

ORIGINAL RESEARCH ARTICLE

Comparative Analysis of Biodiesel Production by Transesterification and Interesterification of *Rothmannia longiflora* Seed oil using a Heterogeneous Catalyst

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ABSTRACT

Biodiesel is a promising alternative fuel with better characteristics over petro-based fuels. It is conventionally produced via transesterification of vegetable oil with methanol. Chemical interesterification is another alternative route for conversion of vegetable oil to biodiesel. It yields a value-added co-product, triacetin as against glycerol formed as by-product by transesterification. In this study transesterification and chemical interesterification of *Rothmannia* seeds oil was investigated using calcined swan mussel shell (*Anodantacygnea*) as solid base catalyst. The catalyst was prepared by hydrothermal treatment and characterized using FT-IR, XRF and XRD analysis. Reaction parameters were optimized, with optimal methanol/oil ratio of 1:6, catalyst amount of 1.0g, reaction time of 60 minutes and reaction temperature of 55°C, achieving maximum yield of 93.47 % via transesterification while for interesterification, an optimum yield of 87.50 % was achieved at catalyst amount of 1.5g, reaction temperature of 60°C, reaction time of 90 minutes and methyl acetate/oil of 9:1. Transesterification of the seed oil shows better conversion of triglycerides than the chemical interesterification. Some important fuel properties were determined and compared with ASTM standard. The study has revealed good potentials of *Rothmannia longiflora* seed oil as feedstock for biodiesel production.

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INTRODUCTION

With the increase in population and the development of technology, the demand for fuel is increasing; however, oil reserves from fossil sources are decreasing. Hence, efforts have been made for sustainable alternative energy sources. One of the alternative energy sources currently being developed is biodiesel. Biodiesel is a promising renewable fuel produced by reaction of vegetable oils or animal fats with short-chain alcohols. Compared to diesel fuel, biodiesel is more environmentally friendly, renewable, biodegradable, sustainable and capable of neutralizing the greenhouse effect (Daryono *et al.*, 2020). Research on biodiesel production has gained a major boost as it emerged as a good alternative to conventional fuel. Biodiesel causes less environmental pollution and is better in terms of sulphur content, flash point and cetane number compared to petroleum-based fuel (Martini and Schell, 1998). The major contributor to the biodiesel manufacturing cost is the cost of the raw materials. The

conversion of methyl fatty acids by homogeneous catalysis also increases the cost of biodiesel due to non-reusability of the catalyst, soap formation and problems in handling and separation. The use of heterogeneous catalysts in transesterification of vegetable oils overcomes these problems. These catalysts are easily separated from the reaction mixture; they are non-corrosive and have appreciable catalytic activity in reuse (Hara, 2009).

Biodiesel production carried out via transesterification of triglycerides is accompanied by production of crude glycerol, which is usually disposed of as waste, thereby reducing the efficiency of the process. Furthermore, production of glycerol exceeds current demand for chemical production (Viana *et al.*, 2014). Therefore, Biodiesel production may be more efficient if the glycerol by-product is eliminated. An alternative to this route has been developed over the few years (Galia *et al.*, 2014). This

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new process involves the reaction of triglycerides with methyl acetate, producing the biodiesel and a molecule of triacetin. The triacetin produced can be employed as valuable biodiesel additive.

Nigeria is endowed with vast arable land where different plant grows. These plants produce a lot of fruit seeds which are left to rot annually untapped. However, economics or energy prospect of many of these fruit seeds were not evaluated. One of these identify plants that grows on a marginal land is *Rothmannia longiflora*. In our previous study, the seeds of *Rothmannia longiflora* were evaluated for its potential as feedstock for biofuels production (Muhammad *et al.*, 2022). The results have shown that the seeds contains reasonable amount of oil that could make it viable for biodiesel production. Identifying other seed oil plants like *Rothmannia longiflora* will diversify the oil sources for biodiesel production. In spite of the appreciable oil content of the plant no information was found in the literature to the best of the authors knowledge that utilizes *Rothmannia longiflora* seed oil for biodiesel production. Hence, this study aimed to report for the first time transesterification and interesterification of *Rothmannia longiflora* seeds oil into biodiesel using calcined Swan Mussel Shell (*Anodonta cygnea*) as heterogeneous base catalyst.

MATERIALS AND METHODS

Sample Collection and Treatment

The fruits seed of *Rothmannia longiflora* were collected using a method described by Ayaz *et al.*, (2002) at Wanke bush along Lungu road in Shagari Local Government Area of Sokoto State, Nigeria. The fresh fruits samples were authenticated at the Herbarium Botany unit (Voucher No: UDUS/ANS/0246) of the Biological Science Department of Usmanu Danfodiyo University, Sokoto, Nigeria. The seed were separated manually from the peel and air dried for 14 days, then mechanically grinded into fine powder using blender.

Oil Extraction

The powdered *Rothmannia longiflora* seed (30g) was placed in the thimble and n-hexane (300 cm³) was poured into the round bottom flask. The apparatus was heated at temperature (60°C) for six (6) hours of continuous extraction using Soxhlet apparatus. The experiment was repeated for the same weights of the sample. The solvent was removed from the oil using rotary evaporator. The percentage oil yield was calculated using equation (1)

$$\% \text{ Oil Yield} = \frac{\text{Weight of extracted oil(S)}}{\text{Weight of sample}} \times 100 \quad (1)$$

Preparation and Characterization of the Catalyst

The catalyst was prepared through hydrothermal treatment of swan mussel shells (*Anodonta cygnea*). The powdered shell was calcined in a muffle furnace under static air conditions at temperature (800°C) for three (3) hours and then refluxed in distilled water at 60°C for six

(6) hours. The solid particles was filtered and dried in hot air oven at 105°C overnight. The solid product was recalcined at 800°C for three (3) hours to change the hydroxide formed to oxide (Yoosuk *et al.*, 2010). The catalyst was characterized using X-Ray Diffraction (XRD), FT-IR and X-Ray Fluorescence (XRF).

Design of Experiment

The experiments were designed using Box-Behnken, a response surface design on MINITAP 17 Statistical software. In the optimization process, the influence of solvent to oil molar ratio, catalyst concentration, reaction temperature, and reaction time on the yield of *Rothmannia longiflora* seed oil biodiesel were investigated. Solvent to oil molar ratio and catalyst concentration were selected from 6:1 to 12:1, and 1.0 to 2.0 g respectively, while reaction temperature and time were varied from 50°C to 60°C, and 30 to 90 minutes. The design generated a total of twenty seven (27) runs (Table 3)

Table1: Independent Parameters and their Levels

Factors	Low levels	High levels
Reaction Temperature (°C)	50	60
Reaction Time (minute)	30	90
Solvent to Oil Ratio	6	12
Catalyst Amount (g)	1.0	2.0

Transesterification of the Seed Oil

Oil sample (30cm³) was measured in beaker (100cm³) and placed on a hot magnetic plate and heated at (100°C) to remove any water present, then cooled to 60°C. The catalyst (0.3g) was weighed and mixed with methanol (6 cm³) into a beaker (100cm³). The mixture of (methanol and CaO) was covered and preheated at temperature (50°C) for two (2) minutes. The mixture was added to the oil and heated in a water bath at temperature 60°C for three (3) hours with constant stirring. After the reaction time, the mixture was allowed to settle under gravity for twenty four (24) hours in a separating funnel. Two layers were formed; the upper layer consisted of methyl ester and residual catalyst while lower layer consisted of glycerol, and excess catalyst. After separation, the methyl ester layer was centrifuged to remove residual catalyst (Nurhayati, *et al.*; 2017). The same method was adopted for the transesterification of each run based on the design of experiment (Table 1). The percentage biodiesel yield was calculated using equt. 2:

$$\% \text{ Biodiesel Yield} = \frac{\text{Weight of Biodiesel}}{\text{Weight of Oil}} \times 100 \quad (2)$$

Interesterification of the Seed Oil

The interesterification reactions were performed in a beaker (100 cm³) with methyl acetate. The control reaction

was carried using the oil (30 cm³) with methyl acetate (6 cm³) and the catalyst (0.3 g) at 60°C for 3 hrs. After completing of the reaction, the products were poured into separating funnel for products separation. The same method was adopted for the interesterification of each run (Ratna *et al.*, 2016).

Analysis of the FAME

Fuel properties such as specific Gravity, Kinematic viscosity, Moisture content, Sulfated ash, Flash point, Pour point, Cetane number and Copper strip corrosion of were analyzed using standard test methods and compared with ASTM standard. The biodiesels were characterized using a method reported by Muhammad *et al.*, (2021); 0.5 cm³ of the sample was transferred into a 15 cm³ plastic centrifuge tube and to it, n-hexane (1.0%) was added and then vortex mixed for 10 min. The mixture was centrifuge at 3500 rpm for 10 min. The supernatant (1.0 uL) was injected into GC. The oven temperature was initially set at 80°C, hold time 0 min with an equilibration time 0.5 min and maximum temperature of 325°C.

RESULTS AND DISCUSSION

Characterization of the catalyst

The composition of the prepared catalyst revealed 95.710 wt% CaO, with other oxides (Al₂O₃, SiO₂, P₂O₅, SO₃, K₂O, TiO₂, Mn₂O₃, Fe₂O₃ and SrO) found in trace

amount (Table 2). The XRD pattern of the catalyst (Figure 1), shows diffraction peaks at $2\theta = 32.3^\circ, 37.57^\circ, 54.0^\circ, 64.5^\circ$ and 67.9° indicating formation of CaO. Peaks due to Ca(OH)₂ also appeared at $2\theta = 18^\circ, 34.0^\circ$ and 51.0° , formation of Ca(OH)₂ may be attributed to reaction of CaO with water vapour. Similar diffraction peaks were also reported for white bivalve calm shell (Natarajan *et al.*, 2013). The result also shows the presence of CaCO₃ at $23^\circ, 29^\circ, 39^\circ, 43^\circ$ and 47° due to incomplete conversion of CaCO₃ material in the shell at 800° because of its hard nature.

Table 2 Composition by XRF of Calcined Swan Mussel Shell (*Anodonta cygnea*)

Oxides	Composition (wt%)
Al ₂ O ₃	0.794
SiO ₂	2.294
P ₂ O ₅	0.108
SO ₃	0.086
K ₂ O	0.094
CaO	95.710
TiO ₂	0.041
Mn ₂ O ₃	0.300
Fe ₂ O ₃	0.417
SrO	0.132

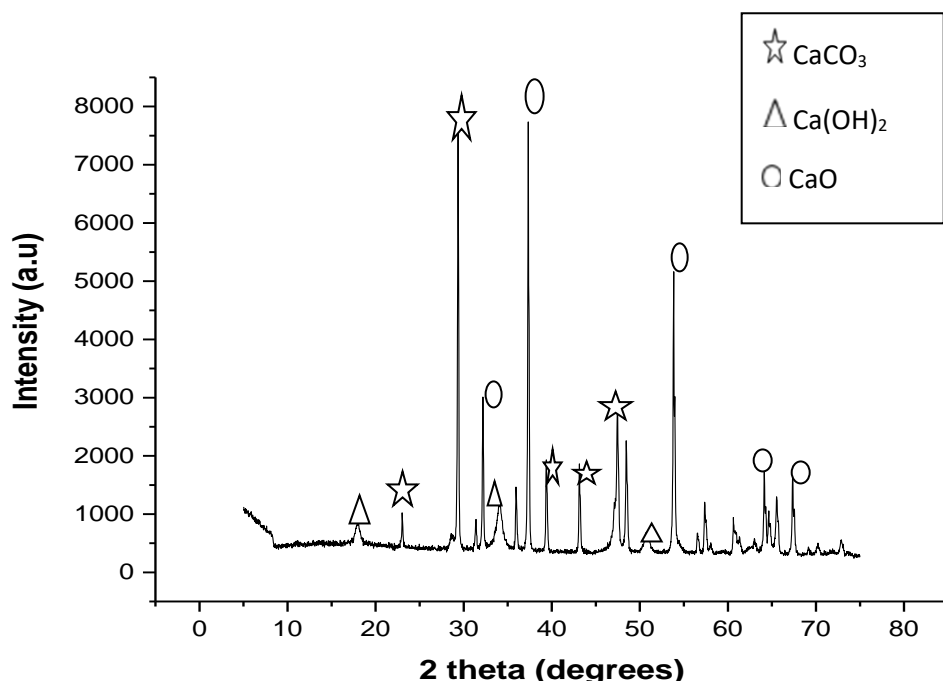


Figure 1: XRD Diffractogram of Calcined Swan Mussel Shell

FTIR spectrum of the uncalcined and calcined Swan Mussel Shell was shown in (Fig. 2 & 3). The spectrum revealed the presence of carbonate ions at 1468.6 cm^{-1} , 711.9 cm^{-1} and 857.3 cm^{-1} , attributed to out-of-plane and in-plane vibrations of CO₃²⁻ (Gjoshi *et al.*, 2015). The presence of CO₃²⁻ ions decreases with increasing heat

treatment (Anjaneyulu *et al.*, 2015). This is in evidence by the IR-spectrum in which the band at 1468.6 cm^{-1} is broader in uncalcined shell and weaker (1408.9 cm^{-1}) in the calcined shell. Likewise, the appearance of a new peak at 3641.6 cm^{-1} in the spectrum of the calcined shell indicates the formation of Ca(OH)₂ due to hygroscopic nature of

CaO (Ikbalet *et al.*, 2018), very similar absorption peaks were also reported by Rajesh *et al.*, (2013) and Muhammad *et al.*,

(2021) for fresh water bivalve mollusk and snell shell respectively.

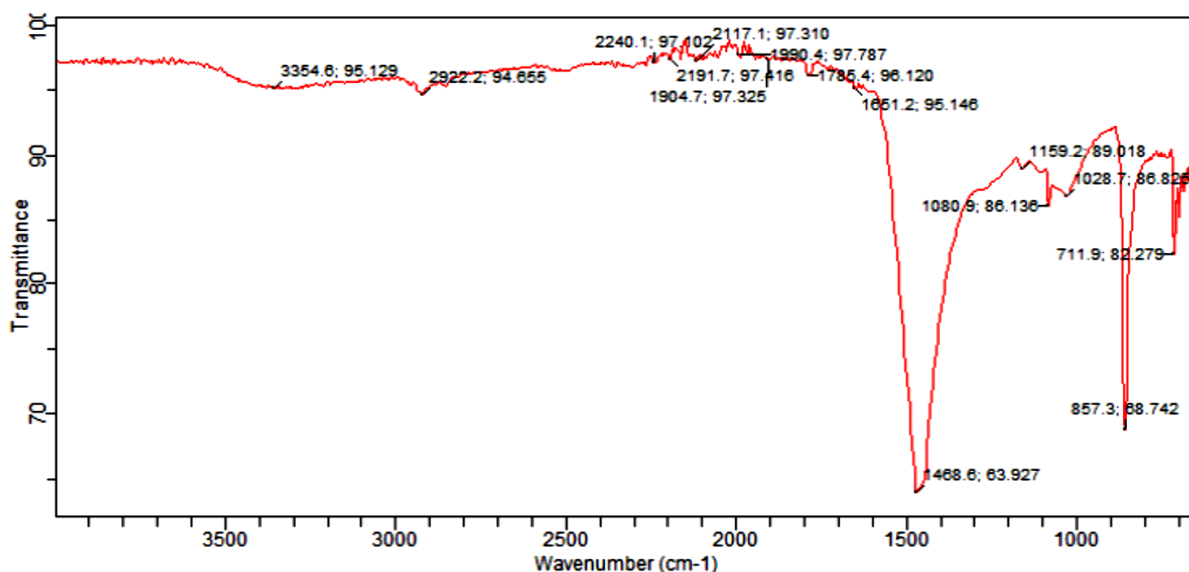


Figure 2: FTIR Spectra of Uncalcined Swan Mussel Shell

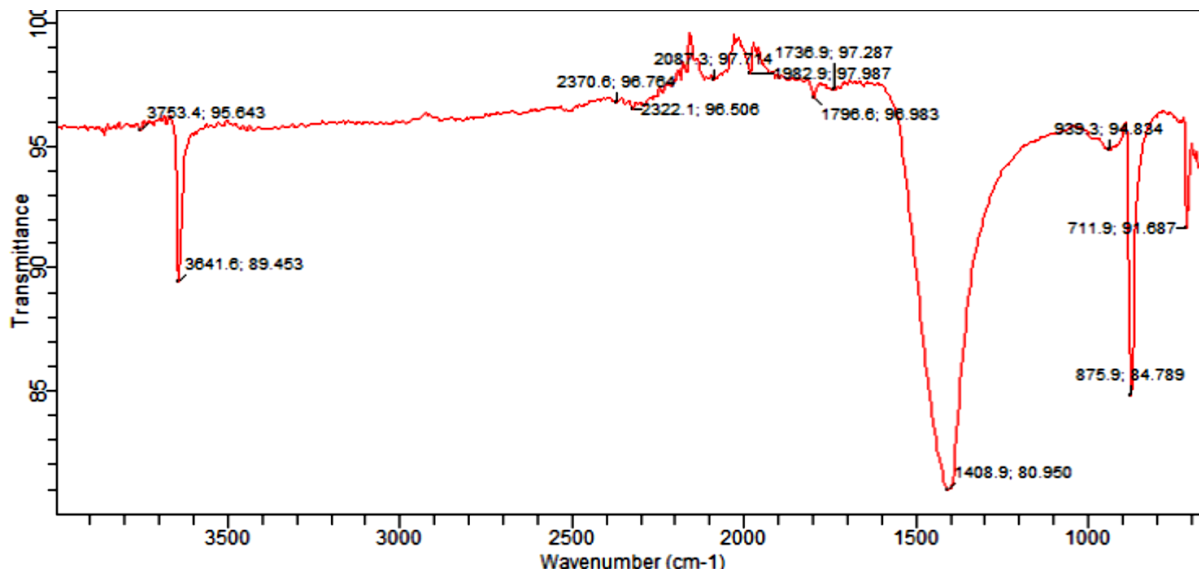


Figure 3: FTIR Spectra of Calcined Swan Mussel Shell

Optimization Process and Statistical Analysis

The result of analysis of variance (ANOVA) which was carried out to determine the model fitness as well as the significant effect of individual terms and their interaction on the biodiesel yield were presented in Table 4 & 5. The goodness of fit of the experimental data in the chosen model can be explained by the R² value (0.9550). This value is close to unity, implying that the experimental data fit the model equation linearly. The predicted and adjusted R² values are 0.7600 and 0.9025, respectively. These values are in good agreement with each other, with a difference of 0.1425, which is below the maximum allowed difference of 0.2. The lack of fit was insignificant relative

to the pure error (p-value = 0.366). With exception of solvent/oil ratio (C), quadratic terms, and two interaction terms (AC & BD), all other terms have a statistical significant effect on the biodiesel yield. Equ. (3), is the model equation developed when insignificant terms were eliminated. Solvent/oil ratio (C) was included in the equation to make the model hierarchical

$$Y_1 = -1694 + 61.15 A + 3.400 B + 2.52 C + 23.7 D - 0.5066 A^2 - 0.00332 B^2 + 15.10 D^2 - 0.0597 AB - 0.096 AC - 1.400 AD + 0.0406 BC \tag{3}$$

Where A: reaction temp (°C), B: reaction time (min), C: solvent/oil ratio and D: catalyst amount (g).

Table 3: Experimental Runs and Results of Biodiesel Yield

Reaction Temperature (°C)	Reaction time (min)	Oil to solvent ratio	Catalyst amount (wt%)	BT YIELD (%)	BI YIELD (%)
55	90	1:6	1.5	75.03	75.10
55	60	1:12	2	80.22	59.20
55	90	1:9	1	87.87	66.00
50	60	1:6	1.5	75.97	62.80
55	60	1:9	1.5	87.5	67.00
50	60	1:9	2	78.75	52.70
60	60	1:9	2	60.67	71.86
50	60	1:12	1.5	77.31	67.50
50	90	1:9	1.5	85.21	76.00
50	60	1:9	1	80.96	58.37
60	90	1:9	1.5	60.22	87.50
55	90	1:9	2	83.94	67.32
55	60	1:9	1.5	82.5	66.00
60	60	1:9	1	76.88	62.60
55	60	1:6	2	85.15	54.82
50	30	1:9	1.5	58.89	66.29
55	90	1:12	1.5	84.65	72.50
60	30	1:9	1.5	69.72	75.00
60	60	1:6	1.5	63.27	74.43
55	30	1:12	1.5	74.29	69.20
55	60	1:12	1	85.32	57.40
60	60	1:12	1.5	58.85	79.90
55	60	1:6	1	93.47	54.00
55	30	1:6	1.5	79.28	63.60
55	30	1:9	1	88.75	55.90
55	30	1:9	2	76.25	57.80
55	60	1:9	1.5	86.5	66.20

Table 4: Results of Analysis of Variance for Biodiesel Produced by Transesterification

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	14	2723.55	194.539	19.08	0.000
Linear	4	552.03	138.006	13.54	0.000
A	1	314.98	314.983	30.90	0.000
B	1	73.71	73.706	7.23	0.000
C	1	0.20	0.018	0.00	0.967
D	1	163.32	163.319	16.02	0.002
Square	4	1451.73	362.932	35.60	0.000
A ²	1	865.98	865.980	84.94	0.000
B ²	1	111.26	111.264	10.91	0.006
C ²	1	55.60	55.599	5.45	0.038
D ²	1	144.14	144.144	14.14	0.003
Interaction	6	719.80	119.966	11.77	0.000
AB	1	320.77	320.768	31.46	0.000
AC	1	28.94	28.944	2.84	0.118
AD	1	272.25	272.250	26.70	0.000
BC	1	53.36	53.363	5.23	0.041
BD	1	18.36	18.361	1.80	0.204
CD	1	26.11	26.112	2.56	0.135
Error	12	122.34	10.195		
Lack-of-Fit	10	111.67	11.167	2.09	0.366
Pure Error	2	10.67	5.333		
Total	26	2845.89			

S = 3.09284 R-sq = 95.50%, R-sq(adj) = 90.25%, R-sq(pred) = 76.00%

Interaction Effect of Process Parameters on the Biodiesel Yield (Transesterification)

Figure (3b) showed the contour plot for the interaction effect between reaction time and temperature on the biodiesel yield. Methanol/oil ratio and catalyst amount kept at 9:1 and 1.5 g, respectively. The figure shows that increasing the reaction time from a low level (30 min) to a high level (90 min) resulted in a corresponding increase in methyl ester yield. Similarly increase in reaction temperature leads to increase in the yield, with a yield greater than (> 85%) being achieved between 52° to 55°. Further increases in reaction temperature were observed to reduce ester content at high levels of reaction time. This may be due to the evaporation of methanol in the longer reaction time. The dependence of biodiesel yield on reaction temperature and catalyst loading is shown in (Fig. 3a). Reaction time (B) and methanol/oil (C) molar ratio were fixed at 60 mints and 9:1. The figure demonstrates that catalyst loading has minor effect on the yield. Interaction of reaction temperature and catalyst loading shows negative effect on the esters yield (Equ. 3), which means influence of reaction temperature and catalyst loading reduces the esters yield.

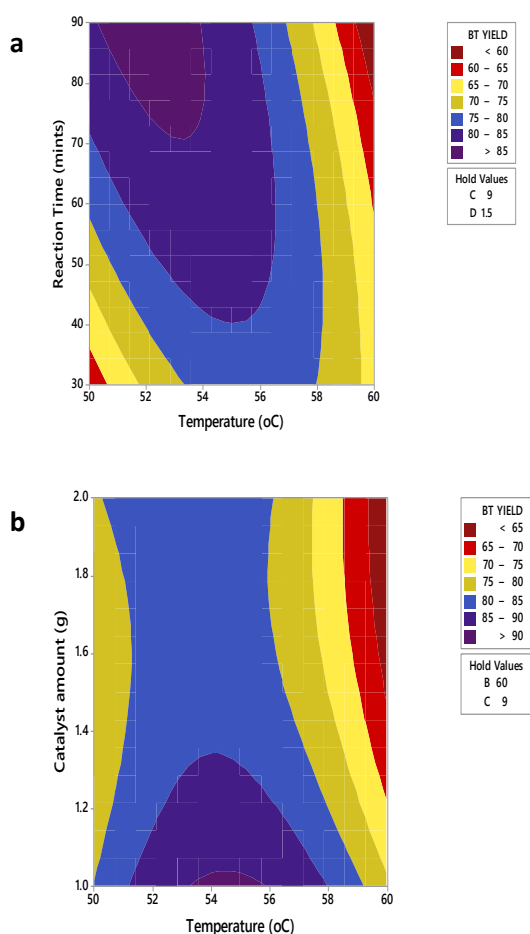


Figure 4: Contour Plot Showing Interaction Effect of Reaction Time with Temperature (a) And Catalyst Amount with Temperature (b) On Biodiesel Yield.

Table 5 Results of Analysis of Variance for Biodiesel Produced by Interesterification

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	11	1881.69	171.063	124.17	0.000
Linear	4	692.7	173.175	125.71	0.000
A	1	381.36	381.358	276.83	0.000
B	1	267.34	267.337	194.06	0.000
C	1	36.57	36.566	26.54	0.000
D	1	7.44	7.438	5.4	0.035
Square	3	1114.32	371.44	269.63	0.000
A ²	1	161.85	161.854	117.49	0.000
B ²	1	141.08	141.081	102.41	0.000
D ²	1	533.18	533.184	387.04	0.000
2-Way Interaction	4	74.67	18.668	13.55	0.000
AB	1	1.96	1.955	1.42	0.252
AC	1	0.15	0.148	0.11	0.748
AD	1	55.76	55.76	40.48	0.000
BC	1	16.81	16.81	12.2	0.003
Error	15	20.66	1.378		
Lack-of-Fit	13	20.12	1.548	5.71	0.159
Pure Error	2	0.54	0.271		
Total	26	1902.36			

S = 1.17371 R-sq = 98.91%; R-sq(adj) = 98.12%; R-sq(pred) = 94.99%

The results of analysis of variance (ANOVA) for interesterification of the seeds oil is shown in (Table 5). The terms included in the Model F-value of 124.17 having p-value of 0.000 shows that the model is significant at the chosen confidence level (95%). Positive coefficients of C, D, B² indicates the terms linearly influenced the esters yield while linear effect of reaction temperature (A), reaction time (B), square effect of reaction time (B), catalyst amount and interaction effect of AD & BC have negative influence on the yield. The model R² value of 0.9891 explained 98.91% of the variability in the data. The predicted and adjusted R² values are 0.9499 and 0.9812 respectively. The values are in good agreement with each other with difference of 0.0392 which is below the maximum allow difference of 0.2. Equ. (4) is the model equation generated after removal of insignificant terms.

$$Y_2 = 660.8 - 23.97 A - 0.2842 B + 1.949 C + 32.6 D + 0.2078 A^2 + 0.005388 B^2 - 37.71 D^2 + 1.493 AD - 0.02278 BC \tag{4}$$

Interaction Effect of Process Parameters on Biodiesel Yield (Interesterification)

Catalyst amount and reaction temperature shows significant interaction effect on the esters yield (Fig. 4a). The highest conversion of triglycerides was obtained at catalyst amount between 1.4 – 1.8 g, where over 75.0 % conversion was achieved at high reaction temperature.

Further increase in the catalyst loading above 1.8g, appeared to cause declined in the yield. As seen in (Fig. 4b), yield increases with an increase in both parameters. The reaction rate increases with reaction time. At low levels of reaction time, the reaction is slow resulting in low conversion. Over time, the reaction starts to accelerate and biodiesel yield increases. This observation suggests that a longer reaction time enhances the rate of the conversion.

Product Analysis and Characterization

The data are mean ± standard deviation of triplicate result. BT: biodiesel produced by transesterification; BI: biodiesel produced by interesterification

The fuel properties of the esters produced are shown in (Table 6). Both the properties of transesterified and interesterified seed oil were highly comparable and fall within the limit of ASTM specification with exception of flash point for the FAME produced by transesterification of the oil. Kinematic viscosity is one of the most important fuel properties of biodiesel. Kinematic viscosities of $3.70 \pm 0.23 \text{cst}$ and $4.5 \pm 0.05 \text{cst}$ were recorded for transesterification and interesterification of the oil, and it can be observed that the values are within the range (1.9-6) specified by ASTM D6751. The presence of water can affect fuel energy content, form bacteria in the fuel tanks and lead to deterioration of fuel chemical structure, etc. (Deka 2011). The moisture content of the produced biodiesels was low ($0.02 \pm 0.00\%$) an indication that the diesel produced is clean, may not form free fatty acid due to hydrolysis and may have minimal microbial growth during storage (Gerpen *et al*, 2004). The acid value of the produced esters was found to be less than 0.5, an indication of minimal corrosion. The pour point, which is the temperature at which the fuel contains some agglomerated crystals that it no longer flow, was found to be $-5.0 \text{ }^\circ\text{C}$, suggesting that it can be used as a fuel at low temperatures.

9-hexadecenoic, hexadecanoic and stearic acid methyl esters were found to be the dominant fatty acids esters (Fig. 6). Others esters detected are octanoic, eicosanic, cis-11-eicosenoic and 7, 10-octadecadienoic esters but in little quantities in all the methods. On comparison it can be observed that more methyl esters (86.93%) were obtained by transesterification than the chemical interesterification with total esters yield of 85.03%, this could be as a result of higher reactivity of methanol with triglyceride than methyl acetate with the triglyceride.

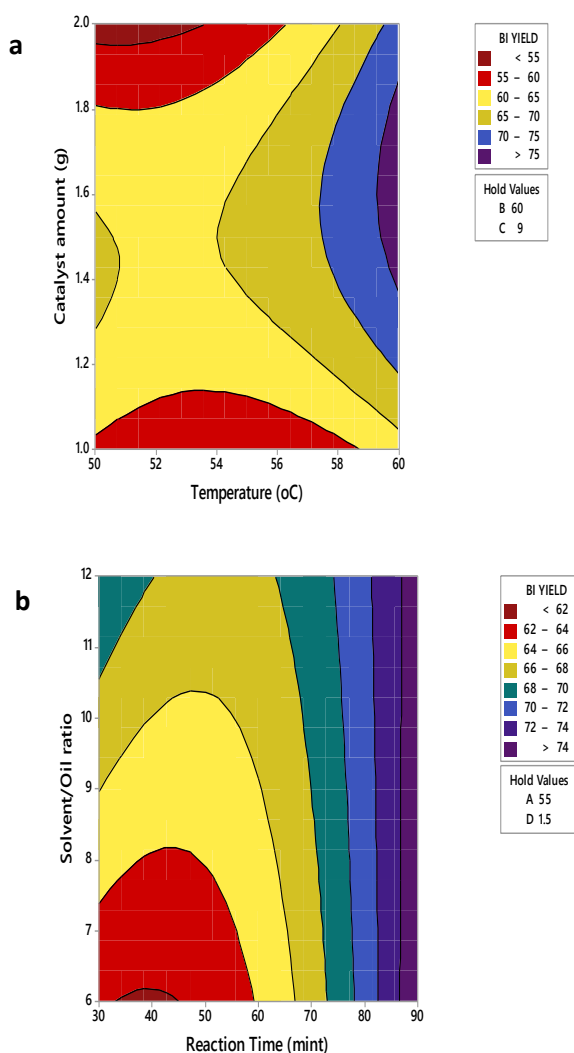


Figure 5: Contour Plot Showing Interaction Effect of Catalyst Amount with Temperature (a) And Solvent/Oil Ratio with Reaction Time (b) On Biodiesel Yield.

Table 6: Physicochemical Properties of *Rothmannialongiflora* Methyl Esters

Parameters	BT	BI	ASTM
Methyl Ester yield (%)	87.20	75.14	
Specific Gravity	0.85±0.01	0.84±0.01	0.81-0.90
Kinematic viscosity (cst) at 40°C	3.70±0.23	4.5±0.05	1.9-6.0
Moisture content (%)	0.02±0.00	0.02±0.00	0.05% max
Suphated ash (%)	0.05±0.01	0.05±0.01	
Flash point (°C)	180±0.58	170±0.55	100-170
Pour point (°C)	-5±0.00	-5±0.00	-15-10
Acid value(mgKOH/g)	0.43±0.01	0.44±0.012	0.8
Iodine value(gI ₂ /100g)	44.43±0.01	44.74±0.01	
Cetane number	63.81±0.03	63.86±0.008	48-65
Copper strip corrosion	1b	1b	No.3
High Heating Value (MJ/kg)	42.06±0.013	41.51±0.013	

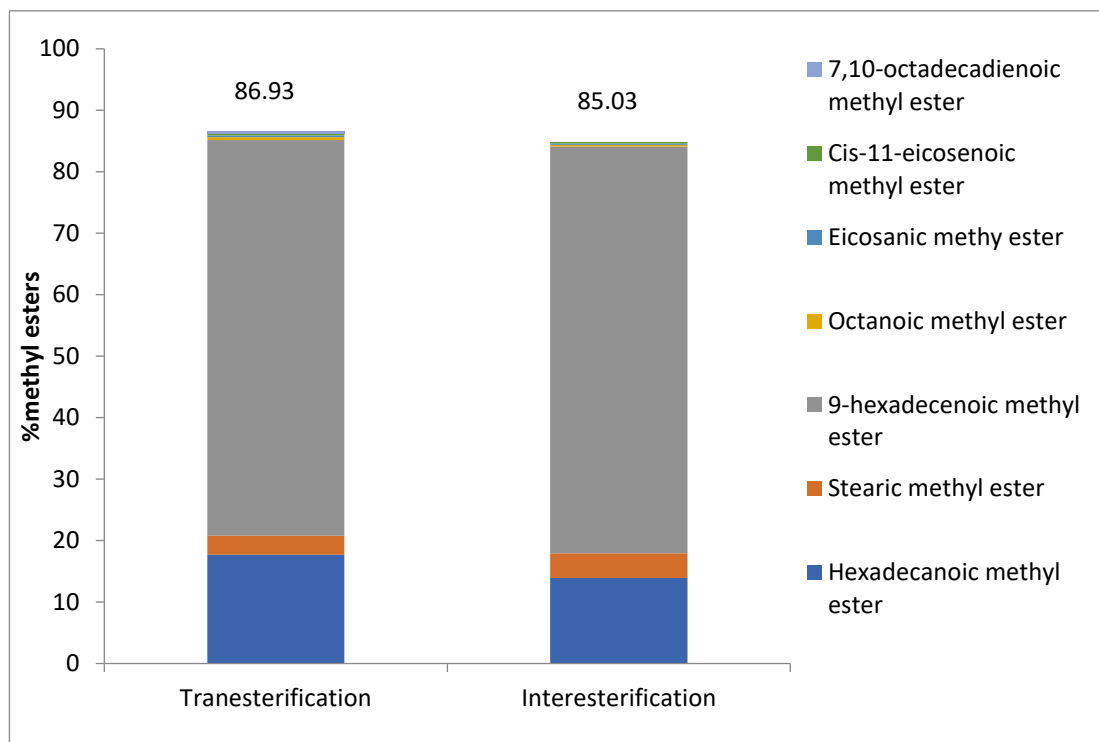


Figure 6: Fatty Acids Methyl Esters Composition

CONCLUSION

In this study, we transesterified and transesterified *Rothmannia longiflora* seed oil using calcined swan mussel shells. The seed oil was successfully converted to biodiesel. The experimental results revealed that the reaction time, reaction temperature, solvent/oil ratio and catalyst amount have significant effects on the biodiesel yield. The optimum conditions for the biodiesel production are; 1:6, methanol/oil ratio, 1.0g, catalyst amount, 60 minutes, reaction time and 55°C, reaction

temperature, achieving maximum yield of 93.47 % for transesterification while for interesterification, the optimum yield of 87.50 % was obtained at catalyst amount of 1.5g, reaction temperature of 60°C, reaction time of 90 minutes and methyl acetate/oil ratio of 9:1. The fuel characteristic of the produced esters shows no significant variation and was within the specified range of ASTM standard. Therefore, *Rothmannia longiflora* Seed oil may be considered as good raw material for biodiesel production.

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