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Alkali-catalyzed Transesterification of *Hibiscus sabdariffa* (*Roselle*) Seed Oil for Biodiesel Production

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Authors' contributions

This work was carried out in collaboration among all authors. Authors EHA and Azhari H. Nour designed the study, conduct the experimental work, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors OAOI and Abdurahman H. Nour managed the analyses of the study and managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The need of energy never comes to an end so; the challenge is to procure power source sufficient to offer for our energy needs. Besides, this energy source must be dependable, renewable, recurring and non-contributing to climate change.
Aims: This study was aimed to produce biodiesel from *Roselle* seed oil and to investigate its quality.
Methodology: The *Roselle* seeds were clean from dirt, milled to proper size and the oil was extracted using soxhlet with n-hexane as solvent. The extracted oil was subjected to physiochemical analysis tests and then transesterified using methanol and potassium hydroxide as catalyst; with ratio of oil to alcohol 1:8 at 65°C. The quality of produced biodiesel was investigated and compared to international standards. The fatty acid composition of the produced biodiesel was determined by GC-MS.

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Results: Based on the experimental results, the yellow with characteristic odor oil was obtained from the seeds had the following physicochemical properties: yield, 12.65%; refractive index (25°C), 1.467 ms^{-1}; free fatty acids, 5.5%; saponification value, 252 mg KOH/g of oil; density, 0.915 g/mL and ester value, 241 mgKOH/g. Also the biodiesel yield achieved was 96%, with density, 0.80 g/mL; API, 44.63; Kinematics viscosity @ 40˚C, 0.742; Pour point, < -51˚C; and Micro Carbon Residual (MCR), 0.65%; which conformed to the range of ASTM D6751 and EN 14214 standard specifications. However, the GC-MS analysis result revealed that the biodiesel produced was methyl ester and free other undesired products such as linoleic acid (33%), elaidic acid (29%) and palmitic acid (17%) and other biomolecules.

Conclusion: Based on the obtained results, *Roselle* seed oil had potential for biodiesel production due to its high contains of free fatty acids. Therefore, in the future, more investigations in alcohol: oil ratio and the concentration of catalyst may be warranted to increase the yield much more.

Keywords: Biodiesel; Hibiscus sabdariffa (Roselle); methanolysis; linoleic acid; transesterification.

1. INTRODUCTION

The present-day global energy demand, diminishing fossil fuel reservoirs and rise in global warming because of the increase in greenhouse gases discharge encouraged human's responsiveness toward substitutes to petroleum-based fossil fuels. It is predicted that world's total energy utilization and CO_2 production are expected to be 80% higher by 2030 than the current levels [1]. With the current energy consumption, the demand for finding an alternative feedstock for the energy needs is increasing [2,3]. Biodiesel is receiving more and more attention recently because it is renewable, biodegradable, non-toxic and environment-friendly [4,5]. Biodiesel, mixture of fatty acid methyl esters (FAME), is generally produced from a varied range of edible and non-edible vegetable oils, animal fats, used frying oils and waste cooking oils by transesterification with methanol in presence of a catalyst (acid, base or biocatalyst) [5,6,7] as in Fig. 1. Biodiesel is quite similar to conventional diesel fuel in its physical characteristics and can be used alone or mixed in any ratio with petroleum based diesel fuel in most existing modern four-stroke combustion ignition diesel engines with very few technical adjustments or no modification. Biodiesel as a neat can be used as a direct substitute for petro diesel and is technically called B100 [6]. The preferred ratio of mixture ranges between 5% (B5) and 20% (B20). The blending ratio has been investigated by various authors on CI engines. Up to 20% blending of biodiesel with diesel has shown no problems in engine parts [6,7]. The international standards (EN 14214 and ASTM D 6751) for quality of biodiesel were shown in Table 1 below [6]. These standards will be used to quality of biodiesel produced from vegetable oil. Researchers have investigated for cheaper and economically viable non-edible oils as alternative feedstocks for use in biodiesel production [6]. In India, biodiesel is produced mainly from non- edible oils like jatropha, pongamia, neem oil etc. However, there are alternative edible oil-yielding crops which can be utilized as feedstocks which are new and low-cost. A new candidate *Hibiscus species* plants are emerging as potential feedstock which are not yet been extensively studied, but is gaining a lot of attention as alternative fuel for diesel engines since it is renewable, non-toxic, environmentally acceptable and can be domestically produced [7]. *Hibiscus sabdariffa*, is the plant which belong to Malvaceae family, known worldwide by many different common names such as hemp, ambadi, *Roselle*, Jamaica sorrel, and India in kannada as Pundi. The Hibiscus genus contains more than 300 species [7]. The research during 2000 to 2015 showed its usage in industrial and

Table 1. ASTM D6751 and EN 14214 biodiesel (B100) specifications

Property	ASTM D6751	EN 14214
Density (15°C, G/CM^3)	NS	0.86-0.90
Kinematic viscosity (40°C, MM^2/S)	1.9-6.0	3.5-5.0
Pour point (°C)	NS	NS
Carbon residue (%)	NS	NS
API	36.95	NS

NS: not specified; Basumatar [6]

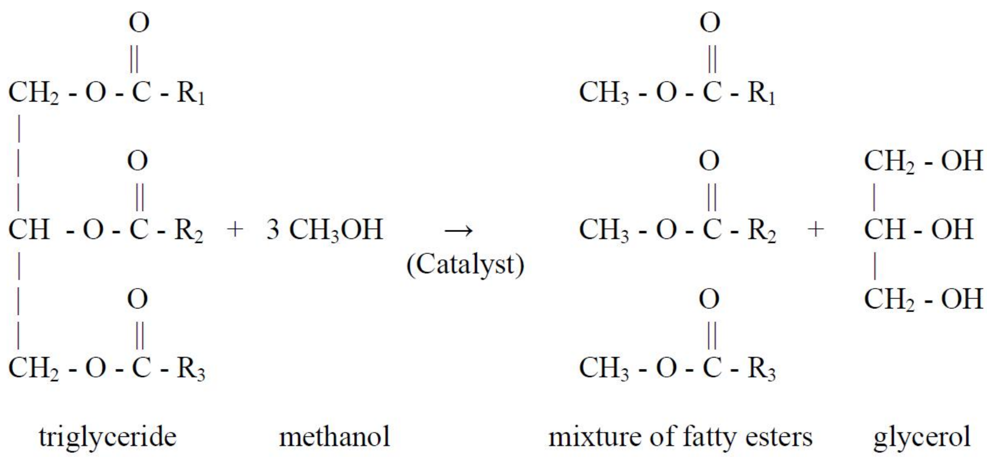

Fig. 1. Transestrifcation process (Pilar, et al. [10])

Fig. 2. *Roselle* seed sample

medicinal properties; however, the seeds (Fig. 2) are normally left unused as waste [8]. The *Roselle* seed oil is rich in fatty acids and can be used as edible oil and as an industrial feedstock [9]. This study was aimed to produce biodiesel from Roselle seed oil and to investigate its quality in term of density, kinematic viscosity, pour point, API and micro carbon residual.

2. MATERIALS AND METHODS

2.1 Plant Material

Roselle seed sample used in this study was collected from local market, Khartoum, Sudan in June 2019. The sample was washed, heat-dried, crushed and milled to proper size; then kept for further studies.

2.2 *Roselle* Seed Oil Extraction

The seed oil was obtained according to method described by Erwa et al. [11] with slight modification. In this respect, about 100 g of *Roselle* prepared seed sample were extracted by soxhlet extractor; n-hexane was used as the extraction solvent for a period of six hours. The method repeated until obtained sufficient amount of oil. The solvent was evaporated using rotary evaporator under reduced pressure and temperature; further dried under open air in a dark area. The oil yield was calculated using the following equation.

$$\text{Oil yield (\%)} = \frac{\text{Weight of oil (g)}}{\text{Weight of sample (g)}} \times 100\,\%$$

2.3 Physiochemical Properties of *Roselle* Seed Oil

2.3.1 Determination of colour, density and refractive index of extracted seed oil

Colour, density and refractive index of extracted *Roselle* seed oil were determined according to method described by Idris et al. [12] with slight modification. Colour of oil was observed through visual observation at room temperature (25°C). For oil density, About 1 mL of extracted oil was injected into a clean dry Density meter, and then reading was recorded until fixed reading was obtained. Refractive index of oil was measured at (25°C) via pen Refractometer (Atago, Japan) with resolution and accuracy value of 0.1% and ± 0.2% at 10-60°C. The measurement was repeated in triplicate and the average value was reported.

2.3.2 Acid value, peroxide value and free fatty acid of *Roselle* seed oil

The Acid Value (AV) was determined through direct titration method of oil in an alcoholic medium against standard potassium hydroxide via AOCS Official method Cd 3a-63 (2009). The Free Fatty Acids (FFA) were analysed according to standard titration methods described by AOCS Official Method Ca 5a-40 (2009). Peroxide Value (PV) was analysed according to standard methods of AOAC Official Method 993.20 (1999) by Wij's reagent, 965.33 (2002) and Ca 6a-40 (1998), respectively [11]. The calculations according to the following equations;

$$AV = \frac{[56.1][\text{Titrattion of standard (mL)}][\text{Molarity of standard (M)}]}{\text{Weight of sample (g)}}$$

$$\text{FFA as Oleic (\%)} = \frac{[28.2][\text{Titrattion volume of standard (mL)}]}{\text{Weight of sample (g)}}$$

$$PV = \frac{[\text{Titration of standard (mL)}][\text{Molarity of standard (M)}\ [100]}{\text{Weight of sample (g)}}$$

2.3.3 Determination of oil saponification value

About 2.0 g of sample was transferred into a 200 mL conical flask; 20 mL of 0.5 mol/L alcoholic potassium hydroxide was added. The flask was gently heated and occasionally shaken for 30 min and cooled. The unreacted KOH was then back-titrated with (0.5 mol/L) HCl. A blank titration was performed [13].

2.3.4 Determination of oil ester value

Ester number is number of milligrams of potassium hydroxide required to neutralize the acids liberated by the hydrolysis of esters present in 1 g of the oil [14]. It calculated as the difference between the values of saponification and frees fatty acids by:

$$\text{Ester value} = \text{Saponification value} - \text{Free fatty acids value}$$

2.4 Biodiesel Production from *Roselle* Seed Oil

The transesterification of crude *Roselle* oil with methanol by using potassium hydroxide (KOH) as catalyst was carried out in a laboratory-scale setup as method described by Nadir et al. [2] with slight modification. The ratio 1:8 of oil to alcohol, and the concentration of potassium hydroxide was 1% at 65°C reaction temperature. The produced biodiesel was analyzed using GC-MS and the properties were also investigated.

2.5 GC/MS Analysis of Produced Biodiesel

The produced biodiesel was analysed using shimadzu Gas Chromatograph, GC-17A linked to a QP 5000 mass spectrometry detector. The gas chromatograph contained a Restek RXi-5ms column of length and inner diameter of 30 m and 0.25 mm respectively. The carrier gas was Helium, with a 1mL/min continuous flow rate. A (class-5000) application software controlled the instrumentation system on the computer. The column oven starting temperature was set at 150˚C, then ramped to 190°C at a rate of 5°C/min, then ramped to 210°C at a rate of 2°C/min. lastly; the oven temperature was brought to 310°C at a rate of 20°C/min with a final hold of 10 min. NIST 107 and 21 libraries were used to identify the compounds. A split ratio of 100:1 was used. The split ratio is explained as follows: 100 parts of sample is thrown out while 1 part is allowed to enter the system from the total quantity injected.

2.6 Properties of Produced Biodiesel

The produced *Roselle* biodiesel properties were determined according to ASTM standard methods as follow.

2.6.1 Density

Small amount (about 0.7 mL) of sample was introduced into the clean dried sample tube of the instrument using a suitable syringe (The sample can also be introduced by siphoning) the external TFE fluorocarbon capillary tube was plugged into the lower entry port of the sample tube. Immersed the other end of the capillary in the sample and applied suction to the upper entry port using a syringe or vacuum line until the sample tube is properly filled, the illumination light was turned on and examined the sample tube carefully. Made sure that no bubbles are trapped in the tube, and that it is filled to just beyond the suspension point on the right-hand side. The sample must be homogeneous and free of even the smallest bubbles. The illumination light was turned off immediately after sample introduction, because the heat generated can affect the measurement temperature. After the instrument displayed a steady reading to four significant figures for density and five for T values, indicating that temperature equilibrium has been reached, the density or T-value was recorded [15].

2.6.2 Pour point

After preliminary heat, the sample is cooled at specified rate and examined at intervals of 3°C for flow characteristics. The lowest temperature at which movement of the specimen is observed recorded as the pour point [16].

2.6.3 Micro carbon residual (MCR)

A weighed quantity of sample is placed in a glass vial and heated to 500°C under an inert (nitrogen) atmosphere in a controlled manner for a specific time. The sample undergoes coking reactions, and volatiles formed are swept away by the nitrogen. The carbonaceous-type residue remaining is reported as a percept of the original sample as “carbon residue (micro) when the test result is expected to be below 0.10% (m/m), the sample can be distilled to produce a 10% (v/v) bottoms, prior to performing the test [17].

2.6.4 Kinematic viscosity

The time is measured for a fixed volume of liquid to flow under gravity through the capillary of a calibrated viscometer under a reproducible driving head and at a closely controlled and known temperature. The kinematic viscosity (determined value) is the product of the measured flow time and the calibration constant of the viscometer. Two such determinations are needed from which to calculate a kinematic viscosity result that is the average of two acceptable determined values [18].

2.6.5 American Petroleum Institute (API)

The American Petroleum Institute (API) measure was calculated by using the following equation:

$$API = \frac{147.5}{S\,g} - 131.5$$

API: American Petroleum Institute [19].
S g: Specific gravity

3. RESULTS AND DISCUSSION

3.1 Physiochemical Properties of *Roselle* Seed Oil

The extracted oil of *Roselle* seeds was subjected to physiochemical analysis and the results obtained were shown in Table 2.

The oil content of *Roselle* seed obtained in this study was 12.65%, where it was low compared to yield obtain by Mohamed et al. [20] (19 ± 0.25%), Hasni et al. [8] (17.5%) and Ahmed et al. [1] (19.11%). The physiochemical properties results of *Roselle* seed oil obtained in this study were consider high for acid value, free fatty acid and saponification value; while its low in peroxide value and closed in refractive index compared to results obtained by Rashmi and Naik [7]; where their acid value, free fatty acid value, saponification value, peroxide value and refractive index were 2.8 mgKOH/g, 0.65%, 170.8 mgKOH/g, 26.6 meq/Kg and 1.45, respectively. However, their obtained results of density, and refractive index were found closed to results obtained by Mohamed et al. [20] where their results were 0.9171 ± 0.0004 and 1.477 ± 0.000, respectively; while their peroxide value (8.63 ± 0.08) was found higher than the result obtained in this study. In addition, Hasni et al. [8] reported the refractive index result (2.16 ms^{-1}) which is higher than the result obtained in this study, while the acid value in this study was found higher than their result (5.48 mgKOH/g). Variation of physiochemical properties and oil yield of *Roselle* seed perhaps due to environmental and soil related factors. Moreover, Eltayeib and Elaziz [21] in their study on two of Roselle oils, they reported the obtained results as refractive index (1.467 and 1.466), saponification value (189.7 and 189.1) peroxide value (4.6 and 4.7) and acid value (3.57 and 3.55), their refractive index was found closed to result obtained in this study, while in both, saponification and peroxide values were higher but had a low acid values compared to the results obtained in this study; these variations may be happened due to environmental and soil issues.

Table 2. Physiochemical properties of *Roselle* seed oil

Property	Units	Value
Density	g/mL	0.915
Refractive Index (40°C)	ms^{-1}	1.467
Acid value	mgKOH/g	11
Free Fatty acid	%	5.5
Saponification	mgKOH/g	252
Peroxide value	meq/kg	2
Ester value	mgKOH/g	241

3.2 Biodiesel Produced from *Roselle* Seed Oil

The transesterification process of *Roselle* oil with methanol by using potassium hydroxide (1%) as catalyst and 1:8 ratio of oil to alcohol at 65°C temperature resulted in high biodesel yield of 96%. Our obtained result consider lower than the result obtained by Nakpong and Wootthikanokkhan [22], where they achieved 99.4% biodiesel from *H. sabdariffa* seed oil using same methanol: oil ratio and catalyst (KOH 1.5%) at temperature 60°C; this different may due to the difference of catalyst concentration and reaction temperature. Also our result lower than the result of Al-Tabbakh et al. [23] where they achieved highest productivity of biodiesel 98% by molar ratio of (1:7) (oil: methanol) at a reaction temperature of (60°C) in the presence of NaOH (1%) as a catalyst. Also lower to Betiku et al. [5] obtained biodiesel yield 99.23% (w/w) at methanol/oil molar ratio of 6.21, catalyst (NaOH) amount of 1.03 wt% and reaction temperature of 51°C. In addition it's slightly higher than the result obtained by Hasni et al. [8] they achieved 95.01% biodiesel yield; by methanol to oil ratio 6:1, temperature 67.5ºC, 1% calcium oxide (CaO) catalyst derived from waste egg shells. The difference in our result with Al-Tabbakh et al., Hasni et al. and Betiku et al. results may be due to the difference in the oil: methanol ratio, reaction temperature and the type of catalyst used. In general our obtained biodiesel yield considers very well in this condition and more optimization in the parameters may be needed to increase it.

In addition after transesterfication process the resulted biodiesel was subjected to GC/MS analysis, the obtained results were shown in Table 3.

The major components in the GC/MS results were shown in Table 3 and the spectrum of biodiesel was shown in Fig. 3. The Linoleic acid was the highest, and then Elaidic acid and Palmitic acid. In the result obtained by Betiku et al. [5] Oleic acid was higher (58.337%), and then Linoleic acid (21.194%) and Palmitic acid (18.280%); where their results were disagreed the result obtained in this study. However, the results obtained in this study was disagreed with results obtained by Hasni et al. [8] where their result indicated that stearic acid (49.74%) was higher, and then Linoleic acid (25.3%) and Palmitic acid (6.57%). While in the study by Atta et al. [24] indicated that the GLC analysis proved that the Palmitic (17.3±0.8%) was similar and linoleic (45.3%), oleic (27.2±0.7%) were higher that is due to the concentration of catalyst and molar ratio used in this study.

Table 3. Components of biodiesel produced from *Roselle* seed oil

Fatty Acid	Systematic name	Formula	Area%
Myristic acid	Tetradecanoic	$C_{14}H_{28}O_2$	0.21
Lycopodic acid	11-Hexadecenoic acid	$C_{16}H_{30}O_2$	0.23
Palmitic acid	Hexadecanoic acid,	$C_{16}H_{32}O_2$	17.8
Malvalic acid	2-octylcyclopropene-1-heptanoic acid	$C_{18}H_{32}O_2$	0.42
Linoleic acid	9,12-Octadecadienoic acid	$C_{18}H_{32}O_2$	33.0
Elaidic acid	9-Octadecenoic acid	$C_{18}H_{34}O_2$	29.8
Oleic acid	9-Octadecenoic acid(z)	$C_{18}H_{34}O_2$	0.46
Linoleic acid	10,13-Octadecadienoic acid	$C_{18}H_{32}O_2$	3.54
Linoleic acid	10,13-Octadecadienoic acid	$C_{19}H_{34}O_2$	3.17
α-Linoleic acid	9,12,15-octadecatrienoic acid	$C_{19}H_{32}O_2$	0.14
α-eleostearic acid	9.cis,11.trans.t,13.trans-Octadecatrienoic	$C_{18}H_{30}O_2$	1.95

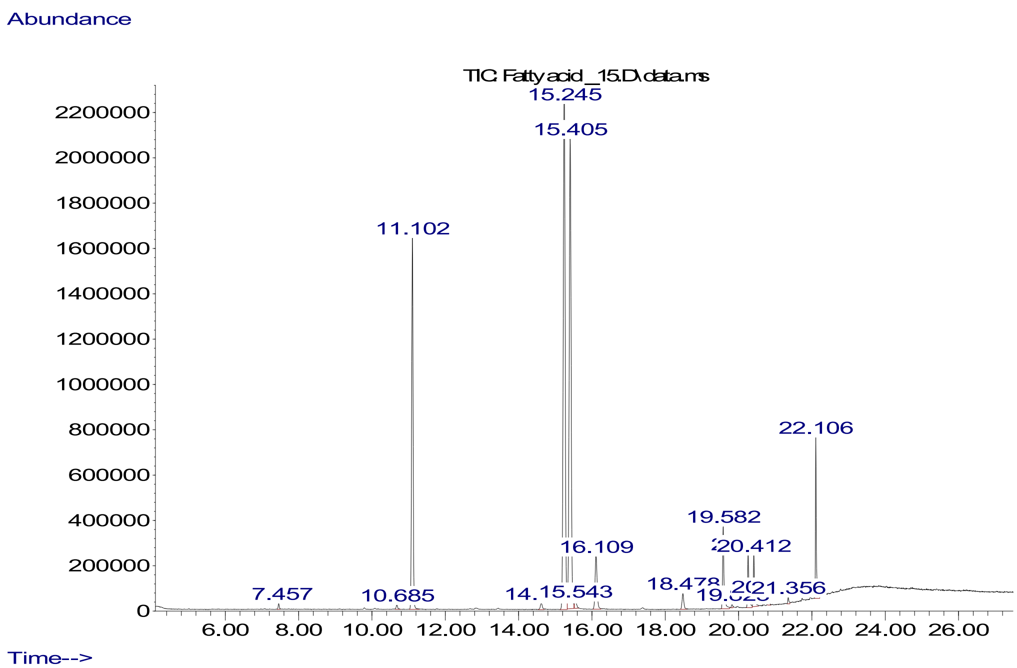

Fig. 3. GC-MS spectrum of biofuels produced from *Roselle*

Table 4. Properties of biodiesel produced from *Roselle* seed oil

Test name	Test method (ASTM)	Unit	Result
Density@ 15˚C	D4052	g/mL	0.80261
API	D4052		44.63
Pour point	D97	˚C	<-51
MCR	D4530	%wt	0.65
Kinematics viscosity @ 40˚C	D445	CSt	0.742

API: American Petroleum Institute Gravity
MCR: Micro Carbon Residue

3.3 Properties of Produced *Roselle* Biodiesel

The properties of produced *Roselle* biodiesel was investigated to test the quality of biodiesel and the results were shown in Table 4.

The obtained results were compared to previous studies as well as to international standard Table 3. Compared to the standard density was in the range of ASTM D6751and EN 14214 standards; while kinematics viscosity and API were higher than the standards. Pour point and Carbon residue (MCR) had no specified international standards. Betiku et al. [5] results indicated that density (0.92), kinematics viscosity (5.80) and pour point (-15) were higher than the results obtained in this study; while its API (32.65) was lower than the result in this study. Nakpong and Wootthikanokkhan [22] results of kinematics viscosity (4.588) and carbon residue (0.84) were found higher than the results obtained in this study, while density (0.880) was closed to the result in this study and pour point (-1) was low. Nadir et al. [2] results compared to the results obtained in this study, where their density (0.8829) was closed, while kinematics viscosity (5.320) and pour point (+3.0) were found higher than the results obtained in this study. Rashmi and Naik [7] their density result (0.856) was closed to result obtained in this study, while kinematics viscosity (4.936) was found higher than the obtained results in this study.

4. CONCLUSION

This study discusses the production of biodiesel from *Roselle* seed oil by transesterification process. The used parameters for converting *Roselle* oil into biodiesel was methanol to oil ratio

8:1, temperature 65°C with potassium hydroxide as a catalyst. The yield achieved was 96%.Then produced biodiesel studied and revealed that density is 0.80 g/mL, API is 44.63, Kinematics viscosity @ 40°C is 0.742 and pour point was < -51°C which conformed with the range of ASTM D6751 and EN 14214 standard specifications. The results indicated some suitable fuel properties of biodiesel and satisfactory yield by using potassium hydroxide.

Based on obtained results we recommended that:

- Production of Biodiesel from *Roselle* seeds oil due to high free fatty acid contains
- Also we recommend using biodiesel for diesel engines due to its lower emission of carbon monoxide and air toxins that produced when engines depending on petroleum based diesel fuel operated.
- Utilization of methanol for biodiesel production due to its low cost and short – chain molar size.
- Care must be taken to determine empirically ideal molar ratios to employ otherwise excess methanol will result in severe difficulty in biodiesel refining process.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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