8:0}; 7.25 wt.%), methyl caprate (C_{10:0}; 6.38 wt.%), methyl palmitate (C_{16:0}; 11.01 wt.%), methyl stearate (C_{18:0}; 3.2 wt.%), and methyl oleate (C_{18:1}; 9.99 wt.%). Saturated fatty acid methyl ester (FAME) comprised 89.22 wt.% of biodiesel, with others (0.79 wt.%) constituting the remaining content.

The fatty acid profile of coconut oil used in this work was summarized in Table 2. There are two main types of fatty acids that can be present in a triglyceride: saturated (C_{n:0}), monounsaturated (C_{n:1}). According to this composition, two parameters based on the type of fatty acids were defined as Allylic Position Equivalent (APE) and Bis-Allylic Position Equivalent (BAPE). These parameters were obtained from empirical Eq. (4) and Eq (6) in Ref. (Knothe *et al.*, 2002), taking into account the amount of monounsaturated and poly-

unsaturated fatty acids (wt.%) present in coconut oil:

$$\text{APE} = 2 \times (\text{C}_{18:1} + \text{C}_{18:2} + \text{C}_{18:3}) = 19.98 \text{ and}$$

$$\text{BAPE} = \text{C}_{18:2} + 2\text{C}_{18:3} = 0.$$

Stability of fatty compounds is influenced by factors such as the presence of air, heat, traces of metal, peroxides, light, or structural features of the compounds themselves, mainly the presence of double bonds. The oxidation stability decreased with the increase of the contents of polyunsaturated methyl esters. It is well known that the autoxidation of unsaturated fatty compounds proceeds at rates depending on the number and position of the double bonds. The positions of allylic to double bonds are especially susceptible to oxidation. The bis-allylic positions are even more prone to autoxidation than allylic. Therefore, coconut oil rich in esters of saturated fatty acids such as palmitic (C_{12:0}) and stearic (C_{14:0}) acids, was the oil with a low iodine value (11.02 g I₂/100g) and APE index (19.98) resulting in good oxidation stability.

Average calculated molecular weight (MW, gmol⁻¹) of coconut oil of 680.06 gmol⁻¹ can be obtained from a weighted average method utilizing the FA profiles depicted in Table 2. This value is in good agreement with that of measuring saponification value (SV) from $\overline{M}_{oil} = \frac{3 \times 56.106}{SV}$.

Because SV and M.W. are inversely related, the use of average M.W. is more straightforward than the use of the SV.

3.2 Optimization of the Transesterification by RSM

The relationship between response coconut methyl esters and three reaction variables (i.e. methanol content, catalyst loading and reaction time) were evaluated using RSM. Twenty experiments were performed and regression analysis was employed to fit the empirical model with the generated re-

sponse variable data (Table 3). The response obtained in Table 3 was correlated with the three independent variables using the polynomial equation (Eq. (3)).

Multiple regression analysis of experimental data gives the following second-order polynomial equation:

$$Y = 79.25 + 0.37X_1 + 39.5X_2 - 0.09X_3 - 0.01 X_1^2 - 23.21 X_2^2 + 8.84 \times 10^{-4} X_3^2 + 0.01X_1X_2 + 1.32 \times 10^{-3} X_1X_3 - 0.14X_2X_3 \quad (3)$$

Y is the response factor, fatty acid ester content (wt.%). X₁, X₂, and X₃ are the values of the three independent factors: methanol content, catalyst loading and reaction time, respectively.

Table 3: Experiment matrix with coded factors of CCD and RSM

Run	Variable			Biodiesel yield, %	
	X ₁ , Methanol content (%)	X ₂ , Catalyst loading (%)	X ₃ , Reaction time (min)	Observed yield(wt. %)	Predicted yield(wt. %)
1	30	1	90	87.11	87.01
2	30	1	90	87.09	87.01
3	30	1	90	87.10	87.01
4	30	1	90	87.09	87.01
5	30	1	90	87.10	86.01
6	30	1	90	87.10	87.01
7	30	1.84	90	52.84	54.76
8	13.18	1	90	80.84	86.78
9	30	1	140.5	85.19	87.76
10	46.82	1	90	83.68	80.88
11	30	0.16	90	85.22	86.44
12	30	1	39.5	90.19	90.76
13	20	1.5	120	74.58	69.81
14	20	1.5	60	77.90	76.49
15	40	0.5	60	84.38	86.93
16	20	0.5	60	94.99	91.35
17	40	1.5	120	65.80	67.22
18	20	0.5	120	94.84	92.88
19	40	1.5	60	72.57	72.31
20	40	0.5	120	90.84	90.04

The data obtained from the CCD were also subjected to the analysis of variance (ANOVA) and the F-test (confidence level 95%), and the results are shown in Table 4. At 95% confidence level, the model was found significant as the computed F value (F = 19.01) with very low probability value (P = 0.0001) indicated the high significance of the fitted model showing the reliability of the regression model for predicting the model yield.

To test the fit of the model, the regression equation and determination coefficient (R²) were evaluated.

In this case, the value of the determination coefficient (R² = 0.9448) indicates that the sample variation of 94.48% for biodiesel production is attributed to the independent variables, and 5.52% of the total variations are not explained by the model (Yuan et al., 2008, Rashid *et al.*, 2011).

At the same time, a relatively lower value of the coefficient of variation (CV=3.94%) indicates a better precision and reliability of the experiments carried out. As shown in Table 4, among all of fac-

tors, only catalyst loading is significant at the 95% confidence level.

A high value of the correlation coefficient r ($=0.97199$) justifies a good correlation between the independent variables.

The yield obtained by putting the respective values of X_i in Eq. (3) is: methanol content of 23.67 wt.%, catalyst loading of 0.5 wt.%, reaction time of 120 min. It can be predicted from the model that the maximum conversion to biodiesel obtained under the above optimum conditions of the variables is 93.03%.

The significance of each coefficient was determined by P -values which are listed in Table 4. The

Table 4: Analysis of variance (ANOVA) for response surface quadratic model

Source of variation	Sum of square	Degree of freedom	Mean squares	F value	P value
Model	1818.88	9	202.10	19.01	< 0.0001
X_1	41.98	1	41.98	3.95	0.0749
X_2	1212.03	1	1212.03	114.03	< 0.0001
X_3	10.88	1	10.88	1.02	0.3355
X_1^2	18.21	1	18.21	1.71	0.2198
X_2^2	485.09	1	485.09	45.64	< 0.0001
X_3^2	9.12	1	9.12	0.86	0.3761
X_1X_2	0.031		0.031	2.940E-003	0.9578
X_1X_3	1.25	1	1.25	0.12	0.7389
X_2X_3	33.62	1	33.62	3.16	0.1057
Residual	106.29	10	10.63		
Lack of fit	106.29	5	21.26	3.751E+005	<0.0001
Pure error	2.833E-004	5	5.667E-005		
Total	1925.17	19			

larger the magnitude of smaller the P -value is, the more significant the corresponding coefficient is. This implies that the variable with the largest effect was the catalyst loading (<0.0001). This great importance of catalyst concentration in the conversion to biodiesel was also emphasized by Vicente et al. (1998). However, at a high concentration, soap formation is an undesirable side reaction, which lowers methyl ester yield. Complete transesterification is assumed for 97% (Lay, 2009) of triglyceride that forms methyl ester. Therefore, the concentration interaction effect was found to be negative, probably due to side reactions, such as soap formation.

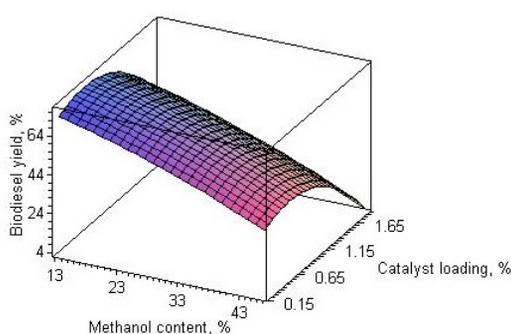


Fig. 2: Response surface plots representing the effects of methanol content and catalyst loading, and their reciprocal interaction on coconut biodiesel synthesis. Other factors are constant at a level of zero

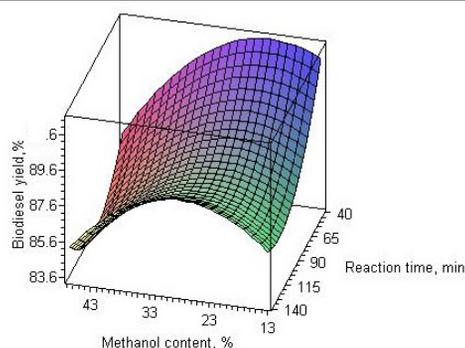


Fig. 3: Response surface plots representing the effects of reaction time, methanol content and their reciprocal interaction on castor biodiesel synthesis. Other factors are constant at zero level

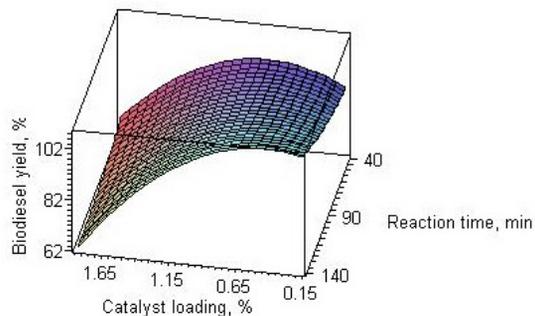


Fig. 4: Response surface plots representing the effects of catalyst loading, reaction time and their reciprocal interactions on castor biodiesel synthesis. Other factors are constant at zero level

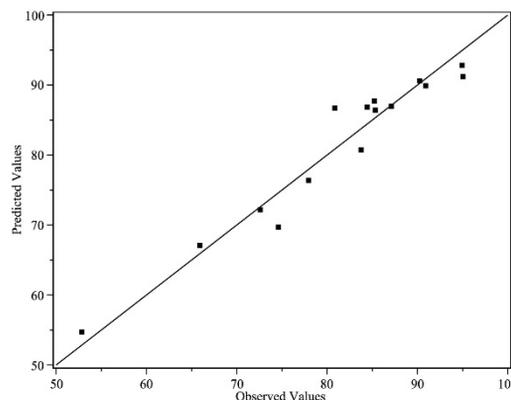


Fig. 5: Plot of observed vs. predicted yield of coconut oil biodiesel, $r = 0.97199$

3.3 Analysis of coconut oil biodiesel

The FT-IR spectrum was used to determine the functional groups of coconut oil biodiesel. The esters show the characteristic carbonyl absorption bands (C=O) at 1743 cm^{-1} , the antisymmetric axial stretching vibrations of C-O bands around 1245 cm^{-1} , and the asymmetric axial stretching vibrations of O-C-C bands around 1197 cm^{-1} . In addition, the observation of absorption peaks around 3008 and $2925\text{--}2854\text{ cm}^{-1}$ may be assigned to the stretching vibrations of CH_3 , CH_2 , and CH , while the peaks around 1461 , 1170 , and 722 cm^{-1} may be assigned to the bending vibration (ρCH_2) of these groups.

Asymmetrical and symmetrical stretching vibration of methylene (CH_2) group at frequency of 2925 and 2854 cm^{-1} .

Ester carbonyl functional group of the triglycerides at frequency of 1743 cm^{-1} .

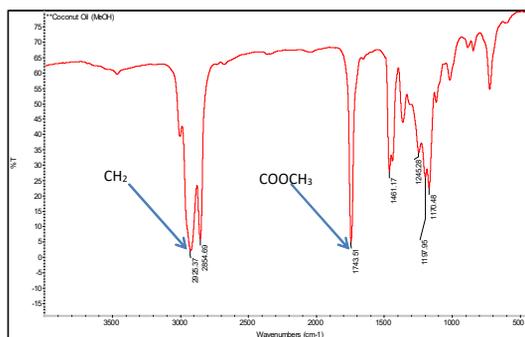


Fig. 6: FTIR spectra of coconut oil biodiesel

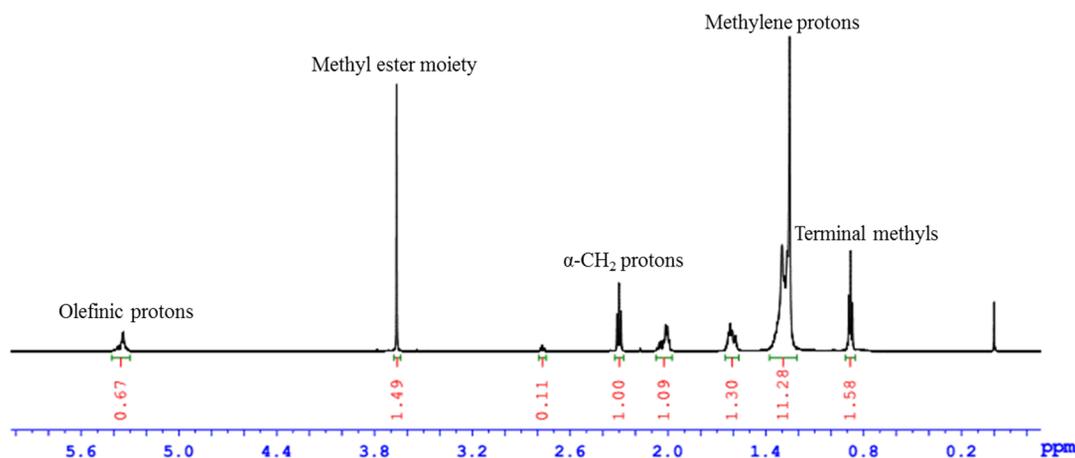


Fig. 7: ^1H NMR spectrum of coconut oil biodiesel

The presences of methoxy groups at δ_{H} 3.66 (s) and a triplet of $\alpha\text{-CH}_2$ at δ_{H} 2.30 (t, $J = 7.2\text{ Hz}$) con-

firmed the successful transformation of coconut oil into biodiesel. Other observed proton signals at δ_{H}

0.88 (t, $J=6.6$ Hz, terminal methyls), 1.30 (m, CH₂ of chain) and 5.35 (m, olefinic protons) were assigned for the long chain of fatty acid methyl esters.

The ¹H NMR and FT-IR spectra of coconut oil biodiesel were qualitatively similar to the spectra of FAME reported elsewhere (Knothe *et al.*, 2004; Moser, 2007; Rashid *et al.*, 2008). Coconut oil biodiesel contained a methyl ester moiety that was prominently indicated in the ¹H NMR spectrum (Fig. 7) by a strong singlet at around 3.67

ppm and in the FT-IR spectrum by a strong carbonyl signal at 1742 cm⁻¹.

3.4 Biodiesel characterization

Coconut biodiesel was tested for their fuel properties. The fuel properties of biodiesel are summarized in Table 5. The table shows that the biodiesel had comparable fuel properties with those of diesel and the values and parameters were within the limits prescribed in latest current standards for biodiesel.

Table 5: Physicochemical properties of biodiesel in the present work

Property	Standards			Coconut oil biodiesel
	JIS	ASTM	EN	
Acid value, mg KOH/g	0.5 max	0.5 max	0.5 max	0.06
KV at 40 °C, mm ² /s	3.5–5.0	1.9–6.0	3.5–5.0	3.12
eroxide value, meq/kg	–	–	–	143.7
Iodine value, g I ₂ /100 g	130 max.	130 max.	130 max.	10.83

4 CONCLUSIONS

The effects of three reaction variables, namely methanol content, catalyst loading and reaction time on biodiesel yield were evaluated by RSM during alkali-catalyzed transesterification of coconut oil. The maximum biodiesel conversion yield, as predicted by the quadratic polynomial model, from coconut oil was established to be 93.03 % under the optimum reaction conditions of 23.67 wt.% of methanol content, 0.5 wt.% of catalyst loading and 120 min of reaction time. Further research on the quality control of biodiesel product has been carefully investigated.

REFERENCES

- Bezerra, M.A., Santellia, R.E., Oliveiraa, E.P., Villara, L.S., Escaleraa, L.A., 2008. Response surface methodology (RSM) as a tool for optimization in analytical chemistry. *Talanta*. 76: 965–977.
- Demirbas, A., 2007. Importance of biodiesel as transportation fuel. *Energy Policy*. 35(9): 4661–70.
- Jeong, G.T., Park, D.H., 2009. Optimization of biodiesel production from castor oil using response surface methodology. *Applied biochemistry and biotechnology*. 156, 431–441.
- Knothe G., 2002. Structure Indices in FA Chemistry. How Relevant Is the Iodine Value?. *Journal of the American Oil Chemist's Society*. 79 (9): 847–854.
- Knothe, G., Kenar, J. A. *Eur. J.*, 2004. *Lipid Science and Technology*. 106, 88–96.
- Lay, L., Myint, Mahmoud, M., El-Halwagi, 2009. Process analysis and optimization of biodiesel production from soybean oil. *Clean Technologies and Environmental Policy*. 11: 263–276.
- Maeda, Y., Thanh, L.T., Imamura, K., Izutani, K., Okitsu, K., Boi, L.V., Lan, P.N., Tuan, N.C., Yoo, Y.E., Takenaka, N., 2011. New technology for the production of biodiesel fuel. *Green Chemistry*. 13: 1124–1128.
- Moser, B.R., Haas, M.J., Winkler, J.K., Jackson, M.A., Erhan, S.Z., List, G.R., 2007. *European Journal of Lipid Science and Technology*. 109: 17–24.
- Rashid, U., Anwar, F., Moser, B.R., Knothe, G., 2008. *Bioresource Technology*. 99: 8175–8179.
- Rashid, U., Anwar, F., Ashraf, M., Saleem, M., Yusup, S., 2011. Application of response surface methodology for optimizing transesterification of *Moringa oleifera* oil: Biodiesel production. *Energy Conversion and Management*. 52: 3034–3042.
- Vicente, G., Coteron, A., Martinez, M., Aracil, J., 1998. Application of the factorial design of experiments and response surface methodology to optimize biodiesel production. *Industrial Crop Productions*. 8:29–35.
- Yuan, X., Liu, J., Zeng, G., Shi, J., Tong, J., Huang, G., 2008. Optimization of conversion of waste rapeseed oil with high FFA to biodiesel using response surface methodology. *Renewable Energy*: 33, 1678–1684.