Journal of
Renewable Energy Research and Applications (RERA)

Vol. 4, No. 1, 2023, 1-12 *DOI: 10.22044/rera.2022.11955.1127*

Hybridization of *Moringa stenopetala* and *Azadirachta indica* Seed Oil to Synthesize Biodiesel with Improved Quality

D. Genene[1,*], S. Kebede[2] and D. Gebeyehu[3]

1. Ethiopian Forestry Development, Forest Products Innovation Center, Addis Ababa, Ethiopia.
2. Addis Ababa University, Addis Ababa Institute of Technology, School of Chemical and Bioengineering, King George VI St., Addis Ababa, Ethiopia.
3. Ethiopian Forestry Development, Forest Products Innovation Research Directorate, Addis Ababa, Ethiopia

Received Date 29 May 2022; Revised Date 02 July 2022; Accepted Date 14 July 2022
**Corresponding author: dgeneneeth12@gmail.com (D. Genene)*

Abstract
Biodiesel has been considered as a biodegradable, green, cleaner, alternative, renewable, and eco-friendly energy source. It can supersede petrol-diesel, and help to solve challenges accompanied with energy crisis, socio-economic, environmental pollution, climate change, and global warming. Hybridization of vegetable oils to synthesize biodiesel while improving fuel quality has not been studied extensively. Thus the aim of this work is hybridization of *M. stenopetala* and *A. indica* seed oil to synthesize biodiesel, and thereby, improve the fuel quality. Response surface methodology, Box-Behnken, design is employed in the experimental design and the result analysis. Oil mixing composition, reaction time, and catalyst dose are selected as the factors of study with three levels: low (-1), medium (0), and high (+1). The other parameters, temperature, alcohol to oil molar ratio, and mixing speed are kept constant. The oil hybrid compositions are $M_{75}N_{25}$ (75% v/v *M. stenopetala* oil and 25% v/v *A. indica* oil), $M_{50}N_{50}$ (50% v/v *M. stenopetala* oil and 50% v/v *A. indica* oil), and $M_{25}N_{75}$ (25% v/v *M. stenopetala* oil and 75%v/v *A. indica* seed oil) with their corresponding biodiesel, $BM_{75}N_{25}$, $BM_{50}N_{50}$, $BM_{25}N_{75}$, respectively. The catalyst doses of 1, 1.5, and 2% w/v potassium hydroxide flake, and the reaction times of 20 min, 40 min, and 60 min are considered as the variables of study. Fifteen experimental runs are conducted with three levels for each factor. The quadratic model is developed with statistical significance, *P- value < 0.0001*. Analysis of variance (ANOVA) and determination of coefficients are used to evaluate the model quality, where the main comparison is conducted at 5% least significant difference.

Keywords: *Azadirachta indica, Biodiesel, Hybridization, Moringa stenopetala, Transesterification.*

1. Introduction

Energy is an indispensable commodity, and the principal driver of socio-economic growth and development of all nations across the globe. Any aspects of human activity are associated with energy. The operation of heavy duty machines, power generating plants, transport vehicles, and mechanized agriculture induce the production and utilization of a large amount of energy [1]. The geometric increase in the number of world population has caused a subsequent increase in the demand of energy, which, in turn, leads to an inadequate supply of energy [2]. The consequence of inadequate energy supply can be detrimental to the economy of the globe [3].

Fossil fuels (i.e. crude oil, coal, and natural gas) have been a principal source of energy supply for some years now [4]. Nevertheless, these fossil fuels are non-renewable sources of energy, limited in their supply, pose several environmental and health problems, and unevenly distributed across the world (i.e. more concentrated in some countries than the others). Hence, the countries that do not have such fossil fuel sources are imposed to import crude oil while encountering a number of challenges, for instance, high cost of procurement and foreign exchange crisis accompanied by importing petroleum fuels [5]. Moreover, the depletion of crude oils, global energy crisis, unstable energy prices, harmful gaseous emission, and environmental pollution are the main problems induced by utilization of conventional fossil fuels [6, 7]. The consumption of about 89 million barrel of petroleum was anticipated per day worldwide in 2012 [8]. At this consumption rate, the crude oil resources are estimated to run out within the next fifty years [9].

Therefore, with the geometric population growth and coping with energy demand, several countries are striving to search for alternative, cleaner, renewable, environmentally friendly, and greener energy sources [8, 10]. To this end, production and utilization of biomass energy or biofuels (i.e. biodiesel, bio-ethanol, and bio-oil) have been regarded as alternative and eco-friendly energy sources, thereby, substituting crude oil [11].

Furthermore, Ethiopia, as one of the developing countries, has had vision for greening its economy or Climate Resilient Green Economy (CRGE) strategy based on its national Growth and Transformation Plan (GTP), in which the country seeks to reach a middle-income status by 2025 [12]. CRGE aims to strengthen the improvement of agriculture, sustainable management of natural resources, and poverty reduction. The ambitious CRGE strategy rests on the four major pillars: First, improving the agricultural practices to enhance food security, secondly, protecting and re-establishing forests for ecosystem services including enhanced carbon stocks and for direct economic benefits, thirdly, expanding electricity generation from renewable sources, and fourthly, leapfrogging inefficient technologies and instead implementing modern, energy-efficient technologies in transport, industry, and infrastructure. Incorporated in the fourth pillar of the CRGE strategy, the country is striving toward decarbonizing transport fuel via production and utilization of biofuels (i.e. biodiesel and ethanol). As per planned strategy, the implementation of 5% biodiesel and 15% ethanol blends by 2030 would substitute 0.28 billion L of diesel and 0.09 billion L of gasoline. During the second Growth and Transformation Plan (2015/16-2019/20), for development of biodiesel in Ethiopia, it was planned to identify about 23 million hectares of land for Biofuel production. Hence, about 16.6 million hectares of land is partially planted and being planted with non-edible biodiesel production feedstocks such as Jatropha, Castor, Moringa and Neem (*Azadirachta indica*) tree.

Biodiesel synthesis by blending feedstocks has not been studied extensively all over. Blending or hybridization of feedstocks before Transesterification process improves the quality of the resulting fuel. Therefore, in compliance with CRGE of Ethiopia, the rationale of this study was biodiesel synthesizing by hybridization of oils from locally available feedstocks (i.e. *Moringa stenopetala*, and *Azadirachta indica* seeds) as cleaner, alternative, biodegradable, domestic fuel, renewable and eco-friendly energy source with improved quality.

2. Biodiesel as renewable energy source

Biodiesel is a liquid biofuel produced from vegetable oils and animal fats by the Transesterification process [13]; and it can be employed either alone or blended with diesel oil in diesel engines [14, 15]. According to the definition of American Society for Testing Materials (ASTM), ASTM-D6751 [16], biodiesel is a mixture of long-chain of fatty acid mono-alkylic esters obtained from renewable resources (i.e. triglycerides of fatty acids-vegetable oil) in the presence of alcohol and suitable catalyst with glycerol as by-product [17, 18]. In twenty-first century, biodiesel has received attention as renewable, biodegradable, cleaner, and environmentally safe fuel source [19]. Sustainable utilization of biodiesel will diminish dependence on fossil fuel [20] and pollutants contributing to environmental pollution and global warming [21]. Moreover, biodiesel utilization does possesses a number of advantages like its substitution for conventional diesel fuel [15], lower emission of contaminants such as poly aromatic hydrocarbons (PAH), aldehydes, carbon monoxide, and hence, low toxicity in comparison with conventional diesel fuel, degrades more rapidly and its excellent lubricant properties [14].

The utilization of non-edible vegetable oils for production of biodiesel is important because of the great need of edible oil for food. The non-edible oils of *Moringa* seed, *A. indica* seed, Jatropha seed, Mahua seed, castor seed, linseed, cotton seed, rubber seed, and oils from microalgae are locally available and accessible in developing countries, and are economical compared to edible oil [22]; and they are considered as highly promising reliable feedstock possessing seeds with high oil content. The prospects of utilizing oils of these non-edible seeds as alternative fuel source has vided scope and very good understanding of biodiesel production from non-edible seeds will make significant contribution supporting countries economy by diminishing the import of crude oil and commercializing the biodiesel product [23]. The *M. stenopetala* and *A. indica* seeds have been considered as a reliable raw materials and locally available oilseeds for the intended study.

3. Materials and methods

3.1 Sample collection and preparation

The sample of *M. stenopetala* seeds was collected from Sidama Region Agricultural Center, with geographical location of latitude 7° 3’ North (N), Longitude 38° 28 East (E) and elevation of 1,708

m above sea levels. The sample of *A. indica* seeds was collected from Afar Region, Awash Arba (Amibara District), in Northeastern part of Ethiopia. The collected samples were transported to the Addis Ababa Institute of Technology (AAiT), School of Chemical and Bioengineering Laboratory, to conduct the intended study. Then the samples were cleaned and free from foreign materials such as weed seed, molds, stones, and other contaminants. The collected seeds were first de-hulled (i.e. decorticated). Following the decortication, the outer husk was separated from the kernels by winnowing. Then the samples of seed kernels were dried in oven drier at 60 °C for 24 hours to remove its moisture content. The sample drying was continued until the moisture content was below 5%. The dried samples of *M. stenopetala* and *A. indica* seed kernels were grinded into the paste at particle size in the range of 0.25–0.425 mm, 0.425–0.60 mm, 0.60–0.85 mm turn-by-turn using grinder to provide a higher surface area of particles for the ease of extraction. The range of desired particle size was obtained through sieving using the Vibrated sieve shaker. The milling operation ruptures the cell wall, and releases the solute for direct contact with the solvent during oil extraction process (i.e. by Soxhlet extraction method). The grinded samples were put into a Low Density Poly Ethylene (LDPE), labeled, and stored until dispatch.

3.2. Oil extraction and purification

The Soxhlet extraction method, with *n*-Hexane as a solvent, was employed for the extraction of oil from the considered species [24]. A weighed 120 g of grinded sample was placed in thimble paper. Then the thimble paper containing the sample was inserted into the Soxhlet apparatus. A measured 600 mL of the solvent (n- Hexane) was poured into a 1000 mL round bottom flask; and adjusted in the set-up of the extraction vessel. The extraction temperature was set at 72 °C, and the extraction process was allowed to continue for 5 hours to obtain the desired crude oil. As the extraction temperature increased and process of heating continued, the solvent commenced to evaporate and condensed back to the thimble containing the sample. The extracted crude oil containing the solvent was recycled and refluxed back to the round bottom flask, and this process was continued until the extraction hour was reached. The solvent was recovered from the crude oil-solvent mixture using a rotary evaporator at a temperature of 72 °C. This extraction process was continued until a reasonable amount of crude oil was obtained [25]. Finally, the amount of the extracted crude *M. stenopetala and A. indica* oil was recorded at the end of each steps of extraction process, and the percentage extracted oil was determined as follows:

$$\textbf{OIL YIELD (\%)} = \frac{\textbf{Mass of crude extracted oil}}{\textbf{Total Mass of Seed kernel}} * \mathbf{100} \qquad (1)$$

The extracted crude oils of each species were subjected to filtration, and the removal of potentially unwanted particles was carried out using centrifuge. For further purification or removal of trace solvent, the oil and solvent mixture was placed in the boiling water bath until the trace n-hexane was liberated completely. Then the extracted oils (i.e. crude *M. stenopetala* and *A. indica* seed oil) were subjected to degumming and oil neutralization steps prior to the transesterification reaction.

3.3. Production of biodiesel by base-catalyzed transesterification process

Experimental design

The experiment was designed according to Response Surface Methodology (RSM), Box-Behnken Design (BBD) method. The selected variables or factors of study were oil mixing composition (A), catalyst dose (B), and reaction time (C), and three levels for each factor (i.e. low, medium and high). The interaction effects of factors and their influences on response were studied while optimizing the required response (i.e. biodiesel yield). Factors and levels were provided below (Table 1) and total number of experiment was computed according to the BBD method [26]:

$$\mathbf{N = K^2 + K + C_p} \qquad (2)$$

where Nis the number of experiments, Kis the number of factors, and C_p is a central replication point.

Table 1. Experimental levels of considered variables of study using BBD method.

Symbols	Levels				
	Variables	Units	-1 (Low)	0 (Medium)	+1 (High)
A	Oil mixing composition	% v/v	25	50	75
B	Catalyst dose	% w/v	1.0	1.5	2.0
C	Reaction time	Min	20	40	60

The mixture of *M. stenopetala* and *A. indica* seed oils were subjected to the base-catalyst transesterification reaction at various operation parameters including oil hybridization ratio (i.e. oil mixing composition), catalyst dose, and reaction time. The considered oil hybrid compositions were $M_{25}N_{75}$ (25% Moringa oil: 75% Neem oil), $M_{50}N_{50}$ (50% Moringa oil: 50% Neem oil) and $M_{75}N_{25}$ (75% Moringa oil: 25% Neem oil) with the corresponding biodiesel products: $BM_{25}N_{75}$, $BM_{50}N_{50}$, $BM_{75}N_{25}$; catalyst dose of 1%, 1.5%, and 2%; and reaction time of 20 min, 40 min, and 60 min; whereas, the other operation parameters (alcohol to oil ratio, temperature and mixing speed) were kept constant as per provided below (Table 2). According to BBD, a total of 15 experimental runs were conducted with 3 levels for each numeric factor.

Biodiesel (FAME) synthesis via bBase-catalyzed transesterification reactio:

The refined oil was trans-esterified to mono-esters of fatty acids using methanol (CH_3OH) and potassium hydroxide (KOH) as the catalyst. The base-catalyzed transesterification was carried out using three-necked round bottom flask (i.e. glass reactor) containing 60 mL of hybridized oils of *M. stenopetala* and *A. indica.* KOH was dissolved in CH_3OH forming a mixture of methoxide. Then the methoxide solution was added to the glass reactor containing oil sample at 6:1 (alcohol to oil molar ratio). The reaction was allowed to take place at 60 $^{\circ}$C and 300 rpm speed of stirrer for each trials as per specified in the experimental design (Table 2 below). After the reaction took place, 3 drops of aqueous hydrochloric acid (HCl) was added to the solution to quench the reaction in order to stop further reaction from occurring. Then the solution was transferred to a separating funnel for glycerol removal. The lower glycerol layer was drained off from the bottom of the separator funnel and the remaining upper layer (i.e. biodiesel or mono-methyl ester) was obtained. For further purification, the biodiesel was added into separator funnel, and allowed to settle for 12 hours to remove traces of KOH, glycerol, and unreacted methanol.

The remaining trace materials and unreacted methanol induces corrosion in engine components, and trace glycerol in the fatty acid methyl ester (biodiesel) diminishes lubricity of the fuel causes injector coking and safety risks [27]. The resulting hybridized biodiesel product was washed (4 times) with distilled water (1:2 biodiesel to water ratio) at 45 $^{\circ}$C by spraying hot water over the biodiesel in order to remove these impurities. Finally, the pure biodiesel was heated at 110 $^{\circ}$C to get rid of trapped traces of water; and the biodiesel was subjected to characterization and physico-chemical determinations.

Evaluation of physico-chemical properties of produced fatty acid methyl ester

The Fourier Transform-Infrared Radiation (FT-IR) spectroscopy was employed to examine the functional groups of *M. stenopetala* seed oil, *A. indica* seed oil and biodiesel produced by hybridization of *M. stenopetala* and *A. indica* seed oil. The analysis of FT-IR was conducted using 5 mL of each sample (i.e. *M. stenopetala* seed oil, *A. indica* seed oil, and biodiesel synthesized from hybridized oil) at the resolution of 1 cm^{-1} for the wave number in the range of 400.0000-4000.0000 cm^{-1}. In addition, determination of the physico-chemical properties of the produced biodiesel follows the ASTM D6751-09 [16] and European's EN14214 standards [28] including specific gravity [29], flash point [30], calorific value [31], kinematic viscosity [32], Cetane number [33], acid number [34], pour points [35], free glycerin, and total glycerin [36]. Moreover, other quality parameters for the desired biodiesel such as moisture content, carbon residue, water and sediments, oxidation stability, methanol content, sulfur and phosphorus content, and calcium and magnesium content were determined using the standard test methods.

4. Results and discussion

4.1. Statistical analysis of FAME produced from hybridized M. stenopetala and A. indica seed oil using RSM

The Response Surface Methodology, BBD, was employed for analysis of variance (ANOVA), analysis of regression and estimation of coefficients of the model equation of fatty acid methyl ester (biodiesel) synthesized by hybridization of *M. stenopetala* and *A. indica* seed oil. The analysis of variance (ANOVA) was also employed to evaluate the adequacy of the model equation. The statistical significance and the quality of fit of the model equation was expressed using prediction coefficients of determination (Pred- R^2), coefficient of determination (R^2), and adjusted coefficients of determination (adj- R^2), F-test, and coefficient of variation (CV), where the main comparison was conducted at 5% levels of the least significant difference.

Table 2. Results of effect of operation variables on yield of fatty acid methyl ester (biodiesel)

Run	Factor 1	Factor 2	Factor 3	Alcohol to oil Molar ratio	Temperature (°C)	Speed of stirrer (rpm)	Response	
	A: Oil mixing Composition (M:N) (% v/v)	B: Catalyst dose (KOH) (% w/v)	C: Reaction time (min)				BMN-Biodiesel yield (%)	
							Actual value	Predicted value
1	$M_{50}N_{50}$	2.00	60.00	6:1	60	300	89.00	90.00
2	$M_{50}N_{50}$	2.00	20.00	6:1	60	300	72.00	71.00
3	$M_{50}N_{50}$	1.50	40.00	6:1	60	300	88.00	87.67
4	$M_{50}N_{50}$	1.00	60.00	6:1	60	300	73.00	74.00
5	$M_{50}N_{50}$	1.00	20.00	6:1	60	300	60.00	59.00
6	$M_{75}N_{25}$	1.00	40.00	6:1	60	300	74.00	73.75
7	$M_{25}N_{75}$	1.50	20.00	6:1	60	300	70.00	70.75
8	$M_{25}N_{75}$	1.50	60.00	6:1	60	300	86.00	84.75
9	$M_{50}N_{50}$	1.50	40.00	6:1	60	300	87.00	87.67
10	$M_{50}N_{50}$	1.50	40.00	6:1	60	300	88.00	87.67
11	$M_{75}N_{25}$	2.00	40.00	6:1	60	300	90.00	89.75
12	$M_{25}N_{75}$	2.00	40.00	6:1	60	300	84.00	84.25
13	$M_{25}N_{75}$	1.00	40.00	6:1	60	300	72.00	72.25
14	$M_{75}N_{25}$	1.50	20.00	6:1	60	300	70.00	71.25
15	$M_{75}N_{25}$	1.50	60.00	6:1	60	300	92.00	91.25

M- M. stenopetala oil; N- A. indica Oil; BMN- Biodiesel of mixed M and N

$$\textbf{Yield of fatty acid methyl ester (biodiesel)} = \frac{\textbf{Mass of obtained biodiesel}}{\textbf{Mass of refined oil input}} * 100 \quad (3)$$

The resulting actual yield of fatty acid methyl ester (biodiesel) synthesized by hybridization of *M. stenopetala* and *A. indica* seed oil at various operational variables (i.e. oil mixing composition, catalyst dose and reaction time) were computed according to equation 3. The results provided above (Table 2) showed that the maximum yield of biodiesel (92 %) was obtained from experiment number 15 at Oil mixing composition of $M_{75}N_{25}$ (i.e., 75 %v/v *M. stenopetala* seed oil and 25 %v/v *A. indica* seed oil), catalyst dose of 1.5%w/v and reaction time of 60 min. Whereas, the minimum yield of biodiesel (60%) was obtained from experiment number 5 at oil mixing composition of $M_{50}N_{50}$ (50% v/v *M. stenopetala* oil and 50% v/v *A. indica* oil), catalyst dose of 1% w/v and, reaction time of 20 min.

4.2. Fatty acid methyl ester (biodiesel) modeling and analysis of model

The RSM, Box-Behnken Design method, was employed for the analysis of variance (ANOVA) of the produced fatty acid methyl ester; and thereby providing the corresponding model equation for the desired product.

Table 3. Results of ANOVA for synthesized biodiesel at various operational variables.

Response 1	Biodiesel (FAME) Yield					
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	1356.17	9	150.69	82.19	< 0.0001	significant
A- Oil mixing composition	24.50	1	24.50	13.36	0.0147	
B- Catalyst dose	392.00	1	392.00	213.82	< 0.0001	
C- Reaction time	578.00	1	578.00	315.27	< 0.0001	
AB	4.00	1	4.00	2.18	0.1997	
AC	9.00	1	9.00	4.91	0.0776	
BC	4.00	1	4.00	2.18	0.1997	
A^2	2.56	1	2.56	1.40	0.2901	
B^2	172.41	1	172.41	94.04	0.0002	
C^2	198.56	1	198.56	108.31	0.0001	
Residual	9.17	5	1.83			
Lack-of-fit	8.50	3	2.83	8.50	0.1071	not significant
Pure error	0.67	2	0.33			
Cor Total	1365.33	14				

The Model F-value of 82.19 indicated that the model was significant (*P- value < 0.0001*). There is only a 0.01% chance that a "Model F-Value" is large, which could occur due to noise. Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case, A, B, C, B^2, and C^2 are significant terms of the developed model. Values greater than 0.1000 show that the model terms are not significant statistically. Thus AB, AC, BC, and A^2 are insignificant terms of the

model. If there are many insignificant model terms (i.e. not counting those required to support hierarchy), model diminution would meliorate the model. The "lack of fit F-value" of 8.50 indicates the lack of fit is not significant (*P- value = 0.1071*) relative to the pure error. There is a 10.71% chance that a "lack of fit F-value" is large, occur due to noise.

After evaluation of a model fit summary of the obtained experimental data, polynomial expression of the highest degree (i.e. second order) with significant terms of the model was selected, while insuring the model is not aliased. Therefore, a quadratic model was suggested as the statistically significant model for the required response of biodiesel (fatty acid methyl ester) yield.

Table 4. Ssuggested model for produced biodiesel from hybridized M. stenopetala and A. indica seed oil at various operational parameters.

Response 1	Biodiesel (FAME) yield					
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	95201.67	1	95201.67			
Linear vs. Mean	994.50	3	331.50	9.83	0.0019	
2FI vs. Linear	17.00	3	5.67	0.13	0.9407	
Quadratic vs. 2FI	344.67	3	114.89	62.67	0.0002	**Suggested**
Cubic vs. Quadratic	8.50	3	2.83	8.50	0.1071	Aliased
Residual	0.67	2	0.33			
Total	96567.00	15	6437.80			

The " Pred-R^2" of 0.8993 was in a reasonable agreement with the "Adj-R^2" of 0.9812. "Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. Thus the ratio of 29.171 indicated an adequate signal for the developed model. Hence, the developed model can be used to navigate the design space. The quality or strength of produced biodiesel (FAME) model can be determined by taking coefficient of correlation into consideration.

Table 5. Measures of adequacy of produced biodiesel (FAME) Model

Std. Dev.	1.35	R^2	0.9933
Mean	79.67	Adj-R^2	0.9812
C.V. %	1.70	Pred-R^2	0.8993
Press	137.50	Adeq Precision	29.171

The coefficient of determination (R^2) value for the developed model was 0.9933. This indicated that 99.33% of the overall variation in fatty acid methyl ester (biodiesel) yield was associated with the operational variables of the intended study. In addition, the lower value of coeffficient of variation (1.70%) obtained from ANOVA showed the reliability and precision of the conducted experiment. This coefficient of variation (i.e. the ratio of standard error of estimate to average value of obtained biodiesel yield) measured the reproducible tendency of the selected model (i.e. quadratic model).

Final equation of the produced biodiesel (FAME) yield in terms of coded factors

$$\text{Biodiesel yield, } Y = 87.67 + 1.75 * A + 7.00 * B + 8.50 * C + 1.00 * A * B + 1.50 * A * C + 1.00 * B * C - 0.83 * A2 - 6.83 * B2 - 7.33 * C2 \quad (4)$$

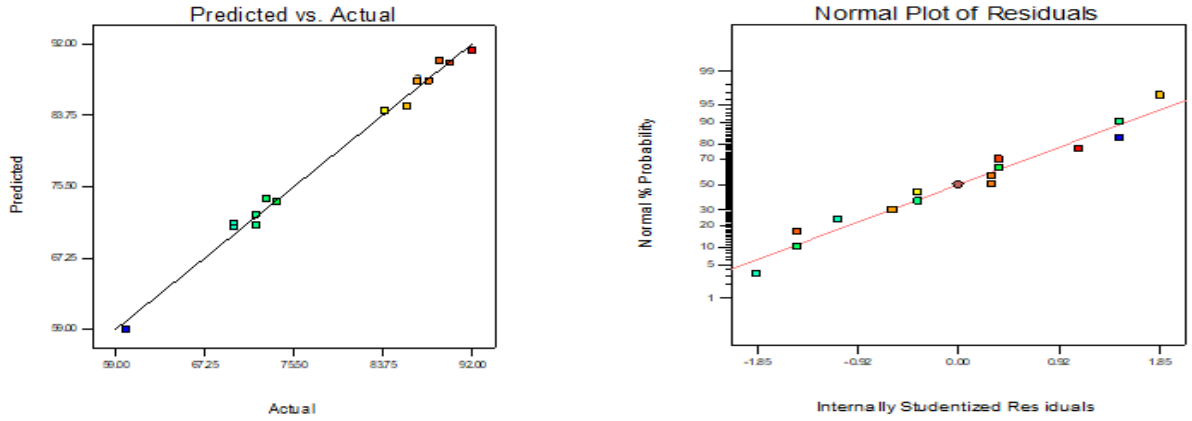
Therefore, from the developed quadratic model equation, it was observed that significant terms affecting the response (i.e. yield of biodiesel) were oil mixing composition (A), catalyst dose (B), reaction time (C), the square of catalyst dose (B^2), and the square of reaction time (C^2), whereas the insignificant terms of the model were the square of oil mixing composition (A^2), the interaction effects of oil mixing compositon with catalyst dose (AB), the interaction effects of oil mixing composition with reaction time (AC), and the interaction effects of catalyst dose with reaction time (BC) (Table 3).

Diagnostics case statistics of biodiesel yield:

To evaluate the model property of statistical distribution, the Box-Behenken Design (BBD) normal probability versus residual plot was employed (Figure 1b below). The plot showed that the residual errors were distributed normally in linear pattern. Thus the data points in the plot were estimated to be in straight line, and there was no abnormality sign in the developed model. The relationship between the actual and the predicted values of biodiesel yield was observed from the high correlation coefficient, R^2 = 0.9933, which was close to unity. Hence, the predicted values of the biodiesel yield were in reasonable agreements with the actual obtained values (Table 6) and the experimental data fit with the developed model providing the precise estimate of the required response for the transesterification reaction process in the considered range of operational variables (Figure 1a).

Table 6. Diagnostics case statistics of biodiesel (FAME) yield

Response 1	Biodiesel (FAME) yield					
Standard order	**Actual value**	**Predicted value**	**Residual**	**Leverage**	**Internally studentized residual**	**Externally studentized residual**
1	72.00	72.25	-0.25	0.750	-0.369	-0.335
2	74.00	73.75	0.25	0.750	0.369	0.335
3	84.00	84.25	-0.25	0.750	-0.369	-0.335
4	90.00	89.75	0.25	0.750	0.369	0.335
5	70.00	70.75	-0.75	0.750	-1.108	-1.141
6	70.00	71.25	-1.25	0.750	-1.846	-2.928
7	86.00	84.75	1.25	0.750	1.846	2.928
8	92.00	91.25	0.75	0.750	1.108	1.141
9	60.00	59.00	1.00	0.750	1.477	1.760
10	72.00	71.00	1.00	0.750	1.477	1.760
11	73.00	74.00	-1.00	0.750	-1.477	-1.760
12	89.00	90.00	-1.00	0.750	-1.477	-1.760
13	88.00	87.67	0.33	0.333	0.302	0.272
14	88.00	87.67	0.33	0.333	0.302	0.272
15	87.00	87.67	-0.67	0.333	-0.603	-0.560



(a) **(b)**

Figure 1. a) Predicted values versus actual values of aiodiesel yield b) Normal % probability versus internally studentized residuals.

4.3. Effect of operational variables on yield of bBiodiesel

Effect of oil mixing composition and catalyst dose on yield of biodiesel

The result of ANOVA showed that oil mixing composition (A) and catalyst dose (B) affected the yield of fatty acid methyl ester (biodiesel) significantly, *P- value* < *0.05* (Table 3). The reaction time (C) was fixed at 40.00 min. The maximum value of fatty acid methyl ester (biodiesel) percentage yield (90 %) was achieved when the oil mixing composition was $M_{75}N_{25}$ (i.e. 75% v/v *M. stenopetal*a seed oil and 25% v/v *A. indica* seed oil) and the catalyst dose of 2% w/v (Figure 2). The 3D plot of Box-Behnken Design (BBD) indicated that for the considered range of these operational variables, increasing the oil mixing composition increased the yield of FAME, and as the catalyst dose increased from 1 to 2% w/v, the yield of FAME was also increased, and vice-versa. Adequate catalyst dose during the transesterification reaction enhanced the forward rate, thereby achieving completion of the reaction and enhancing the yield of FAME.

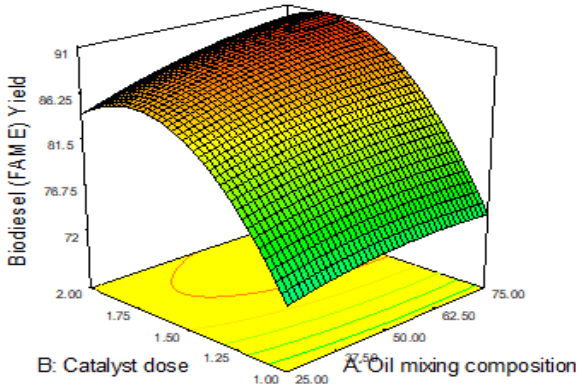


Figure 2. Effect of oil mixing composition (A) and catalyst dose (B) on yield of biodiesel.

Effect of oil mixing composition and reaction time on yield of biodiesel

For the data obtained from 15 experimental runs, the result of ANOVA indicated that oil mixing composition and reaction time were significant operational variables with *P-value* < *0.05*, and

hence, they had significant effect on the yield of biodiesel. The catalyst dose was kept constant at 1.5% w/v. From the provided 3D plot of the BBD, it was observed that the percentage yield of Biodiesel increases with increased oil mixing composition from 25 - 75 %v/v and reaction time increases from 20 - 60 min. The maximum yield of biodiesel (92%) was obtained when the oil mixing composition was $M_{75}N_{25}$ (i.e. 75% v/v *M. stenopetal*a seed oil and 25% v/v *A. indica* seed oil) and the reaction time of 60 min (Figure 3). The optimum and sufficient reaction time enhanced the conversion of reactants and completion of the transesterification reaction while maximizing the yield of biodiesel. However, longer reaction time beyond the optimum induces the reverse reaction (i.e. hydrolysis of FAME into triglycerides), and thereby, diminishing the yield of the FAME.

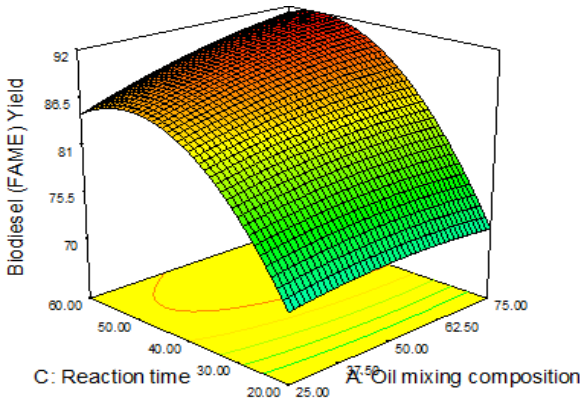


Figure 3. Effect of oil mixing composition (A) and reaction time (C) on yield of biodiesel.

Effect of catalyst dose and reaction time on yield of biodiesel

To observe the effect of catalyst dose (B) and reaction time (C) on the yield of biodiesel, the oil mixing composition (A) was kept constant at 50.00% v/v (i.e. 50% v/v *M. stenopetala* seed oil and 50% v/v *A. indica* seed oil). From the result of ANOVA, both operational variables (i.e. catalyst dose and reaction time) had *P-value* < *0.0001* (Table 3 above); and hence, these variables had significant effects on the yield of fatty acid methyl ester. From the 3D plot of Box-Behnken Design (BBD) provided below (Figure 4), was been indicated that the yield of biodiesel increased significantly with increasing catalyst dose (% w/v) and reaction time (min). The maximum value of 89% biodiesel yield was obtained at the maximum catalyst dose of 2% w/v and maximum reaction time of 60 minutes, whereas the minimum value of 60% biodiesel yield was obtained at the minimum catalyst dose of 1% w/v and reaction time of 20 minutes. Thus during the base-catalyzed transesterification reaction, optimum catalyst dose and reaction time promoted the forward rate of reaction and derived the reaction system to reach the equilibrium while maximizing the yield of the desired product (FAME).

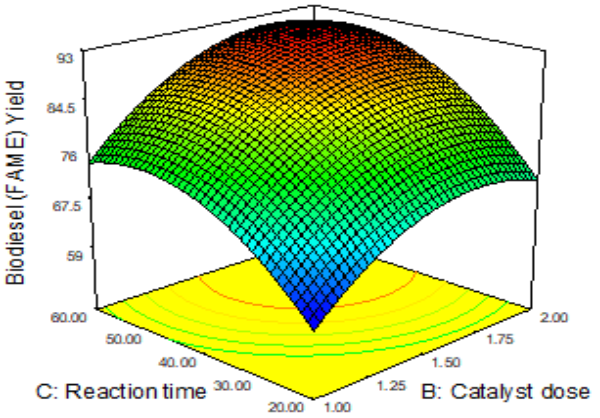


Figure 4. Effect of catalyst dose and reaction time on yield of biodiesel.

4.4. Evaluation of physico-chemical properties of optimized yield of biodiesel

Results of Fourier transform Infrared Radiation (FTIR) spectrometer

The FT-IR spectroscopy was employed to examine the functional groups of *M. stenopetala* seed oil, *A. indica* seed oil and biodiesel produced by hybridization of *M. stenopetala* and *A. indica* seed oil. The analysis of FT-IR was conducted using 5 mL of each samples (i.e. *M. stenopetala* seed oil, *A. indica* seed oil, and biodiesel synthesized from hybridized oil) at the resolution of 1 cm^{-1} for the wave number in the range of 400.0000-4000.0000 cm^{-1} (Figure 5).

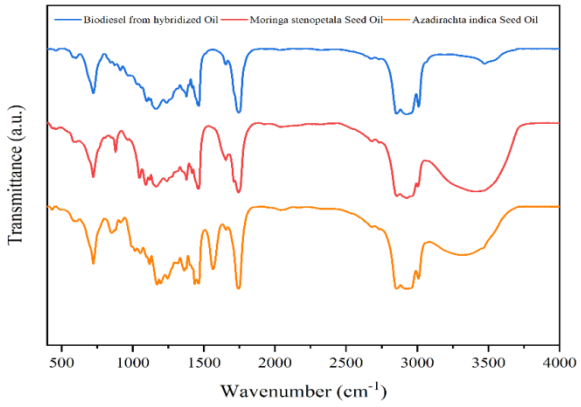


Figure 5. FT-IR spectrum of M. stenopetala seed oil, A. indica seed oil, and biodiesel produced from hybridized oil

The analysis of peak points of infrared spectrum for each sample indicated that there was a significant difference between the spectrum of feedstocks and biodiesel produced in the

considered range of wave number, which, in turn, signified the existence of different functional groups in each sample. This variation in infrared spectrum occurred due to the transition of triglyceride esters in the non-edible oils into fatty acid methyl ester (biodiesel) via the transesterification reaction. The peak of infrared spectrum in the range of 400-750 cm^{-1} wave number indicated that the existence of functional groups of C-H–aromatic bending vibration in the feedstocks (*M. stenopetala* and *A. indica* seed oil) and the presence of alkyl (methyl)–functional group in the fatty acid methyl ester (FAME) [37]. The infrared spectra peak in the range of 750-1470 cm^{-1} wave number showed the presence of C -H bending vibration of alkane (- CH_2 -) in the feedstocks and in biodiesel. These results were in agreement with the results obtained by Banik S. K. *et al*. [38]. Moreover, the peak of spectrum in the range of wave number from 1500-1740 cm^{-1} indicated the existence of functional group of C-H stretching vibration of alkenes (= CH_2) in fatty acid methyl ester (biodiesel) and feedstocks [39]. The transmission band of feedstocks and fatty acid methyl ester were similar in the range of wave number 1750- 2850 cm^{-1}. The functional group of C = O stretching vibration of esters (i.e. carboxylic acid group) occurred in *M. stenopetala* seed oil, *A. indica* seed oil and biodiesel at the peak of spectrum in the region of 1750-2850 cm^{-1} wave number. This indicated that fatty acid methyl ester (biodiesel) could be prepared from the considered feedstock successfully via the transesterification reaction [40]. The range of infrared spectrum peak with wave number of 3000-4000 cm^{-1} showed that the presence of water and –OH group in *M. stenopetala* and *A. indica* seed oil; and from the spectra of biodiesel, it was observed that there was no presence of moisture or water content in the biodiesel (Figure 5 above) signifying that the synthesized biodiesel can be utilized in the diesel engine as alternative fuel or energy source.

Results of physico-chemical determination of biodiesel

Table 7. Results of physico-chemical properties of biodiesel produced from hybridized M. stenopetala and A. indica seed oil.

Properties		Unit of measurement	Standard method used	Experimental value	Standard Values	
					EN 14214 Standard [28]	ASTM Standard [16]
Specific gravity		-	ASTM D1298 [29]	0.87 ± 0.01	0.86 - 0.90	-
Flash point		˚C	ASTM D93 [30]	163 ± 1.54	> 130	≥ 120
Pour point		˚C	ASTM D5853 [35]	4.36 ± 0.50	*	*
Cloud point		˚C	ASTM D2500 [41]	8.68 ± 1.12	*	*
Higher heating value		MJ/Kg	ASTM D240 [31]	41.64 ± 0.33	-	40 - 42
Kinematic viscosity	at 22 ˚C	mm^2/s	ASTM D445 [32]	5.64 ± 0.35	3.5 - 5.0	1.9 - 6.0
	at 40 ˚C			3.35 ± 0.15		
	at 60 ˚C			2.42 ± 0.56		
Cetane number		-	ASTM D613 [33]	53 ± 0.58	> 51	≥ 47
Acid number		mg KOH/g	ASTM D664 [34]	0.134 ± 0.12	≤ 0.5	≤ 0.5
Water and sediment		%vol.	ASTM D2709 [42]	0.067 ± 0.06	-	≤ 0.05
Carbon residue		%mass	ASTM D4530 [43]	0.03 ± 0.01	-	< 0.05
Methanol content		%	EN 14110 [44]	0.01 ± 0.005	< 0.2	-
Free glycerin		%mass	ASTM D6584 [45]	0.013 ± 0.005	< 0.02	< 0.02
Total glycerin		%mass	ASTM D6584 [45]	0.015 ± 0.01	< 0.25	< 0.24
Oxidation stability		Hours	EN 14112 [46]	7.20 ± 0.50	8 hours	3 hours
Phosphorous		mg/Kg	ASTM D4951 [47]	0.03 ± 0.01	< 4.0	< 0.001
Sulfur content		mg/Kg	ASTM D5453 [48]	0.06 ± 0.03	< 10.0	< 0.05
Calcium and magnesium		mg/Kg	EN 14538 [49]	3.13 ± 0.04	< 5	≤ 5

The evaluation of physico-chemical properties of the resulting product was carried out for the maximum yield of biodiesel (92 %) that was obtained from experimental run 15 (Table 2). The physico-chemical characteristics of the optimized fatty acid methyl ester or biodiesel (i.e. specific gravity, flash point, pour point, cloud point, higher heating value, kinematic viscosity, Cetane number, acid number, water and sediment, carbon residues, methanol content, free glycerin, total glycerin, oxidation stability, phosphorus and sulfur content, and calcium and magnesium content) were evaluated according to the procedures provided in their respective standard test method, ASTM [16] and EN 14214 standards [28] (Table 7 above). The obtained results were in agreement with the standard limit of biodiesel quality specification parameters, and hence, the synthesized FAME from hybridized *M. stenopetala* and *A. indica* seed oil was suitable for application in the diesel engine. In this study, biodiesel production by hybridization of considered feedstocks involves mixing of *M. stenopetala* and *A. indica* seed oil at various

percentage of mixing composition (% v/v), and thereby, obtaining the final product with improved quality. Moreover, the presence of high percentage of mono-unsaturated fatty acids (i.e. predominantly, oleic acid) in each feedstocks (i.e. *M stenopetala* and *A. indica* seed oil) induces the improvement of biodiesel quality parameters. Thus the presence of 76.00% of oleic acid ($C_{18}H_{32}O_2$) in *M. stenopetala* seed oil [50] and 51.00% oleic acid ($C_{18}H_{32}O_2$) in *A. indica* seed oil [51] enhances and improves the specific gravity (0.87 ± 0.01), flash point (163 ± 1.54 $^{\circ}$C), higher heating value (41.64 ± 0.33 MJ/Kg), Cetane number (53 ± 0.58) and other properties of the produced biodiesel (Table 7). Therefore, biodiesel obtained from hybridized or blended *M. stenopetala* and *A. indica* seed oil exhibits better fuel quality than biodiesel produced from individual feedstocks.

5. Conclusion

A fatty acid alkyl ester (biodiesel) is a long-chain of fatty acid mono-alkyl esters synthesized by the transesterification reaction of triglycerides of vegetable oils and animal fats with alcohol in the presence of a suitable catalyst. It has been taken into consideration as cleaner, alternative, renewable, biodegradable, and environmentally benign energy source. Upon its sustainable utilization, biodiesel can replace conventional diesel fuel and solve challenges accompanied with environmental pollution due to emissions of harmful gaseous and particulate matters from conventional diesel fuel, climate change, socio-economic, energy crisis, and global warming. This study aimed at synthesizing biodiesel with improved quality by hybridization of *M. stenopetala* and *A. indica* seed oil to substitute conventional diesel fuel with greener, alternative and eco-friendly energy source.

In the biodiesel production process, the statistical software package Design-Expert® RSM, Box-Behnken Design (BBD) method, was employed in the experimental design and the result analysis. The considered factors of the study were oil mixing composition, catalyst dose, and reaction time with three levels for each factor, low (-1), medium (0) and high (+1); and the other operational parameters, alcohol to oil molar ratio, temperature and mixing speed, were kept constant. The interaction effects of factors and their influences on response (biodiesel yield) were studied. The considered oil hybrid compositions subjected to the transesterification reaction were $M_{25}N_{75}$ (25% v/v *M. stenopetala* seed oil : 75% v/v *A. indica* seed oil), $M_{50}N_{50}$ (50% v/v *M. stenopetala* seed oil:50% v/v *A. indica* seed oil), and $M_{75}N_{25}$ (75% v/v *M. stenopetala* seed oil:25% v/v *A. indica* seed oil) with their corresponding biodiesel, $BM_{25}N_{75}$, $BM_{50}N_{50}$, $BM_{75}N_{25}$; catalyst dose of 1% w/v, 1.5% w/v, and 2% w/v; and reaction times of 20 min, 40 min, and 60 min. A total of 15 experimental runs were conducted with 3 levels for each numeric factor. The quadratic model was developed for conducted experiment with statistical significance, *P-value < 0.0001*. The analysis of variance (ANOVA) and coefficients of determinations were used to evaluate the quality of the model, where the main comparison was conducted at 5% least significant difference. The model adequacy was expressed using, $R^2 = 0.9933$, $Adj\text{-}R^2 = 0.9812$, $Pred\text{-}R^2 = 0.8993$, and lack of fit test with *P-value = 0.1071* (i.e. statistically not significant). The value of $Pred\text{-}R^2 = 0.8993$ was adequately in agreement with the value of $R^2 = 0.9933$, signified that the developed quadratic model equation can be used to navigate the design space. The maximum 92% biodiesel yield was obtained at the operating variables, oil mixing composition (75% v/v of *M. stenopetala* and 25% v/v of *A. indica* seed oil), catalyst dose (1.5 %w/v KOH) and reaction time (60 min). The physico-chemical characteristics of the optimized fatty acid methyl ester or biodiesel (i.e. specific gravity, flash point, pour point, cloud point, higher heating value, kinematic viscosity, Cetane number, acid number, water and sediment, carbon residues, methanol content, free glycerin, total glycerin, oxidation stability, phosphorus and sulfur content, and calcium and magnesium content) were evaluated according to the procedures provided in their respective standard test methods, ASTM [16] and EN 14214 standards [28], and the obtained results were in agreement with the standard limit of biodiesel quality.

6. Acknowledgments

The authors are grateful and thankful to Ethiopian Forestry Development and Addis Ababa Institute of Technology (AAiT) of Addis Ababa University (AAU) for the materials and financial support during the study. In addition, the authors would like to thank Laboratory Technicians in the School of Chemical and Bioengineering, AAiT, who have made valuable and fruitful contributions, for their active participation and all rounded support over the course of the study.

7. References

[1] "Organization of the Petroleum Exporting Countries (OPEC)," World Oil Outlook, Vienna, Austria, 2011, ISBN 978-3-9502722-1-5.

[2] A.B. Yahya, "Biodiesel production from Moringa Oleifera seeds using heterogeneous acid and alkali catalyst," Diss. UMP., 2013.

[3] A.A. Ayoola, K.F. Hymore, and C.A. Omonhinmin, "Optimization of biodiesel production from selected waste oils using response surface methodology," Biotechnol, Vol. 16, pp. 1-9, 2017.

[4] C. Carraretto, A. Macor and A. Mirandola, "Biodiesel as alternative fuel: Experimental analysis and energetic evaluation," J Energy, Vol. 29, pp. 2195-2211, 2004.

[5] S. Singh and D. Singh, "Biodiesel production through the use of different sources and characterization of oils and their esters as the substitute of diesel: A review." Renewable and Sustainable Energy Reviews, Vol. 14, pp. 200-216, 2010, doi: 10.1016/j.rser.2009.07.017.

[6] B.K. Barnwal and M.P. Sharma, "Prospects of Biodiesel production from vegetables oils in India," Journal of Renewable Energy and Sustainable Energy Review, Vol. 9, pp. 363-378, 2005.

[7] P.V. Amish, N. Subrahmanyam, and A.P. Payal, "Production of biodiesel through trans- esterification of jatropha oil using KNO_3/Al_2O_3," Fuel, Vol. 88, pp. 625-628, 2009.

[8] M. Balat, "Political, economic and environmental impacts of biomass-based hydrogen," Int J Hydrogen Energy, Vol. 34, pp. 3589-3603, 2009.

[9] Y. Arifn, E. Tanudjaja, A. Dimyati, and R. Pinontoan, "A second generation biofuel from cellulosic agricultural by-product fermentation using clostridium species for electricity generation," Energy Procedia, Vol. 47, pp. 310-315, 2014.

[10] E. Kirtay, "Recent advances in production of hydrogen from biomass," Energy Convers Manage, Vol. 52, pp. 1778-1789, 2011.

[11] S.A. Hossain, B.M. Salleh, A. Boyce, and A.N. Chowdhury, "Biodiesel Fuel Production from Algae as Renewable Energy, "American Journal of Biochemistry and Biotechnology, Vol. 4, pp. 250-251, 2008.

[12] "Federal Democratic Republic of Ethiopia (FDRE), Growth and Transformation Plan (2010/11-2014/15), Addis Ababa," 2010.

[13] Y. P. Upadhyay and R.B. Sharma, "Biodiesel: An alternative fuel and its emission effect," Journal of Mechanical and Civil Engineering, Vol. 5, No. 3, pp. 1-4, 2013.

[14] V. J. Gerpen, "Biodiesel processing and production," Fuel Processing Technology, Vol. 86, pp. 1097-1107, 2005, doi: 10.1016/j.fuproc.2004.11.005.

[15] S.D. Romano, S. E. González and M.A. Laborde, "Biodiesel In: Combustibles Alternatives, 2nd Edn. Ediciones Cooperativas, Buenos Aires, 2006.

[16] "American Standards of Testing Materials, Standard specification for biodiesel fuel (B100) blend stock for distillate fuels. In: Annual Book of ASTM Standards," ASTM International, West Conshohocken, ASTM D6751-08, 2008a.

[17] Y.D. Domínguez, D.T. García, L.G. Pérez, E.F. Santana, M.R. Macias, T. Fischer and R.P. Rodríguez, "Rheological behavior and properties of biodiesel and vegetable oil from Moringa Oleifera Lam," AFINIDAD, Vol. 76, pp. 587, 2019.

[18] G. Knothe, "Improving biodiesel fuel properties by modifying fatty ester composition," Energy Environment Science, Vol. 2, No. 7, pp. 759-766, 2009.

[19] A.M. Liaquat, A.M. Kalam, H.H. Masjuki, and M.H. Jayed, "Potential emissions reduction in road transport sector using biofuel in developing countries," Atmospheric Environment, Vol. 44, No. 32, pp. 3869-3877, 2010.

[20] U. Rashid, F. Anwar and B.R. Moser, "Moringa Oleifera oil: A possible source of Biodiesel," Bioresour Technol, Vol. 99, pp. 8175-8179, 2008.

[21] G. Kafuku, M.K. Lam, J. Kansedo, K.T. Lee, and M. Mbarawa, "Heterogeneous catalyzed biodiesel production from Moringa Oleifera oil," Fuel Process Technol, Vol. 91, pp. 1525-1529, 2010.

[22] G. Balaji and M. Cheralathan, "Potential of various sources for biodiesel production, Energy Sources, Part A: Recovery, Utilization, and Environmental Effects," Vol. 35, No. 9, pp. 831- 839, 2013.

[23] N.K. Patel, P.S. Nagar, and S.N. Shah, "Identification of non-edible seeds as potential feedstock for the production and application of bio-diesel," Energy and power, Vol. 3, No. 4, pp. 67-78, 2013.

[24] M. Bockish, "Extraction of vegetable oils": Fats and oils handbook Champaign, IL: AOCS Press, 151, 1998.

[25] A.O. Aliyu, J.M. Nwaedozie, and A. Ahmed, "Quality Parameters of Biodiesel Produced from Locally Sourced Moringa oleifera and Citrullus colocynthis L. Seeds Found in Kaduna, Nigeria," International Research Journal of Pure & Applied Chemistry, Vol. 3, No. 4, pp. 377-390, 2013.

[26] N. Aslan, and Y. Cebeci, "Application of Box-Behnken design and response surface methodology for modeling of some," Turkish coals Fuel, Vol. 86, pp. 90-97, 2007.

[27] M. Hanumanth, O.D. Hebbal, and M.C. Navindgi, "Extraction of Biodiesel from Vegetable Oils and Their Comparisons," International Journal of Advanced Scientific Research and Technology, Vol. 2, pp. 2, 2012.

[28] "Liquid petroleum products-Fatty acid methyl esters (FAME) for use in diesel engines and heating applications - Requirements and test methods," European Union's EN 14214 Standards Test Method, 2019-05, 2019.

[29] "American Society for Testing Materials, Standard Test Method for Density, Relative Density (Specific Gravity), or API Gravity of Crude Petroleum and Liquid Petroleum Products by Hydrometer Method," ASTM D1298.

[30] "Standard test method for flash and fire points by Cleveland open cup tester. American Society for Testing and Materials, Pennsylvania," ASTM D92-05, 2005.

[31] "Standard Test Method for Heat of Combustion of Liquid Hydrocarbon Fuels by Bomb CalorimeteR," ASTM D240-17, 2017.

[32] "Standard test method for kinematic viscosity of transparent and opaque liquids (and the calculation of dynamic viscosity). American Society for Testing and Materials, Philadelphia," ASTM, D445.

[33] "Standard Test Method for Cetane Number of Diesel Fuel Oil," ASTM D613.

[34] "Standard Test Method for Acid Number of Petroleum Products by Potentiometric Titration," ASTM D664.

[35] "Standard Test Method for Pour Point of Crude Oils," ASTM D5853.

[36] M. Tariq, M. Sirajuddin, and S. Ali, "Physico-chemical Characterization of Biodiesel from Vegetable Oils," Germany, Saarbrücken: Lambert Academic, 2015.

[37] S. Donnell, I. Demshemino, M. Yahaya, I. Nwadike, and L. Okoro, "A review on the spectroscopic analyses of biodiesel," European International Journal of Science and Technology, Vol. 2, pp. 137-146, 2013.

[38] S. K. Banik, M. A. Rouf, T. Rabeya, M. Khanam, S.I. Sajal, S. B. Sabur, and M.R. Islam, "Production of biodiesel from neem seed oil," Bangladesh J. Sci. Ind. Res. Vol. 53, No. 3, pp. 211-218, 2018.

[39] M.A. Dube, S. Zheng, D.D. Mclean, and M.J. Kates, "Comparison of attenuated total reflectance-Fourier Transformation Infrared (FTIR) spectroscopy and GPC for monitoring biodiesel production," Journal of the American Oil Chemist Society, Vol. 81, pp. 599-603, 2004.

[40] A.Y. Oyerinde and E.I. Bello, "Use of Fourier Transformation Infrared (FT-IR) Spectroscopy for Analysis of Functional Groups in Peanuts Oil Biodiesel and Its Blends," British Journal of Applied Science and Technology, Vol. 13, No. 3, pp. 1-14, 2016.

[41] "Standard test method for cloud point of petroleum products," American Society for Testing and Materials, ASTM, D2500, Pennsylvania, 2002.

[42] "Standard Test Method for Water and Sediment in Middle Distillate Fuels by Centrifuge," American Society for Testing Materials, ASTM D2709-96, 2006.

[43] "Standard Test Method for Determination of Carbon Residue (Micro Method)," American Society for Testing Materials, ASTM D4530, 2003.

[44] "Fat and oil derivatives–Fatty Acid Methyl Esters–Determination of methanol content," European Union's, EN 14110, 2019.

[45] "Standard Test Method for Determination of Total Monoglycerides, Total Diglycerides, Total Triglycerides, and Free and Total Glycerin in B-100 Biodiesel Methyl Esters by Gas Chromatography," American Society for Testing aterials, ASTM D6584, 2021.

[46] "Fat and oil derivatives–Fatty Acid Methyl Esters (FAME)–Determination of oxidation stability," European Union's EN 14112, 2020.

[47] "Standard Test Method for Determination of Additive Elements in Lubricating Oils by Inductively Coupled Plasma Atomic Emission Spectrometry," American Society for Testing Materials, ASTM D4951, 2002.

[48] "Standard Test Method for Determination of Total Sulfur in Light Hydrocarbons," American Society for Testing Materials, ASTM D5453, Spark Ignition Engine Fuel, Diesel Engine Fuel, and Engine Oil by Ultraviolet Fluorescence.

[49] "Fat and oil derivatives–Fatty acid methyl ester (FAME)–Determination of Ca, K, Mg, and Na content by optical emission spectral analysis with inductively coupled plasma,", European Union's EN 14538, 2006.

[50] E. Andinet, A. Araya, A. Nigist, and L. Peter, "Moringa stenopetala seed oil as a potential feedstock for Biodiesel production in Ethiopia," Department of Chemistry, Addis Ababa University, 2010. DOI: 10.1039/b916500b.

[51] A. Adepoju and O. Olawale, "Optimization and Predictive Capability of RSM Using Controllable Variables in Azadiracha Indica Oilseeds Extraction Process," International Journal of Chemistry and Materials Research, Vol. 3, No. 1, pp. 1-10, 2015.