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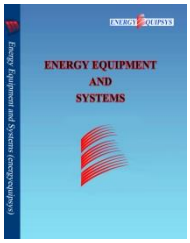

Biodiesel quality improvement by mixing the Azadirachta indica and Moringa stenopetala seed oil (In Situ Hybridization) via transesterification process

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ABSTRACT

Biodiesel is conceived as a renewable, environmentally benign, alternative, cleaner, biodegradable, and greener fuel derived from vegetable oils, animal fats, and algae, superseding non-renewable petroleum products. Hybridization of vegetable oil has not been widely examined to produce biodiesel with ameliorated quality. Hence, this study aims at hybridizing Azadirachta indica and Moringa stenopetala seed oils to produce biodiesel with improved quality. The Box-Behnken Design was used in the design of the experiment and analysis of results. Catalyst dose, oil hybridization ratio, and reaction time were considered experimental factors with 3 levels: -1(low), 0(medium), and +1(maximum); whereas, agitation speed, oil-to-methanol molar ratio, and reaction temperature were kept constant. The oil hybridization ratios were: $A_{25}M_{75}$ (25% A. indica and 75% M. stenopetala oil), $A_{50}M_{50}$ (50% A. indica and 50% M. stenopetala oil), and $A_{75}M_{25}$ (75% A. indica and 25% M. stenopetala oil); reaction times: 30, 60, and 90 minutes; and sodium hydroxide doses: 0.50, 2.50, and 3.50%w/v with their respective biodiesel products. Seventeen experimental runs were conducted and the second-order model was developed with $P < 0.0001$ (significant). The quality and adequacy of the model were determined by analysis of variance (ANOVA) and determination coefficients: R^2 = 0.9986, Pred-R^2 = 0.9830, Adj-R^2 = 0.9968 and lack of fit test with P = 0.1150 (insignificant), at 5% the least significant difference. The quality of synthesized biodiesel was improved and in agreement with the standard specification for biodiesel, and hence, it can be employed in diesel engines.

Keywords: Biodiesel, Fuel Characteristics, Oil Hybridization, Non-Edible Oil, Transesterification

1. Introduction

Energy is a fundamental, vital ingredient and the backbone for economic growth, social and environmental welfare as well and it plays a great role in achieving the civilization of mankind worldwide. The existence of mankind, global civilization, and socio-economic growth necessitated the sustainable production and utilization of energy [1-3]. The extraction and utilization of fossil fuels (i.e., coal, natural gas, liquefied petroleum gas, and nuclear energy) have been regarded as the main source of energy supply for several years

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[4, 5]. Nonetheless, when used, fossil fuels are environmentally unfriendly (harmful gaseous emissions into atmosphere), non-renewable, scarce (limited crude oil reserves), and unevenly distributed across the globe [6-8]. Furthermore, the rise in energy demand, the geometric increase in global population, the markedly decreasing sources of non-renewable energy, environmental pollution, greenhouse gas emissions, gas flaring, fluctuations in fuel prices, the hastened global warming, and the global energy crisis are challenges associated with the use of fossil fuels [9-11]. To overcome these challenges, the world is strongly looking forward to sustainable production and utilization of biofuels thereby superseding the non-renewable petroleum products [12-14].

Biodiesel has been identified as a suitable fuel that is a greener, eco-friendly, cleaner, renewable, and alternative fuel that can replace the non-renewable and limited oil reserves [4, 15, 16]. It is a liquid biofuel derived and synthesized from biological resources like non-edible vegetable oils, algae, and animal fats through the transesterification process [17, 18]. It is a fatty acids mono-alkyl esters mixture produced from potential feedstocks like triglycerides of the non-edible vegetable oils undergoing the transesterification reaction by employing appropriate alcohol and a basic-catalyst providing glycerol ($C_3H_8O_3$) as a byproduct of the reaction process [19-21]. Biodiesel synthesis through hybridization of feedstocks has not been extensively studied all over the globe. The process of hybridization in the transesterification reaction involves a novel combination or mixing of two feedstocks (bi-hybridization), three feedstocks (tri-hybridization), or many feedstocks (poly-hybridization) at various ratios to synthesize a new product with improved attributes derived from the original feedstocks [16, 22]. Thus, blending of feedstocks before the transesterification process (i.e., in situ hybridization) improves the quality of the resulting fuel. This approach is new and hasn't been tried extensively in previous work and paves the way for biofuel processing industries to produce and develop biodiesel products from various feedstocks. Hybridizing feedstocks like Azadirachta indica and Moringa stenopetala seed oils to synthesize biodiesel will enhance fuel stability and engine performance (fuel combustion and compression ignition) during utilization [23, 24]. Moreover, hybridization of appropriate feedstocks results in improved and ameliorated biodiesel quality that is better than the one synthesized from a mono-feedstock [22]. Vegetable oil that constituents sufficient mono-unsaturated fatty acids alongside the fatty acid profiles of oil (a higher percentage of oleic acid) is considered a suitable ingredient to obtain biodiesel exhibiting good fuel quality [23, 25, 26]. For instance, the crude oil obtained from Azadirachta indica seeds constituents about 51% of oleic acid ($C_{18}H_{32}O_2$) [25]; and this will be improved and ameliorated upon blending with the higher oleic acid content of 76% $C_{18}H_{32}O_2$ in the oil extracted from Moringa stenopetala seeds [26]. In light of this explanation, the experiment was conducted to examine the effectiveness and suitability of the hybridized non-edible oils of A. indica and M. stenopetala seeds, thereby synthesizing the improved biodiesel through the transesterification reaction as a new approach to product design, processing, developing, and utilizing the biofuel.

2. Methodology

2.1. Sample collection and preparation

The Azadirachta indica seeds were collected in January 2023 from Amibara, Afar Regional State, in the eastern part of Ethiopia. The sample collection area has a latitude of 09°15′ North, a longitude of 40°10′ East, and an altitude of 740 m a.s.l. The Moringa stenopetala seeds were collected in January 2023 from Sidama Regional State, south part of Ethiopia, situated at the latitude of 7°4′ north, longitude of 38°24′ east, and an altitude of 1,712 m a.s.l. The seeds were taken to the Laboratory of Addis Ababa Institute of Technology. Then, the collected seeds were cleaned of unwanted substances like stones, weed seeds, molds, other potential contaminants, and the seeds were decorticated (i.e., the seed kernels were separated from the husk). The seed kernels were then subjected to drying at about 100±5°C for twenty-four hours using the oven dryer until the moisture in the seed kernels was below five percent [27]. Finally, the amount of moisture content in the seed kernels was computed as

$$\text{Moisture content } (\%) = \frac{W0 - W1}{W0} * 100 \quad (1)$$

$\mathbf{W_0}$ - sample weight (g) before drying; $\mathbf{W_1}$ – sample weight after drying

For the ease of the oil extraction process, the dried seed kernels were subjected to comminution using a hammer mill at various ranges of particle sizes, 0.2-0.4 mm, 0.4-0.6 mm and 0.6-0.8 mm thereby enhancing the surface area-to-volume ratio of the particles. The vibrating sieve shaker was used to obtain the required range of particle size and then the powder of each sample was labeled and kept appropriately until dispatch.

2.2. Extraction and purification of oil for biodiesel synthesis

In the oil extraction process, the Soxhlet extractor was used with the extraction solvent n-Hexane (n-C_6H_{14}). A 150 g sample powder was put into the extraction thimble and placed in the Soxhlet. A measured 750 mL of n-C_6H_{14} was added into a flask of 1000 mL and attached to the Soxhlet apparatus. The reflux condenser was also attached to the upper part of the Soxhlet, and the complete extraction set-up was adjusted and placed in the boiler. The temperature of the boiler was set at 74 °C, which is relatively higher than the boiling point of n-Hexane, and oil extraction was allowed to take place continuously for about four hours until the desired amount of crude oil was obtained. As heating continues, the temperature of extraction rises gradually, and the solvent is vaporized and refluxed to the sample in the thimble by the condenser. Then, the rotary evaporator was employed to separate the mixture of crude oil and the solvent (n-hexane) (Fig. 1).

The yield of oil (%) at every extraction step was computed as [28, 29]

$$\text{Yield of oil } (\%) = \frac{mass\ of\ extracted\ oil(g)}{mass\ of\ seed\ kernel(g)} * 100 \quad (2)$$

The obtained oil was subjected to the oil purification process by employing the centrifuge to remove unnecessary substances like suspended fine particles. The trace solvent or n-hexane was also liberated and removed from the crude oil by heating and boiling the oil. Then, before the transesterification reaction, the crude oils were subjected to the oil degumming, esterification reaction (acid pretreatment), and neutralization processes for further purification and refining of the extracted oil.

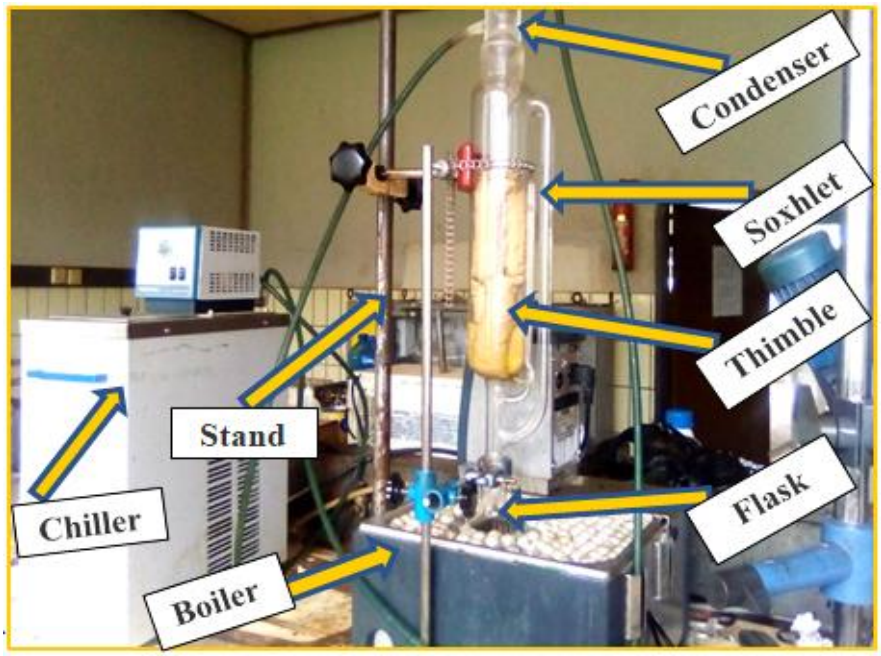


Fig. 1. Oil extraction set-up using the solvent method

In the oil degumming process, phospholipids and gums were removed from A. indica and M. stenopetala oils. A 2 %v/v (distilled water/oil) was poured into extracted oils and stirred for five minutes in a hot water bath at 65 °C. Then, a 1.3 %v/v (phosphoric acid/oil) was added to the oil and distilled water mixture and stirred (i.e., at 200 revolutions per minute) in the hot water bath for 1 hour. The degummed oils were esterified to diminish the amount of free fatty acid (FFA) content. In the esterification reaction, oils were heated for ten minutes at 60 °C in a hot water bath. Then, 45 %v/v methanol was poured into esterified oil and stirred for five minutes. A 1.2 %v/v sulfuric acid was added to the oil mixture and stirred in the hot water bath for an additional 45 minutes. The mixture was added into a separating funnel and allowed to settle down for four hours. The refined oil was obtained from the bottom portion of the mixture in the funnel and stored in an air-tight waterproof container. The refined oil was neutralized by adding 0.1 N sodium hydroxide solution into the esterified oil, heated at 60 °C, and stirred at 250 rpm for about thirty minutes. Finally, the neutralized oil mixture was allowed to settle down using the separating funnel thereby obtaining the neutralized oils for the synthesis of the biodiesel [30].

2.3. Biodiesel synthesis via base-catalyzed transesterification reaction

- Experimental design

The Response Surface Methodology (RSM), Box-Behnken Design (BBD), was employed in the design of the experiment. The operational variables in the study were: oil hybridization ratio (A), reaction time (B), and catalyst dose (C), with three experimental levels for each variable, low (-1), medium (0), and high (+1) and the yield of biodiesel (%) was the response of the experiment (Table 1). The influence of operational variables on the response was examined along with their interaction effects. A total of seventeen experimental runs were conducted (i.e., three factors with three levels and five central replication points) following the BBD method [31]. Thus;

$$N = K^2 + K + C_p \quad (3)$$

where, N is the total experimental run, K is the number of operational variables and C_p is the central replication point.

The refined crude oils of Azadirachta indica (A) and Moringa stenopetala (M) seeds were obtained in various proportions and allowed to undergo the transesterification process at different operational variables, such as oil hybridization ratio, reaction time, and catalyst dose. The considered reaction times (in minutes) were: 30, 60, and 90 minutes; the catalyst doses were: 0.50, 2.00, and 3.50 %w/v; and the oil hybridization ratios (%w/w) were: $A_{25}M_{75}$ (25% A. indica seed oil hybridized with 75% M. stenopetala seed oil), $A_{50}M_{50}$ (50% A. indica seed oil hybridized with 50% M. stenopetala seed oil), and $A_{75}M_{25}$ (75% A. indica seed oil hybridized with 25% M. stenopetala seed oil), with their respective yields of biodiesel (BAM) products: $BA_{25}M_{75}$, $BA_{50}M_{50}$, and $BA_{75}M_{25}$. Besides, the other operational variables like agitation speed (rpm), oil-to-methanol molar ratio, and reaction temperature (°C) were allowed to be constants during experimentation (Table 2).

Table 1. Levels of experiment for the considered factors of study

Symbols	Levels				
	Variables	Units	-1 (Low)	0 (Medium)	+1 (High)
A	Oil hybridization ratio	%w/w	25	50	75
B	Reaction time	minutes	30	60	90
C	Catalyst dose	%w/v	0.50	2.00	3.50

Table 2. The influence of factors of study on yield (%) of synthesized biodiesel from hybridized oils of A. Indica and M. Stenopetala seeds

Run	Factor A	Factor B	Factor C				Response Yield of biodiesel (%)	
	Oil hybridization ratio, A_xM_y (%w/w)	Reaction time (minute)	Catalyst dose (%w/v)	Agitation speed (rpm)	Oil-to-methanol molar ratio	Reaction temperature (°C)	Predicted value	Actual value
1	$A_{50}M_{50}$ (0)	60 (0)	2.00 (0)	300	1:6	60	87.91	88.00
2	$A_{75}M_{25}$ (+1)	90 (+1)	2.00 (0)	300	1:6	60	86.41	86.54
3	$A_{50}M_{50}$ (0)	60 (0)	2.00 (0)	300	1:6	60	87.91	87.42
4	$A_{50}M_{50}$ (0)	30 (-1)	0.50 (-1)	300	1:6	60	60.48	60.96
5	$A_{25}M_{75}$ (-1)	60 (0)	3.50 (+1)	300	1:6	60	89.55	90.00
6	$A_{50}M_{50}$ (0)	60 (0)	2.00 (0)	300	1:6	60	87.91	88.36
7	$A_{25}M_{75}$ (-1)	90 (+1)	2.00 (0)	300	1:6	60	91.97	92.00
8	$A_{75}M_{25}$ (+1)	60 (0)	0.50 (-1)	300	1:6	60	73.10	72.65
9	$A_{75}M_{25}$ (+1)	60 (0)	3.50 (+1)	300	1:6	60	83.65	84.00
10	$A_{75}M_{25}$ (+1)	30 (-1)	2.00 (0)	300	1:6	60	70.27	70.24
11	$A_{50}M_{50}$ (0)	60 (0)	2.00 (0)	300	1:6	60	87.91	87.78
12	$A_{50}M_{50}$ (0)	60 (0)	2.00 (0)	300	1:6	60	87.91	88.00
13	$A_{50}M_{50}$ (0)	30 (-1)	3.50 (+1)	300	1:6	60	72.76	72.44
14	$A_{25}M_{75}$ (-1)	60 (0)	0.50 (-1)	300	1:6	60	75.61	75.26
15	$A_{25}M_{75}$ (-1)	30 (-1)	2.00 (0)	300	1:6	60	73.13	73.00
16	$A_{50}M_{50}$ (0)	90 (+1)	3.50 (+1)	300	1:6	60	90.22	89.74
17	$A_{50}M_{50}$ (0)	90 (+1)	0.50 (-1)	300	1:6	60	78.02	78.34

A_xM_y- hybridized oils at various proportions; x and y were oil mixing ratios; rpm- revolutions-per-minute; A- Azadirachta indica seed oil; M- Moringa stenopetala seed oil; %w/w- percentage of weight-by-weight; %w/v- percentage of weight-by-volume

2.4. Experimental procedure for the transesterification process

The refined oils of A. indica and M. stenopetala were hybridized and trans-esterified into methyl esters by applying the appropriate basic catalyst, sodium hydroxide (NaOH) flakes, and methanol (CH_3OH). A three-necked round-bottom glass reactor was employed to conduct the base-catalyzed transesterification reaction. A 0.50, 2.50, and 3.50 %w/v sodium-methoxide (CH_3ONa) solution were used in the transesterification reaction (Table 1). The 100 g hybridized oil was added into the glass reactor. Then, the prepared basic catalyst, CH_3ONa solution, was poured turn-by-turn into the glass reactor containing a sample of the hybridized oils (%w/w) with a 1:6 oil-to-methanol molar ratio. The mixture was allowed to undergo the transesterification reaction at a temperature of 60 °C and an agitation speed of 300 revolutions per minute (rpm) for 30, 60, and 90 minutes, respectively. To quench the reaction from occurring further (i.e., beyond the prescribed reaction time), about three droplets of hydrochloric acid were mixed with the solution in the glass reactor. This procedure was repeated for all experimental runs, and their corresponding biodiesel was obtained as per the information provided below (Table 2). The trans-esterified solution was transferred into a funnel and kept for 24 hours to remove glycerol. Then, the obtained biodiesel was washed four times at a 3:1 biodiesel-to-distilled water ratio and heated at 60 °C for further purification of the fatty acid methyl ester (FAME) from traces of NaOH and other impurities. Then, the purified FAME was heated at 100±5 °C thereby removing traces of washing water and subjected to the evaluation of physicochemical characteristics and Fourier transform infrared radiation (FTIR) spectrometer.

2.5. Evaluation of the physicochemical characteristics of the synthesized FAME

Determination of the FAME physicochemical properties was conducted according to the American Society for Testing Materials [ASTM] D6751 and the European Committee for Standardization of Biodiesel (EN 14214). These properties include kinematic viscosity, higher heating value, specific gravity, cetane number, flash point, acid number, free glycerin, total glycerin, cloud point, water and sediment, methanol content, oxidation stability, pour point, carbon residues, magnesium, and calcium contents. Besides, the FTIR spectrometer was used to analyze functional groups of A. indica and M. stenopetala oils, and biodiesel synthesized from the hybridized

oils. The FTIR analysis was executed for each sample, using 5 ml of oils and biodiesel at a wave number ranging from 4000-400 with 1 cm^{-1} resolution.

3. Results and Discussion

3.1. The statistical analysis of synthesized biodiesel using the RSM

The RSM, Box-Behnken Design, was employed to analyze and interpret the experimental results. The analysis of variance (ANOVA), determination of coefficients of the developed model equation of the synthesized biodiesel from hybridized oils and regression analysis were carried out using the RSM, BBD method. Results of ANOVA were also used to evaluate the adequacy of the developed model equation. The coefficients of determination (R^2), predicted coefficient of determination (Pred-R^2), coefficient of variations (CV), F-test, and adjusted coefficients of determination (adj-R^2) were used to explain the quality and its statistical significance, provided that the main comparisons were carried out at 5% of the least significant difference (LSD).

The actual yield of biodiesel synthesized from each and every single experimental run (Table 2) was computed using [32]

$$\text{Yield of biodiesel } (\%) = \frac{\textit{Mass of produced biodiesel}}{\textit{Mass of hybridized oil}} * 100 \quad (4)$$

The obtained results indicated that the maximal biodiesel yield, 92.00%, was recorded for experimental run 7 (Table 2) at $A_{25}M_{75}$ (25%w/w Azadirachta indica oil was hybridized with 75%w/w Moringa stenopetala oil), 90 min of reaction time, and 2%w/v of catalyst dose; whereas, the minimal percentage of biodiesel yield, 60.96%, was obtained for the experimental run 4 at $A_{50}M_{50}$ (50%w/w Azadirachta indica oil was hybridized with 50 %w/w Moringa stenopetala oil), 30 min reaction time and 0.5 %w/v catalyst dose.

3.1.1. Development and analysis of the model equation for the synthesized FAME

The ANOVA results were used to develop the model equation and analyze its significance and suitability or fitness using the BBD.
Results of ANOVA showed that the model equation is significant with an F-value of 559.30 and a P< 0.0001. This shows only a 0.01% chance that the model F-value is large, and it could happen due to noise. The model terms with a P < 0.05 are considered significant. Thus, the model terms A, B, C, AB, AC, A^2, B^2, and C^2 are significant as the P-value < 0.05, whereas BC is not a significant term of the model as the P-value > 0.05 (Table 3).

Table 3. Analysis of variance for the synthesized FAME at different factors of study

Response	Yield of FAME					
ANOVA for Response Surface Quadratic Model [Partial Sum of Squares-Type III]						
Source	Sum of Squares	Degree of freedom	Mean square	F-value	P-value Prob > F	
Model	1316.49	9	146.28	559.30	< 0.0001	significant
A- Oil hybridization ratio	35.41	1	35.41	135.38	< 0.0001	
B- Catalyst dose	612.15	1	612.15	2340.59	< 0.0001	
C- Reaction time	299.76	1	299.76	1146.14	< 0.0001	
AB	1.82	1	1.82	6.97	0.0334	
AC	2.87	1	2.87	10.99	0.0129	
BC	$1.6*10^{-3}$	1	$1.6*10^{-3}$	$6.118*10^{-3}$	0.9398	
A^2	5.86	1	5.86	22.41	0.0021	
B^2	166.44	1	166.44	636.39	< 0.0001	
C^2	164.72	1	164.72	629.83	< 0.0001	
Residual	1.83	7	0.26			
Lack of Fit	1.36	3	0.	3.80	0.1150	not significant
Pure Error	0.48	4	0.12			
Cor Total	1318.32	16				

The insignificant terms of the model are not counted and would be diminished to ameliorate the model equation. The F-value of 'lack of fit'= 3.80 shows that 'lack of fit' is statistically insignificant with a P-value= 0.1150 in comparison to pure error. There is only an 11.50% chance that the 'lack of fit F-value' is large, and this could occur because of noise. The model fit summary was evaluated for the obtained experimental results, resulting in the selection of the second-order model equation with statistically significant terms (F-value of 464.53 at P < 0.000), where the model is not aliased (Table 4).

Moreover, the prediction coefficients (Pred-R^2 of 0.9830) were in agreement with the adjusted coefficients of determination (Adj-R^2=0.9968). The value of 'adequate precision' (80.284) measures the signal-to-noise ratio and the model desirability, and hence, the ratio of 80.284 shows an adequate signal for the model as it was greater than four (Table 5); and the model equation can be employed to navigate the design space for the production of biodiesel at various experimental variables.

The obtained value of R^2 (0.9986) shows that about 99.86% of the overall variations that occurred in the experimental responses (yield of FAME) were associated with the factors considered in the study. Besides, the minimum value of the coefficient of variations (0.63%), the ratio of the estimated standard error to the average value of biodiesel yield, indicated that the conducted experiment to synthesize biodiesel via the transesterification reaction was precise, reliable, and reproducible. Therefore, the model equation is considered reproducible and it can be used at various experimental variables with different levels to synthesize the hybridized FAME [31]:

The developed model equation with coded factors to synthesize the biodiesel according to BBD, Yield of biodiesel (Y):

$$Y = -1.18*A^2 - 6.29*B^2 - 6.25*C^2 + 1.67*A*B + 0.85*A*C - 0.020*B*C + 2.10*A + 8.75*B + 6.12*C + 87.91 \quad (5)$$

The diagnostics case statistics of the synthesized FAME:

Table 4. The developed model for the synthesized FAME at various experimental variables

Response	Yield of FAME					
	Sequential Model Sum of Squares [Type I]					
Source	Sum of Squares	Degree of freedom	Mean square	F-value	P-value Prob>F	
Mean vs Total	$1.128*10^5$	1	$1.128*10^5$			
Linear vs Mean	947.31	3	315.77	11.06	0.0007	
2FI vs Linear	4.70	3	1.57	0.043	0.9875	
Quadratic vs 2FI	364.48	3	121.49	464.53	< 0.0001	Suggested
Cubic vs Quadratic	1.36	3	0.	3.80	0.1150	Aliased
Residual	0.48	4	0.12			
Total	$1.141*10^5$	17	6712.42			

Table 5. The developed model adequacy measures for the synthesized FAME

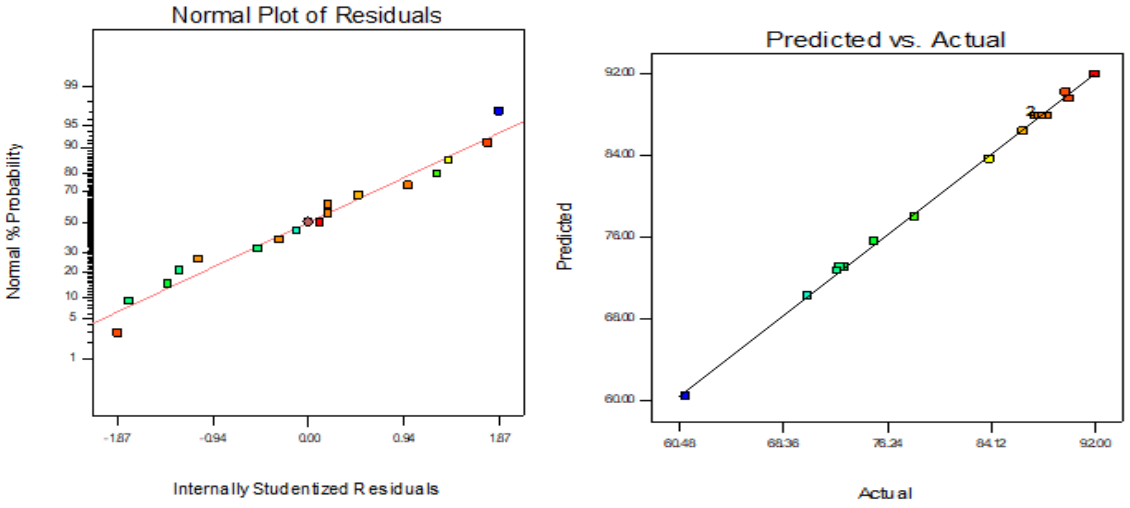
Standard Deviation	0.51	R^2	0.9986
Mean	81.	Adj-R^2	0.9968
C.V. (%)	0.63	Pred-R^2	0.9830
PRESS	22.42	Adeq Precision	80.284

R^2- coefficient of determination; C.V.(% - percentage of coefficient of variations;

To determine the model's properties and the nature of its statistical distribution, the plot of normal probability versus residual error was used following the BBD method. The obtained plot indicated the normal distribution of residual errors were in linear patterns (Fig. 2a). Therefore, for the developed model equation, a set of data points was anticipated to be in a linear pattern with no sign of abnormality involved in the experimental data distributions. In addition, the obtained coefficient of determination, R^2 = 0.9986 (a high correlation coefficient) showed that the predicted FAME yields were in agreement with actual experimental results (Table 6); and a set of obtained data well fits the model, offering a precise prediction of the desired response or yield of biodiesel for the conducted experiment while considering the range of experimental parameters (Fig. 2b).

Table 6. The diagnostics case statistics of synthesized biodiesel

Response				Yield of FAME		
Run order	Actual value (%)	Predicted value (%)	Residual	Leverage	Internally Studentized residual	Externally Studentized residual
1	88.00	87.91	0.088	0.200	0.192	0.179
2	86.54	86.41	0.130	0.750	0.494	0.465
3	87.42	87.91	-0.490	0.200	-1.076	-1.090
4	60.96	60.48	0.48	0.750	1.872	2.3
5	90.00	89.55	0.	0.750	1.760	2.182
6	88.36	87.91	0.0	0.200	0.979	0.976
7	92.00	91.97	0.029	0.750	0.112	0.104
8	72.65	73.10	-0.	0.750	-1.760	-2.182
9	84.00	83.65	0.35	0.750	1.379	1.495
10	70.24	70.27	-0.029	0.750	-0.112	-0.104
11	87.78	87.91	-0.130	0.200	-0.289	-0.269
12	88.00	87.91	0.088	0.200	0.192	0.179
13	72.44	72.76	-0.320	0.750	-1.266	-1.335
14	75.26	75.61	-0.35	0.750	-1.379	-1.495
15	73.00	73.13	-0.130	0.750	-0.494	-0.465
16	89.74	90.22	-0.48	0.750	-1.872	-2.3
17	78.34	78.02	0.32	0.750	1.266	1.335



a b

Fig. 2. a) Normal probability versus residual errors plot b) The predicted values versus actual experimental values plot

3.2. The effect of experimental factors on the yield of the FAME:

3.2.1. The effect of oil hybridization ratio and reaction time on the yield of the FAME

The results of the analysis of variance indicated that oil hybridization or mixing ratio and reaction time significantly affect yield of FAME with 'P-value = 0.0334', which is less than 0.05 (Table 3). The effect was obtained by keeping the catalyst dose at 2.00%w/v. The maximum biodiesel yield (92%) was obtained when oil hybridization ratio of $A_{25}M_{75}$ (mixture of 25 %w/w A. indica oil and 75 %w/w M. stenopetala oil) reacts with 2.00 %w/v of catalyst dose for about 90 minutes; whereas, the minimum biodiesel yield (73%) was obtained when the oil hybridization ratio ($A_{25}M_{75}$) reacted with a 2.00 %w/v catalyst dose for 30 minutes (Fig. 3). The response surface plot showed that the oil hybridization ratio decreases the yield of biodiesel and increasing the reaction time until an optimum point increases the yield of biodiesel and vice-versa. Nonetheless, a longer reaction time beyond the optimum point decreases the yield of FAME yield as the transesterification reaction is reversible.

3.2.2. The influence of oil hybridization ratio and catalyst dose on the FAME yield

The ANOVA result signified that the oil hybridization ratio and catalyst dose affect the yield of biodiesel significantly with 'P = 0.0129' (Table 3). The reaction time was kept constant at 60 minutes. The maximum yield of biodiesel (90%) was obtained when the oil hybridization ratio of $A_{25}M_{75}$ (mixture of 25%w/w A. indica oil and 75 %w/w M. stenopetala oil) reacted with 3.5 %w/v catalyst dose for 60 minutes, whereas, the minimum yield of biodiesel (72.65%) was obtained for the reaction of the oil hybridization ratio $A_{75}M_{25}$ (a mixture of 75 %w/w of A. indica oil and 25 %w/w of M. stenopetala oil) with 0.50 %w/v of catalyst dose (Fig. 4). The 3D plot showed that, for experimental factors taken into account, increasing the oil hybridization ratio diminishes the yield of biodiesel, and as the amount of catalyst rises from 0.50-3.50 %w/v, the FAME yield (%) will also be enhanced, and vice-versa.

3.2.3. The influence of reaction time and catalyst dose on the FAME yield

The experimental variables (reaction time and catalyst dose) affect the FAME yield significantly at $P < 0.0001$ (Table 3). The oil hybridization ratio was kept constant at $A_{50}M_{50}$ (a mixture of 50%w/w A. indica oil and 50 %w/w M. stenopetala oil) in order to observe the effect of the considered variables on the yield of FAME. The 3D plot of BBD signifies that increasing the reaction time and catalyst

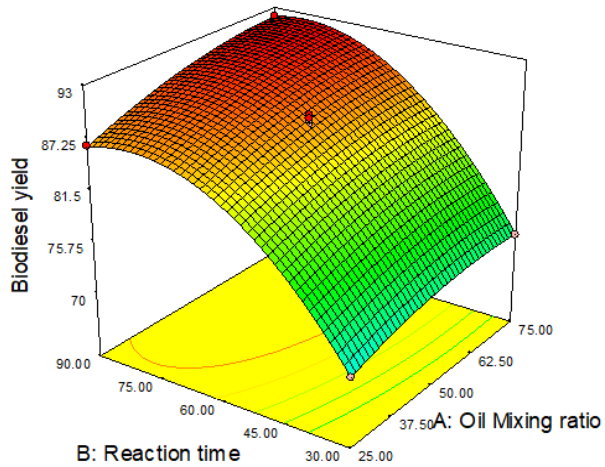


Fig. 3. The plot of oil hybridization ratio and reaction time effects on the FAME yield

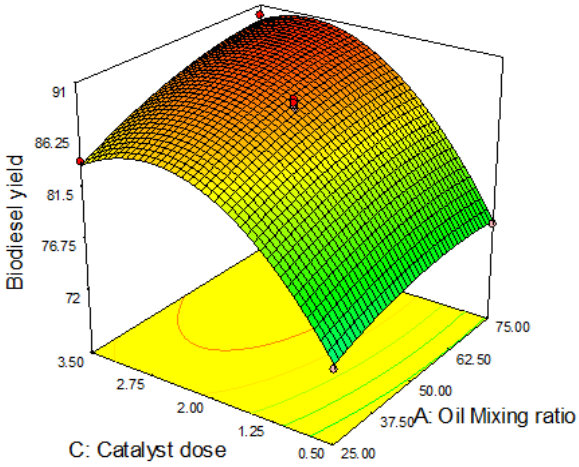


Fig. 4. The plot of oil hybridization and catalyst dose effects on the FAME yield

dose enhances the yield of FAME and vice-versa. The maximum yield of biodiesel (89.74%) was achieved when the transesterification reaction was conducted for 90 minutes with a 3.50 %w/w catalyst dose, and the minimum product yield (60.96%) was recorded when the experiment was conducted with a 0.50 %w/w catalyst for about 30 minutes (Fig. 5).

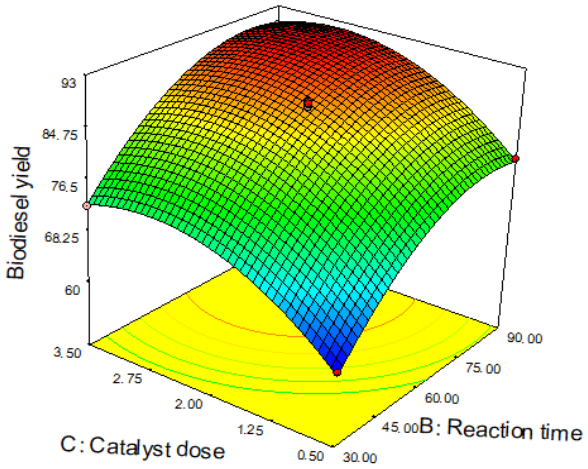


Fig. 5. The effect of reaction time and catalyst dose on the yield of FAME

3.3. Determination of the physicochemical characteristics of the synthesized FAME:

3.3.1. The Fourier transforms infrared radiation (FT-IR) spectrometer

The FT-IR spectrometer was used to analyze the functional group of the synthesized biodiesel or FAME, the A. indica, and M. stenopetala seed oils [33, 34]. The determination of FTIR was carried out at 1 cm^{-1} resolution for the transmission band ranging from 4000-400 cm^{-1} wave number using 5 ml samples of the biodiesel, A. indica, and M. stenopetala seed oils (Fig. 6). The existence of various functional groups in the extracted oils of Azadirachta indica and Moringa stenopetala seeds and the synthesized FAME were analyzed by peak points of the infrared radiation spectrum for the specified range of a wave number [35]. The transesterification reaction induces the transition of triglycerides from precursors (the refined non-edible oils) into their corresponding FAME, which in turn causes the occurrence of variations in the infrared spectrum [34, 36]. The wave number between 4000-3125 cm^{-1} show the presence of a single bond or hydrogen bond (O-H) in the refined oils. This band of the spectrum signifies the presence of moisture or hydrate (H_2O) in the refined oils of A. indica and M. stenopetala. Nonetheless, there is no hydrate in the synthesized biodiesel from hybridized oils due to the absence of a peak point in the specified range of the infrared spectrum. In the region of the infrared spectrum from 3125-1900 cm^{-1} wave number, the transmission band of the refined oils was similar to that of the synthesized biodiesel. In addition, the existence of the carboxylic acid functional group in A. indica oil, M. stenopetala oil, and the synthesized fatty acid methyl ester in the transmission band of 3125-1900 cm^{-1} indicated that the refined oils can be trans-esterified into the alkyl ester (biodiesel) via the transesterification reaction [34, 36].

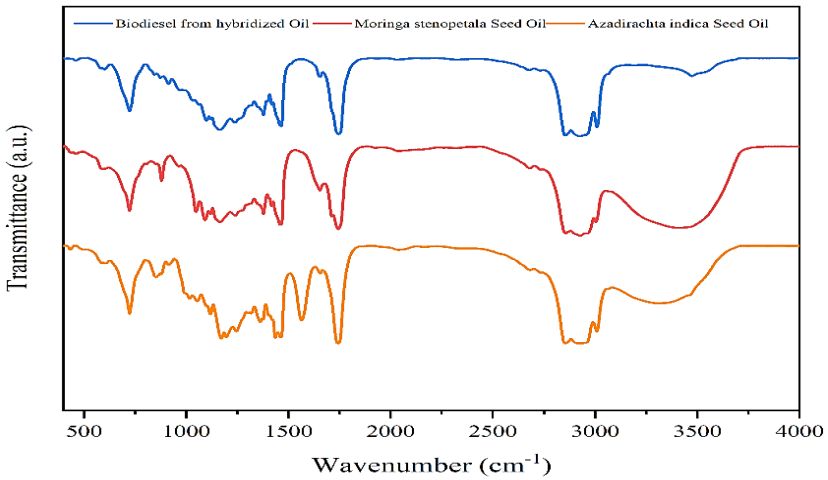


Fig. 6. Analysis of FTIR for the synthesized biodiesel, A. indica and M. stenopetala seed oils

The physicochemical characteristics of synthesized biodiesel from hybridized oils of A. indica and M. stenopetala seeds

The peak of the infrared spectrum in the range of frequencies 1900 - 10 cm^{-1} signifies the existence of C-H stretching vibration of the alkene (= CH_2) functional group in the refined oils and biodiesel [37]. Moreover, the region of transmission band between the frequencies of 10-750 cm^{-1} was considered as a 'fingerprint' region, indicating the existence of the C-H bending vibration of an alkane (- CH_2 -) functional group in the synthesized fatty acid methyl ester and refined oils of A. indica and M. stenopetala seeds (Fig. 6). The obtained result was in compliance with the findings of Vlachos et al.[38]. The presence of a C-H-aromatic bending vibration functional group in refined oils and synthesized fatty acid methyl ester was indicated by a peak in the transmission band with a wave number ranging from 750 to 400 cm^{-1} [39].

The physicochemical characteristics of the synthesized biodiesel were evaluated according to ASTM and EN 14214 standards for the maximum FAME yield (92%) obtained when the oil hybridization ratio of $A_{25}M_{75}$ (a mixture of 25% A. indica and 75% M. stenopetala seed oils) reacted with a 2%w/v catalyst dose for 90 minutes as per experimental run 7 (Table 2). The experimental results were recorded as one standard deviation from the mean value (mean±SD). The evaluated physicochemical characteristics were kinematic viscosity (3.42±0.08 mm^2/s), a higher heating value (41.86±0.12 MJ/kg), specific gravity (0.89±0.01), cetane number (62.48±0.38), acid number (0.18±0.04 mg KOH/g), flash point (172.65±1.42 °C), free glycerin (0.014±0.002 %mass), total glycerin (0.018±0.003 %mass), cloud point (5.24±0.80 °C), water and sediment (0.04±0.01 %vol.), methanol content (0.03±0.01 %), oxidation stability (7.68±0.40 hours), pour point (3.86±0.78 °C), Mg^{2+} and Ca^{2+} (4.12±0.18 mg/kg) and the sulfur content (0.04±0.008 mg/kg) (Table 7). These results signified that the fuel quality of synthesized biodiesel was in compliance with the standard limit of the FAME quality specification. Therefore, biodiesel produced by mixing or hybridizing of Azadirachta indica and Moringa stenopetala seed oil was appropriate fuel that could supersede petroleum and could be utilized as the transport fuel in the engine.

In this experimentation, the production of FAME involves the hybridization of A. indica and M. stenopetala seed oils at various proportions (%w/w) while synthesizing the biodiesel with ameliorated fuel quality than biodiesel obtained from single feedstocks. The improved fuel quality of the newly synthesized biodiesel includes the higher heating value (41.86±0.12 MJ/kg), specific gravity (0.89±0.01), cetane number (62.48±0.38), flash points (172.65±1.42 °C), and other parameters. This improvement is induced by the high amount of mono-unsaturated fatty acids ($C_{18}H_{32}O_2$ - oleic acid) comprised in A. indica and M. stenopetala seed oils. For instance, the presence of about fifty-one percent $C_{18}H_{32}O_2$ in A. indica seed oil [25] and seventy-six percent $C_{18}H_{32}O_2$ in M. stenopetala seed oil [26] made a significant contribution to meliorate the fuel quality of the synthesized FAME from the hybridized or mixed non-edible oils of the considered feedstocks. Hence, the produced biodiesel exhibits improved fuel characteristics better than the FAME synthesized from a single non-edible vegetable oil.

4. Conclusion

Biodiesel is a mixture of mono-alkyl esters produced through a base-catalyzed transesterification of animal fats and vegetable oils in the presence of suitable alcohol. It has been perceived as biodegradable, eco-friendly, greener, cleaner and an alternative source of energy. When synthesized and utilized in a sustainable manner, biodiesel can supersede petroleum, and hence, it can make a significant contribution to overcoming challenges associated with energy and economic crises, hastened global warming and environmental defilement because of the particulate matters, and gaseous emissions during the utilization of conventional diesel fuels. This study aims at producing the FAME with ameliorated fuel characteristics via mixing or hybridization of A. indica and M. stenopetala seed oils (i.e., in situ hybridization) in the presence of CH_3OH and NaOH thereby replacing the conventional diesel fuels.

Table 7. Analysis of Physicochemical characteristics of the synthesized FAME

The physicochemical characteristics	Employed standard methods (References)	Measurement unit	The obtained experimental results (mean ± SD)	Standards	
				ASTM Standard	EN 14214 Standard
Kinematic viscosity at 40 °C	[40]	mm^2/s	3.42±0.08	1.9-6.0	3.5-5.0
Higher heating value	[41]	MJ/kg	41.86±0.12	40-42	-
Specific gravity	[42]	-	0.89±0.01	-	0.86-0.90
Cetane number	[43]	-	62.48±0.38	≥ 47	> 51
Acid number	[44]	mg KOH/g	0.18±0.04	≤ 0.5	≤ 0.5
Flash point	[45]	°C	172.65±1.42	≥ 120	> 130
Free glycerin	[46]	% mass	0.014±0.002	< 0.02	< 0.02
Total glycerin	[46]	% mass	0.018±0.003	< 0.24	< 0.25
Cloud point	[47]	°C	5.24±0.80	*	*
Water and sediment	[48]	% volume	0.04±0.01	≤ 0.05	-
Methanol content	[49]	%	0.03±0.01	-	< 0.2
Oxidation stability	[50]	Hours	7.68±0.40	3 hours	8 hours
Pour point	[51]	°C	3.86±0.78	*	*
Carbon residue	[52]	% mass	0.02±0.01	< 0.05	-
Magnesium (Mg^{2+}) and Calcium (Ca^{2+}) content	[53]	mg/kg	4.12±0.18	≤ 5	< 5
Sulfur content	[54]	mg/kg	0.04±0.008	< 0.05	< 10.0

SD– standard deviation; *- Not determined (it depends on location and seasonal variations)

To conduct the experimentation, the Box-Behnken Design was used in the design of the experiment and evaluation of experimental results. The considered experimental variables were the oil hybridization ratio (%w/w), reaction time (minutes), and catalyst dose (%w/v) with 3 levels for each variable, -1 (low), 0 (medium), and +1 (high). The hybridized oil ratios involved in the base-catalyzed transesterification reaction were $A_{25}M_{75}$ (a mixture of 25 %w/w A. indica and 75 %w/w M. stenopetala oils), $A_{50}M_{50}$ (a mixture of 50 %w/w A. indica and 50 %w/w M. stenopetala oils), and $A_{75}M_{25}$ (a mixture of 75 %w/w A. indica and 25 %w/w M. stenopetala oils) with their respective biodiesel yield, $BA_{25}M_{75}$, $BA_{50}M_{50}$, $BA_{75}M_{25}$, whereas, the reaction times were 30, 60 and 90 minutes, and the catalyst doses (NaOH) of a 0.50 %w/v, 2.00 %w/v, and 3.50 %w/v. The other experimental factors, such as agitation speed (300 rpm), molar ratios of oil-to-methanol (1:6), and reaction temperature (60 °C), were kept constant during the experimentation. Seventeen experimental runs were conducted and the biodiesel yield (%) was obtained for each experimental run. In addition, the effect of experimental variables on the yield of biodiesel was observed. The results of the ANOVA signified that the quadratic model expression was developed for the executed experiment with a $P < 0.0001$ (significant). The quality and adequacy of the developed model were determined by the determination coefficients: $R^2 = 0.9986$, Pred-$R^2 = 0.9830$, Adj-$R^2 = 0.9968$ and a lack of fit test with a $P = 0.1150$ (insignificant) at 5% the LSD. The prediction coefficient, 'Pred-R^2 of 0.9830' is in agreement with the adjusted coefficients of determination, 'Adj-R^2' of 0.9968, showing that the obtained model expression can be employed for the navigation of the design space. The minimum percentage of FAME yield (60.96%) was obtained when the oil hybridization ratio, $A_{50}M_{50}$ (a mixture of 50 %w/w A. indica oil and 50 %w/w M. stenopetala seed oil) reacted with a 0.5 %w/w of NaOH catalyst for 30 minutes whereas, the maximum percentage of the FAME yield (92%) was obtained when the oil hybridization ratio, $A_{25}M_{75}$ (i.e., a mixture of 25 %w/w A. indica oil and 75 %w/w M. stenopetala seed oil) reacted with a 2 %w/w NaOH catalyst for 90 minutes. The analysis of the FTIR (Fig. 6) and evaluation of physicochemical properties were executed following the ASTM and EN 14214 standard methods for the synthesized biodiesel (Table 7). The quality of the biodiesel synthesized from the hybridized oils was in compliance with the standard limits of biodiesel specification showing that the biodiesel can be employed in the diesel engine.

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Declarations

Competing interests

There are no competing interests in the publication and authorship of the Paper.

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