PRINT ISSN 1119-8362
Electronic ISSN 2659-1502

Full-text Available Online at
https://www.ajol.info/index.php/jasem
http://www.bioline.org.br/ja

J. Appl. Sci. Environ. Manage.
Vol. 27 (1) 147-154 January 2023

Optimization of Transesterification Parameters of Biodiesel Produced from Sword Bean (Cavalia gladiate) Seed Oil

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ABSTRACT: The subject of this study was to optimize the transesterification parameters of biodiesel produced from sword bean (C. gladiate) using Response Surface Methodology (RSM). Four transesterification parameters (Reaction temperature 55 0C, Catalyst concentration 0.4 wt%, Methanol to oil ratio 4:1 and reaction time 60 min) were optimized. Data obtained revealed that the optimum reaction conditions for the transesterification of C. gladiata seed oil resulted in 92.88% biodiesel yield. Multiple regression analysis produced a quadratic polynomial equation for methylester and a linear relationship was recorded between the observed and predicted value (R2 = 0.9647) with a significant molar ratio and temperature. The interaction terms of methanol to oil, molar ratio and temperature with reaction time exhibited a positive effect on the methylester yield. The response surface methodology was found to be a suitable technique for optimizing transesterification process and producing biodiesel that meets the ASTM standard.

DOI: **https://dx.doi.org/10.4314/jasem.v27i1.21**

Cite this paper as: SALAWU, F. B; OTORI, A. A; MOHAMMED, K; MATHEW, J. T. (2023). Optimization of Transesterification Parameters of Biodiesel Produced from Sword Bean (Cavalia gladiate) Seed Oil. *J. Appl. Sci. Environ. Manage.* 27 (1) 147-154

Dates: Received: 03 January 2023; Revised: 23 January 2023; Accepted: 25 January 2023;
Published: 31st January 2023

Keywords: Biodiesel; Canavalia gladiate; Methanolysis; Transesterification

The worldwide worry about the protection of the environment and the conservation of non-renewable natural resources has given rise to an alternative development of sources of energy as a substitute for conventional energy consumed worldwide comes from fossil resources (coal and natural gas). However, these sources are limited and was exhausted in the near future. Thus, looking for alternative sources of new and renewable energy such as solar and wind. Alternative new and renewable fuels have the potential to solve many of the current social problems and concerns, from air pollution and global warming to other environmental improvements and sustainability issues (Anitha and Dawn, 2010; Otori *et al.,* 2021). In the precedent scenario, biodiesel has been the main focus of attention being a renewable and biodegradable energy source. Plant seed oils, animal fats, dissipated cooking oils, or possibly additional feed-stock like microalgae are attaining popularity among researchers as potential sources of biodiesel (Yahaya *et al.*, 2016). It has a chemical structure like those of fatty acid alkyl esters and is being manufactured by means of trans-esterification of oils with alcohols having short chains or via the esterification of fatty acids. The trans-esterification process includes the transformation of triglycerides into fatty acid alkyl ester accompanying methanol or ethanol alcohols, and an alkali or acid as a catalyst, giving glycerol as a by-product (Sruthi *et al.,* 2013). Chemical inaction without using catalysts at supercritical conditions has also been observed. As a consequence of retreating petroleum reservoirs and the injurious biological impact of emitted gases from petro-diesels, biodiesel has fascinated consideration for the last couple of years as a renewable and environment-friendly energy source. Biodiesel consists of very modest sulfur, polycyclic aromatic hydrocarbons and few metals. Whereas diesel fuel as a derivative of petroleum may consist of 20 % polycyclic aromatic hydrocarbons. For the corresponding carbon numbers, polycyclic aromatic hydrocarbons have three times greater solubility in

water in comparison to straight-chain aliphatic hydrocarbons. Biodiesel is a harmless substitute for storage arid transportation because of the fact that biodiesel does not have polycyclic aromatic hydrocarbons (Anigo *et al.*, 2013). Biodiesel is obtained from renewable sources, therefore, limiting the reliance over and saving petroleum reservoirs. The other positive aspect of biodiesel is its biodegradability which poses low toxicity due to insignificant sulfur contents (Variya *et al.*, 2013). Elevated cost value may be compensated by using low price feed-stock that has flashed awareness of resources like squandered oils (e.g., non-edible fats and oils, used frying oils etc.). Most of the limitations related to biodiesel may be alleviated via cold flow developer, antioxidant additives, merging with petro-diesel and dropping the storing duration (Sulistyo *et al.*, 2014). To improve the working of biodiesel at lesser temperatures supplementary processes of crystals formation and alcohols of lengthy or branched chains are selected for trans-esterification (Sruthi *et al.*, 2013). Currently, the most developed transesterification reaction work is an alkali catalysis system. Stoichiometrically, the chemical equation needs one mole of triglyceride and three moles of alcohol to form three moles of fatty acid alkyl esters and one mole of glycerol in the presence of a strong base. Glycerin, used in the pharmaceutical and cosmetic industry along with many other applications, is produced as a by-product in this reaction (Obasi *et al.*, 2014; Jimoh et al., 2022). Subsequently, the reaction is reversible, an excess of alcohol is used to shift the reaction equilibrium to the product's side. The key parameters affecting transesterification are the molar ratio of oil to alcohol, catalyst type and its concentration, time, reaction temperature and contents of free fatty acids (FFA) and water in the feedstock oils and fats (Umar, et al., 2021). Alkali-catalyzed transesterification is much faster than acid-catalyzed transesterification and is most often used commercially. Generally, alkaline catalysts (NaOH) are used for oils with low FFA content to catalyze the transesterification reaction (Ofolu, 2017).

The response surface method (RSM) is a suitable statistical technique for the exploration of complex processes. Many studies have been reported in the literature showing the application of RSM for methanolysis of vegetable oils using acid or base catalyst. Otori *et al.* (2018) applied the same method to examine the ethanolysis, optimized the variables and reduce the free fatty acids content of *Afzelia africana* seed oil. In this study, we optimize the transesterification parameters by using RSM and develop a high biodiesel yield with superior quality end product that will meet ASTM standard.

MATERIAL AND METHODS

Collection and Pretreatment of Sample: A non-conventional feed-stock *C. gladiata* seed was selected for this study, as this plant is native to Bida, Niger State, Nigeria. The samples collected from the farm were prepared according to the method described by Edeoga *et al.* (2015). The samples (seeds) collected from the experimental sites were washed with distilled water to remove impurities and were dry at room temperature. These will then be ground into uniform powdered manually. It was sieved, weighed, bottled and kept for laboratory analysis (Mathew *et al*., 2013a, b; Mathew et al., 2014).

Extraction of Oil from the Seeds: One hundred grams of ground seeds were taken in a soxhlet extractor activated on a water bath. Extraction was done in *n*-hexane for 6 h and repeats this three times to get maximum oil. Extracted oil was made free of the solvent under a vacuum in a rotary evaporator. The % yield of oil extracted was calculated by the following formula:

$$\% \text{ Yield} = \frac{\text{Grams of oil produced}}{\text{Grams of Sample used}}\ x\ 100$$

Characterization of Extracted Oil: The extracted oil was characterized for its physicochemical properties such as density, specific gravity, colour, pH, peroxide value, acid value, iodine value, saponification value, refractive index and odour by the methods described by AOAC (2008) and Tanko et al., (2017).

Preliminary Test for C. gladiata oil: All the preliminary tests done for the physicochemical properties of *C. gladiata* oil were according to methods given in AOCS (American Oil Chemist's Society). The tests performed were density, specific gravity, colour, pH, peroxide value, acid value, iodine value, saponification value, refractive index and odour (AOCS, 2008; Tanko et al., 2017).

Production of Biodiesel Using Heterogeneous Catalyst: The transesterification reactions were carried out with 100 g of *C. gladiate* placed in a 500 ml three necked flask. The oil was heated to 60 ºC and then 140 – 165 cm^3 of methanol and 1 - 5 wt% of CaO based catalyst derived from the cow bone was weighed and added into the oil. The reaction was carried out under reflux at a stirring speed of 500 – 800 rpm with a magnetic stirrer for mixing the oil methanol and catalyst. An electronic temperature controller was used to vary the reaction temperature in the range of 25 - 80 °C and a reaction time range of 20 – 70 min was explored. Reaction parameters such as methanol-to-oil ratio, temperature, catalyst and reaction time were varied for optimization. The experimental design was done using Response Surface Methodology (RSM) based on the four variables.

Experimental Design of Trans-esterification Reaction using Response Surface Methodology (RSM): The design of an experiment for biodiesel production was used in conjunction with Response Surface Method (RSM) to optimize the condition for the maximum

conversion of oils to biodiesel. RSM is a combination of a mathematical and a statistical technique used for developing and optimizing the processes and is used to evaluate the relative significance of several factors affecting the system even in the presence of complex interactions.

Optimization of Transesterification Parameters by Heterogeneous Catalyst using Experimental and Response Surface Method (RSM): The biodiesel production was optimized experimentally using the prepared heterogeneous catalyst from cow bones. Four different parameters were optimized namely; methanol-to-oil ratio, temperature, calalyst concentration and reaction time. The response surface method was also used using Design Expert 7.0 Model.

Fuel Properties of Produced Biodiesel from C. gladiata Oil: The produced biodiesel was characterized using the standard method by ASTM for Cetane number, pour point, viscosity, saponification value, flash point, acid value, peroxide value, iodine value and free fatty acid.

Statistical Analysis: The statistical analysis which is Analysis of Variance (ANOVA) was done with the help of Design Expert 8.0 software for the response surface. The mathematical model for experiment design was chosen based on different tests like ANOVA, lack of fit test and high value of R^2. The model was only thought to be acceptable if ANOVA reached the maximum statistical significance, with *F* values within 95% level of confidence and *p* values < 0.05. The validity and significance of different parameters were evaluated using the *F* test and the *I* test. The validity of the model and the best possible value of the variables was checked through diagnostics plots like normal probability plot of residuals, predicted versus actual residuals.

RESULTS AND DISCUSSION

Physicochemical Analysis: The physicochemical analysis of *C. gladiata* oil includes the study of the % yield of *C. gladiata* oil, specific gravity, moisture content, refractive index, pH, colour, viscosity, ash content, iodine value, peroxide value, acid value, saponification, and free fatty acid (FFA)

Table 1: Physicochemical Properties of Oils from *C. gladiata*

Properties	***C. gladiata***
Yield (%)	36.70 ± 0.20
Specific gravity	0.91 ± 0.01
Moisture content (%)	1.02 ± 0.02
Refractive index (40^{o}C)	0.81 ± 0.02
pH	5.32 ± 0.42
Colour	Brown
Viscosity (mm^2/s) at 25^{o}C	22.13 ± 0.32
Ash content	1.22 ± 0.02
Iodine value (g/100g)	120.6 ± 0.15
Peroxide value (meq/kg)	4.53 ± 0.06
Acid value (mgKOH/g)	2.2 ± 0.11
Saponification (mgKOH/1g)	107.10 ± 0.10
FFA	2.1

Optimization using central composite rotatable design for the production of biodiesel from C. gladiate: 5-level -4- factors were employed in this study totaling 30 experiments, consisting of 16 factorial points, 8 axial points and 6 centre points. The level of each was chosen based on the importance of the experiment according to the method described by Razali *et al.* (2010). Central composite design (CCD), a segment of response surface methodology was used to optimize the selected preparation parameters (methanol ratio, catalyst weight, temperature, and reaction time) on *C. gladiata*. The response for this optimization is biodiesel yield. The development of a polynomial regression equation for the analysis of the correlation between these four independent transesterification process variables on biodiesel yield and their corresponding percentage yield was done using the quadratic model as suggested by the central composite design (CCD).

$$y = b_0 + \sum_{i=1}^{4} b_f x_f + \sum_{ij=1}^{4} b_{ij} x_{ij} + \sum_{j=1}^{4} b_{jj} x_{jj}$$

Where *Y* is the predicted yield of methyl esters (mol/mol), x_i and x_j represent the parameters, b_o is the offset term, b_j is the linear effect, b_{ij} is the first order interaction effect and b_{jj} is the squared effect.

Through the designed experimental data the ester phase was retracted by second order polynomial model and it is depicted in the following equation:

$Y_{C.\ gladiata}$= -184.92225 + 39.64029A + 255.86792B + 2.10807C + 1.31045D + 35.03438AB + 0.69044AC – 0.40888AD + 1.01844BC - 2.64521BD + 0.08413CD - 8.27387A^2 - 322.12760B^2 - 0.10594C^2– 4.80046 x $10^{-3}D^2$

Where y = dependent or response variable (biodiesel yield); A = methanol ratio (mol), B = catalyst weight(g),C = Reaction temperature (^{o}C)) and D = Reaction time(min) are all independent variables.

A quadratic model was used as selected by the software for the three responses. The models were selected based on the highest order polynomials where the additional terms were significant and the models were not aliased according to the sequential model sum of squares. The six replicate variables at the centre points run (25- 30) were conducted to determine the experimental error and the reproducibility of the data. The results of actual and predicted values were compared and presented in Table 2. The result signifies that there is a minimal difference between the predicted values generated by the model equations for percentage yield and the experimental data. The result showed that a linear relationship exists between the actual and predicted values thereby indicating that the model equations adequately predict the yield and the difference is within the limit of statistical acceptable.

Among the 30 experiments, 6 experiments were a repetition of the central point (Run 25- 30). The results of the parity plot of predicted and actual are presented in Figure 1-5. These are the experiments in which all the factors are in the centric point of values and the closeness of the responses of these 6 experiments is a piece of evidence that the predicted results are accurate in comparison with the experimental data. Run 13 for *C. gladiata* was found to be the best run as it has the optimum yield of (95.20, 92.88 %). This implies that the conditions at which experimental run 13 was carried out for *C. gladiata* is referred to as optimum conditions. The results of statistical parameters on the yields for *C. gladiata* are shown in Table 3. The results of a statistical analysis of variance (ANOVA) for *C. gladiata* were carried out to study the significance and fitness of the models as well as the effects of significant individual terms and their interactions on Biodiesel yield. The accuracy of the model developed can be understood by the value of R^2, adjusted R^2 and standard deviation. R^2 indicates the ratio between the sum of the squares (SSR) and with the total sum of the square (SST) and it describes up to what extent perfectly the model estimated experimental data points. Determination of the coefficient of variance (CV) value is essential as it indicates the ratio between the standard error of estimate with the mean value of the observed response as a percentage and also helps to measure the reproducibility of the model. The ANOVA for response surface quadratic model for biodiesel yield and statistical parameters was obtained from the analysis. In Table 3, the model had F-value of 35.94 which implied that it was significant and had only a 0.36 % chance that its magnitude could occur due to noise (induced variation under normal operating conditions by uncontrollable factors). Values of Prob> F less than 0.05 indicate that the model terms are significant and the only significant model terms in Table 4.3 are: A, B, C, D, AB, AC, AD, BD, CD, A^2, B^2, C^2. From the model,the factor affecting Biodisel yield could be a single or double effect (interacting effect). The quadratic terms of methanol to oil ratio, reaction temperature and reaction time were significant, while the quadratic term for catalyst weight was insignificant. The terms with a p-value greater than 0.1 were insignificant terms in the models and they were excluded from the final regression equations. It is worth mentioning that the interacting effect of temperature and time has the highest F-values in the model while reaction time has the highest F-Value in the single effect. This illustrates that the maximum yield of Biodiesel production would be obtained with these two process variables or a combination of either catalyst weight or methanol ratio. This result is in agreement with other authors (Mohan, 2015; Aliozo, 2013). Table 4 shows important parameters which determine the acceptability of the model. A rule of thumb suggested by Bas and Boyaci, (2007) is that the adjusted and predicted R^2 should be close to unity and their difference not more than 0.3. In this study, the R^2 value and adj.R^2 for *C. gladiata* is 0.9719 and 0.9440. These values revealed the goodness of the model for Biodiesel yield and thereby make the models fit for the prediction of Biodiesel yield valid. In similar manner, the adequate precision should be greater than 4 as indicated by Design expert Version 7. The values obtained .from the developed model for Biodiesel production are greater than 4, the result implied adequate signal and can be used to navigate design space. A 3-D plot was used to optimize the biodiesel production from the three oils using the response surface method. These plots were employed to show the relationship and effect of two variables on each other as well as in determining their optimal levels keeping other variables at constant levels. The results are presented in Figure 1-5. The plots show the relationship between the four parameters optimizes. The interaction effects of variables on biodiesel production are studied by plotting the three-dimensional response surfaces with the vertical axis representing biodiesel conversion (response) and two horizontal axes representing the coded levels of two independent variables while keeping other variables at their central level (0). Figure 1 indicates the relationship between reaction temperature and molar ratio, there is a direct relationship between these two as the malar ratio increases product yield and also increases with increasing temperature but to a certain extent after which further increase lowers the yield. Figure 2 and 3 also shows that reaction time and catalyst concentration both on molar ratio are directly proportional to each other, product yield gradually increases with increasing reaction time/catalyst concentration and molar ratio but up to an extent. The same interactions are shown in figure 4 -5 with a 3D plot indicating the significance of the interaction between reaction time and temperature, catalyst concentration and reaction time. From all the above discussion, it is clear that catalyst concentration, reaction temperature and time have more influence on product yield. It can be accomplished from all the interpretations made above that the most optimum parameters to get maximum biodiesel yield (78.0%) are catalyst concentration, 5:1 mole ratio, 60 ^{0}C reaction temperature, and 65 min reaction time. This data is also supported by the literature, but different values of parameters are also observed for methanolysis of *pongamia* oil i.e. 12:1 methanol to oil molar ratio, 65 ^{0}C reaction temperature for 3 h. Table 5 describes the fuel properties of optimized *C. gladiata* oil biodiesel compared with ASTM (6751) specification. The fuel properties are of valuable significance as the quality of biodiesel is greatly dependent upon these properties. Cloud point and pour point have implications for the use of biodiesel in cold weather applications. The cloud point is the most common measure of the tendency of a fuel to crystallize and the cloud point of *C. gladiata* was determined as (-7.10 ± 0.10 ^{0}C).

Table 2: Result of Experimental Design Matrix for Biodiesel Yields

Run	Methanol Ratio (moll)	Catalyst Weigh (g)	Temp. (ºC)	Time (min)	% Yield Actual	% Yield Predicted
1	4.10	0.40	30.00	30.00	38.66	43.30
2	6.10	0.40	30.00	30.00	8.34	7.62
3	4.10	0.80	30.00	30.00	34.12	33.99
4	6.10	0.40	30.00	30.00	25.88	24.34
5	4.10	0.40	30.00	30.00	10.12	11.94
6	6.10	0.40	55.00	30.00	11.23	11.89
7	4.10	0.80	55.00	30.00	9.44	7.790
8	6.10	0.80	55.00	30.00	12.88	15.75
9	4.10	0.40	35.00	60.00	89.88	77.83
10	6.10	0.40	35.00	60.00	13.58	15.62
1	4.10	0.80	35.00	60.00	24.05	23.71
12	6.10	0.80	35.00	60.00	0.6	0.40
13	4.10	0.40	55.00	60.00	**95.20**	**92.88**
14	6.10	0.40	55.00	60.00	58.34	60.28
15	4.10	0.80	55.00	60.00	57.44	58.98
16	6.10	0.80	55.00	60.00	58.67	52.41
17	3.10	0.60	45.00	45.00	59.55	56.24
18	7.10	0.60	45.00	45.00	9.88	11.98
19	5.10	0.20	45.00	45.00	21.77	25.75
20	5.10	1.00	45.00	45.00	10.77	12.58
21	5.10	0.60	25.00	45.00	14.99	15.10
22	5.10	0.60	65.00	45.00	35.88	34.56
23	5.10	0.60	45.00	15.00	35.66	33.29
24	5.10	0.60	45.00	75.00	91.32	92.49
25	5.10	0.60	45.00	45.00	68.76	67.21
26	5.10	0.60	45.00	45.00	66.87	67.21
27	5.10	0.60	45.00	45.00	66.81	67.21
28	5.10	0.60	45.00	45.00	66.93	67.21
29	5.10	0.60	45.00	45.00	66.95	67.21
30	5.10	0.60	45.00	45.00	66.93	67.21

Table 3: Statistical Analysis of Variance for *C. gladiata*

Source	Sum of Squares	DF	Mean Square	F Value	p-value Prob> F
Model	22818.75	14	1629.911	35.94138	< 0.0001
A	2936.873	1	2936.873	64.76138	< 0.0001
B	610.3442	1	610.3442	13.45878	0.0523
C	567.9401	1	567.9401	12.52372	0.0030
D	5256.072	1	5256.072	115.9024	< 0.0001
AB	785.5408	1	785.5408	17.32207	0.0008
AC	762.7263	1	762.7263	16.81898	0.0009
AD	601.8436	1	601.8436	13.27133	0.0024
BC	66.38176	1	66.38176	1.463793	0.2451
BD	1007.586	1	1007.586	22.21842	0.0003
CD	2540.916	1	2540.916	56.03009	< 0.0001
A^2	1877.668	1	1877.668	41.40472	< 0.0001
B^2	4553.854	1	4553.854	100.4176	< 0.0001
C^2	3078.302	1	3078.302	67.88005	< 0.0001
D^2	31.99886	1	31.99886	0.705611	0.4141
Residual	680.237	15	45.34914		

This value is within the ASTM standard of (-12 to 13 ^{0}C). This signifies that *C. gladiata* has a tendency of forming cloudy crystals easily in cold temperatures (Van Gerpen *et al.,* 2014). Flash point is the key property in determining the flammability of the fuel (Van Gerpen, *et al.,* 2014). The flash point is the lowest fuel temperature at which the application of an ignition source causes the vapour of the fuel sample to ignite under the prescribed test condition. The result obtained for the produced biodiesel is 150 ^{0}C.

Table 4: Statistical Parameters Obtained from the Analysis of Variance (ANOVA) on Biodiesel Yield

Variables	*C. gladiata*
Standard Deviation	6.73
R^2	0.9719
Adj-R^2	0.9440
Predicted R^2	0.8338
Adequate Precision	20.01
Mean	40.94

This value is within the range of biodiesel fuel (100 to 170^{0}C). The high value indicates that the biodiesel produced in this study is essentially free from methanol as even a small amount of methanol can reduce the flash point, and this affects fuel pump seals (Zhang, *et al.*, 2014). According to Zaku *et al.* (2012) reported that biodiesel with a flash points of less than 100 is classified as non-flammable and hazardous; and this may cause a fire hazard. Therefore, biodiesel is safer than petrol diesel because petrol diesel has a low flash point while biodiesel has a high flash point. Cetane number is a measure of the ignition quality of diesel fuel and is a prime indicator of fuel quality in the realm of diesel engines. The cetane number of a fuel is related to the ignition delay time. The time requires for injection of fuel into the cylinder and the concept of ignition. The results of the Cetane number from the biodiesel study is 50.10±0.10. The value is within the limits of ASTM standard (48 – 65) of biodiesel production. The value was in agreement with the report of Yahaya *et al.* (2016) for jatropha oil at 46.75 and hence the results are within the acceptable limit.

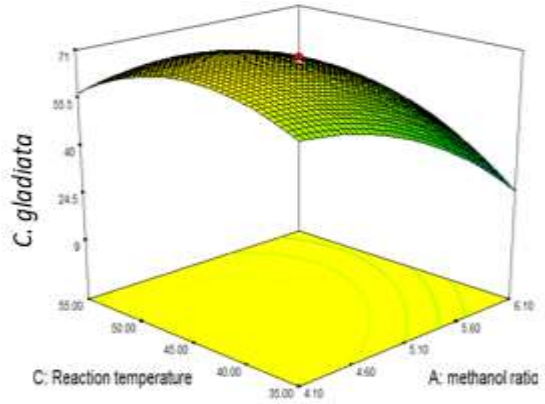

Fig 1: Response Surface Plot showing the Effect of Reaction Temperature and Molar ratio on Biodiesel Conversion

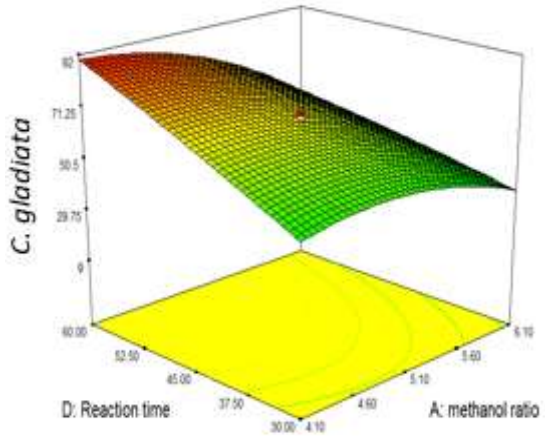

Fig 2: Response Surface Plot showing the Effect of Reaction Time and Mole ratio on Biodiesel Conversion

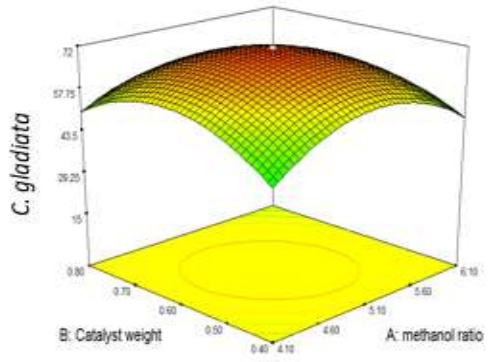

Fig 3: Response Surface Plot showing the Effect of catalyst concentration and mole ratio on Biodiesel Conversion

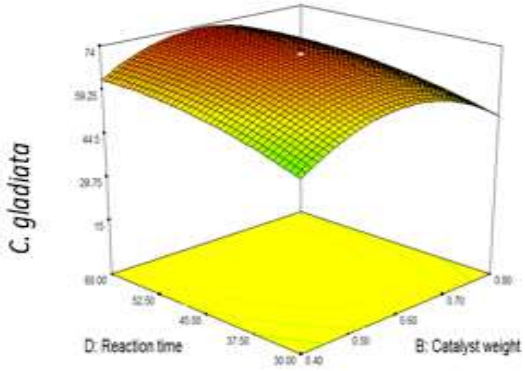

Fig 4: Response Surface Plot showing the Effect of Reaction Temperature and Reaction Time on Biodiesel Conversion

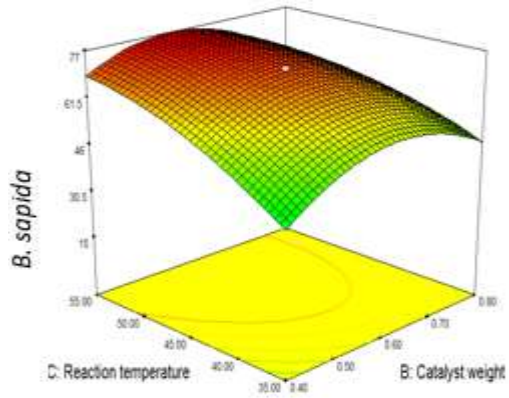

Fig 5: Response Surface Plot showing the Effect of Reaction Temperature and Catalyst concentration on Biodiesel Conversion

Kinematic viscosity which is a measure of the flow capabilities of the oils was measured at 30^{0}C. High velocity is a major problem in using vegetable oil as fuel for diesel engines (Wang *et al.*, 2016). The result of viscosity shows that the kinematic viscosity of the biodiesel produced is within the ASTM standard (1.9 – 6.0 mm^2/s) 3.43±0.06 mm^2/s. Dantas *et al.* (2011) reported the viscosity of soya beans methyl ester (3.8-4.1 mm^2/s) and this value is in agreement with the result obtained from the study. The high value of

viscosity in biodiesel may be attributed to incomplete reaction or insufficient purification steps as reported by Mushrush *et al.,* (2015). The pour point is the temperature at which the amount of wax from biodiesel is sufficient to gel the fuel, from the result it was shown that the pour point of the produced biodiesel is -7.0±0.06 ^{0}C. This result suggests that the biodiesel produced is within the ASTM standard (-1.5 – 10.0^{0}C). The result also suggests that biodiesel from (16 ^{0}C) will coagulate or solidify faster. It can be observed that some of the products have higher pour points than others.

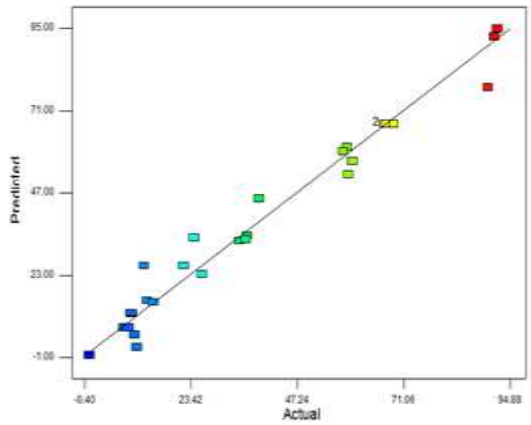

Fig 6: Parity Plot of Predicted and Actual Yield of *C. gladiata*

Table 5: Fuel Properties of Biodiesel Produced from *C. gladiata*

Biodiesel Properties	***C. gladiata***	**ASTM 6751**
Flash point(˚C)	151.70±0.65	100 – 170
Cloud point(˚C)	-7.10±0.10	-13 – 12
Specific gravity	0.90±0.02	
Ash content (Wt %)	0.03±0.01	0.02
Cetane number	46.70±0.58	48 – 65
Water content (%)	0.02±0.01	0.05
Boiling point(˚C)	60.20±0.10	-
Acid value(mgKOH/g)	0.02±0.01	0.05max
Iodine value	105±0.13	-
Pour point(˚C)	-7.0±0.05	-15 – 10
Density(g/cm^3)	0.82±0.01	0.75-0.84
Kinematic viscosity(mm^2/s)	3.43±0.06	1.9 – 6.0

Values are mean ± SD of triplicate determinations

This could be due to variations in the feedstock oil composition. The biodiesel samples could be handled and stored safely without solidifying, but when the fuel is to be used in cold areas, they would have to be gently preheated or blended with some additives that will reduce the pour point (Mushrush *et al.* 2015). The acid value is a measure of the acid constituents in fuel; it is a good indicator of the quality of the fuel. The common acid constituents are fatty acid which has a high tendency for corrosion and is also a symptom of water in the fuel (Zhang *et al.*, 2014). The maximum limit of acid contents in biodiesel was (0.05 – 0.8 mgKOH/g). The result obtained in Table 6 shows that *C. gladiata* (0.02±0.01 mg/KOH/g). The result indicates that the biodiesel produced have less tendency of causing wear in storage tanks and fuel system.

Conclusions: The fuel properties of *C. gladiata* biodiesel were found to be within the ranges of ASTM 6751 standard. The standard specified that *C. gladiata* oil can be used as a potential source for biodiesel production that can be combusted in compression-ignition engines and this way substitution, the common petrol diesel which is being exhausted from the globe. The findings of this study might lead to exploring such *C. gladiata* seed oil for the production of high-yield and better quality renewable and sustainable biodiesel as an alternative to petro-diesel.

Acknowledgments: The authors would like to acknowledge TetFund, the Rector of Federal Polytechnic Bida in the person of Dr. Abdul Dzukogi, all the manage¬ent staff of the institution and the director of institution-based research, Mr. Ahmed Ohizie for the nomination and for providing enabling environment for this research work.

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