Optimization and Characterization of Biodiesel Synthesis from Neem Seed Oil

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Abstract

Bd was produced by transesterification of refined NSO with MeOH and CaO catalyst using batch mode. Physicochemical properties of NSO and Bd as an alternative diesel fuel were characterized using ASTM fuel test standards. The FA composition and functional groups of Bd were investigated using GC-MS and FT-IR, respectively. The effect of basic parameters such as reaction catalyst Ct, MeOH/oil molar ratio, reaction T and time, and SS were determined using batch mode. These process parameters were optimized using RSM and ANOVA. The importance of various process parameters and their combined effects on transesterification performance was determined through CCD. Statistical analyzes of the data led to the development of a second-order quadratic polynomial regression model that established the relationship between Bd yield and process variables. The model was able to adequately predict Bd yield with R² value of 0.9942. The obtained results were in good agreement with published data for Bd from other vegetable oils, as well as with various international standards for this product. An optimal yield of 94% was achieved under optimal conditions of catalyst Ct (4 wt%), MeOH/oil molar ratio of 8:1, T of 55 °C, time of 60 min and SS of 400 rpm. The results show that Bd produced using alcohol in the transesterification process improved CP and CFPP properties.

Keywords: ANOVA; Bd; NSO; oil transesterification; optimization; RSM.

Introduction•

Energy has always been considered as a key source for the sustainable economic growth of any country, and fossil fuels such as coal, natural gas and crude oil have

[•]The abbreviations list is in pages 351-52.

been contributing as major sources for the fulfillment of this need. With the rapid expansion of industrialization and urbanization, by 2035, total world energy consumption is predicted to increase by 33% [1]. The world is concerned about the dependence of human progress on these energy resources and its depletion. As a result, alternative energy sources are becoming increasingly important [2]. Alternate fuels that replace these primary sources of fuel which are fast depleting have to be found. Natural and sustainable fuels from more easily available sources like plants and vegetables are available to be exploited [3], since they are less polluting and more environmentally friendly [4]. Preferably, raw materials should be inedible and have a high content of FFA, which can be transesterified with an alcohol solvent (MeOH/ethanol) in the presence of a suitable catalyst.

In general, Bd are defined as monoalkyl esters of long-chain FA, derived from lipids such as vegetable oils, animal fats and microalgae. Bd can be made from vegetable oil through several processes, including micro-emulsion, pyrolysis (thermal cracking), direct use, blending and transesterification [5]. Their production is generally carried out by the esterification reaction of FA and/or the transesterification reaction of triglycerides in the presence of alcohol, and usually in the presence of a catalyst [6]. Transesterification is the most commonly used method for Bd production [2] in laboratories and on an industrial scale, with cost-effective and environmentally friendly catalysts [7]. Transesterification, the main Bd production process, typically involves the reaction of an alkyl alcohol with a long-chain ester bond, in the presence of a catalyst, to form monoalkyl esters (Bd) and a byproduct, glycerol. Commonly used alcohols are methyl alcohol and ethyl alcohol. MeOH has been steadily used in commercial activities, due to its low cost and physical and chemical benefits [8].

Furthermore, raw material costs account for 60 to 80% of the total Bd production costs, which weigh heavily on its economic viability. An alternative approach to improve the economic feasibility of Bd production and contribute to a sustainable society is to use raw materials that are not suitable for human consumption. Therefore, NSO is gaining importance as an alternative cheap source of raw material for Bd production [7]. Different types of catalysts such as base, acid or lipase are used in transesterification for Bd synthesis, but the base-catalyzed reaction is the most common in the industry, due to easier, faster and cheaper processing [9].

In optimizing the operating parameters, a statistical approach was used, i.e., RSM. RSM approach is a collection of mathematical and statistical techniques used to analyze data, create models, find optimal conditions and determine the importance of factors influencing a process. In Bd industry, efficiency and cost-effectiveness are paramount, so optimizing the production process is a top priority [10]. RSM is used to reduce the number of trials required and to evaluate multiple factors and their interactions, so as to make the experimental process faster and more accurate [11].

The main purpose of this research was to use RSM methodology in combination with CCD, to optimize the operating conditions of Bd production from NSO, by transesterification process, using a low toxic alcohol (MeOH). The novelty of the study lies in its focus on the synthesis of Bd from NSO, and the subsequent characterization of the obtained product. NSO is not commonly used as feedstock for Bd production, so exploring its potential as a source of Bd is an interesting aspect of this study. The optimization process involved in this research aimed to determine the optimal conditions for NSO transesterification reaction, considering variables such as catalyst weight, MeOH:NSO molar ratios, reaction T, RT and SS. Furthermore, the characterization of the synthesized Bd provided valuable insights into its properties and quality. This included analyzing parameters such as Bd yield and its physical and chemical properties, and potentially assessing its compatibility with existing diesel engines and infrastructures.

By focusing on NSO as a feedstock and optimizing the synthesis process, while characterizing the resulting Bd, this study contributed to the broader understanding of Bd production, expanding the knowledge base in the field.

Materials and methods

Materials

Refined NSO was obtained from a local market in Kaduna State. MeOH (analytical grade) at 99.5% purity was procured from Zaria. All chemicals were of analytical grade, and used without further purification.

Methods

The batch process was chosen for Bd production, due to MeOH sensitivity and rapid evaporation. The process involved pre-heating NSO to 60 °C, using a water bath with a T regulator. The transesterification reaction was performed using a 250 mL batch reactor equipped with a reflux condenser and a magnetic stirrer. The reaction mixture consisted of NSO, MeOH and CaO, as catalyst. Several variables were considered during the experiment, including catalyst weight (3-5 wt%), MeOH:oil molar ratios (6:1-10:1), reaction T (45-65 °C), RT (2-6 h) and SS (300-500 rpm). After a suitable period, excess alcohol was evaporated at a mild T, under a moderate vacuum, using a rotary evaporator. The mixture was then transferred to a separating funnel for phase separation. Once separated, the remaining mixture was neutralized, and any traces of catalyst and alcohol were washed out with distilled water, until the hydric layer became completely translucent. To remove any remaining water, the Bd was heated at 100 °C, for 45 min, followed by the addition of anhydrous CaCl. The percentage of Bd yield was determined by comparing the weight of the Bd layer with that of the refined NSO.

Bd yield (%) =
$$\frac{biodiesel weigh}{used oil weig} x100$$
 (1)

Physiochemical characterization of NSO and produced Bd

Physiochemical properties of oil and produced Bd were characterized according to ASTM D6751.

Determination of SG

A 25 mL SG bottle was weighed while empty. The bottle was filled with water and weighed. After draining the bottle, NSO and the Bd sample were poured into the bottles, and the weights were recorded. The experiment was carried out as quickly as possible, at room T. SG of NSO was calculated from the recorded weight using the relation:

$$SG = \frac{sample weight}{weight of equivalent water volume}$$
(2)

Determination of viscosity

The rheological behavior was tested using the absolute viscosity determined in a Brookfield viscometer LV –DVII model, at varying T, using spindle 62. The T of the samples was varied, with a constant T magnetic hot plate. The viscosity measurements were then taken at different shear rates.

Determination of FFA

1 g each of NSO and Bd samples was dissolved in a 25 mL neutral mixture (equal volume of diethyl ether and absolute ethanol). The resultant oil solution was titrated with a 0.1 M KOH solution, with a phenolphthalein indicator added in 3 drops. The titration continued until a permanent pink color appeared, which indicated that the endpoint was reached. FFA was expressed in terms of oleic acid, and computed using the expression below:

$$FFA value = \frac{Tr \times N \times 56.1}{weight of sample}$$
(3)

where Tr is titre value, N is normality and W is the sample weight in g.

Determination of AV

1 g of each sample was dissolved in a 25 mL neutral mixture of solvent (equal volume of diethyl ether and absolute ethanol). The resultant oil solution was titrated with a 0.1 M KOH solution, using a phenolphthalein indicator. The titration continued, until a pink-colored solution was obtained, indicating the endpoint. AV was calculated using the expression below:

AV (KOH mg/g)
$$= \frac{M \times V \times 56.1}{W}$$
 (4)

where M is standard KOH (0.1 M) normality, V is KOH volume in 56.1 mL (molar mass) and W is weight in g of oil sample.

Determination of MC

About 1 g each of NSO and Bd were weighed out in preweighed Petri dishes. The two samples were placed in an oven and dried at 100 °C, for 2 h. The weights of the samples were taken every 30 min. The procedure was repeated until a constant weight was obtained. After each 30 min, each sample was removed from the oven and kept in a desiccator to cool, and it was reweighed. The cooled dishes were

weighed, and the result of each dry sample was determined and recorded. The MC in the samples was calculated from the formula:

$$MC(\%) = \frac{W_1 - W_2}{weight \ of \ sample} \tag{5}$$

where W1 and W2 are the original sample weight before and after drying, respectively.

Determination of IV

1 g of each sample was dissolved in 15 mL carbon tetrachloride. The solution was mixed with 25 mL of Wiji's solution. The flask with the content was kept in the dark, for thirty min, at room T, to enable oxidation to take place. Then, 100 mL distilled water and 20 mL 10% Kl solution were added to the flask content. The resultant mixture was titrated with 0.1 M Na₂S₃O₃ using 10% starch solution (weight by volume). A blank determination was carried out in the same manner under similar conditions. IV was calculated as:

$$IV = \frac{12.6 \times N(W_1 - W_2)}{weight of sample (W)}$$
(6)

where 12.69 is iodine molecular mass, N is normality, V_2 is blank titre value, V_1 is sample titre value and W is sample weight.

Determination of SV

1 g of each sample, NSO and Bd was dissolved in 25 mL 0.5 M ethanolic KOH solution, using a 250 mL quick-fit flask. The flask was heated in a steam bath under reflux, for 30 min, with occasional swirling. The resultant solution was heated with 0.5 M HCl, using a phenolphthalein indicator. A blank determination was carried out under similar conditions. The difference in the above two titer values gave the number in mL of HC1 acid required to neutralize the alkali after saponification. SV was determined as follows:

SV (number) =
$$\frac{56.1 \times N(V_2 - V_1)}{W}$$
 (7)

where 56.1 is KOH molecular mass.

Determination of FAME value

EV of the NSO and Bd samples was calculated as the difference between the SV and the acid value. Therefore:

$$EV = SV - AV \tag{8}$$

Boiling point determination

20 mL Bd were poured into a heating beaker with a thermometer, carefully converted, and heated with a hot plate. As T increased, the point at which the sample started boiling was recorded.

CP and PP determination

A cooling bath containing a freezing mixture of salt and water was used, to obtain a lower T. 50 mL FAME and NSO were placed in a glass jar, and T was lowered, until crystals clouds appeared at the bottom. 20 mL sample each of Bd and NSO were poured into a beaker, corked and kept in a refrigerator, to solidify. When the sample was brought out and kept in the open, the T at which it melted and started flowing was recorded.

Determination of RI

A few drops of NSO and Bd sample were put on the glass slide of the refractometer. Water, at 30 °C, was circulated round the glass slide, to keep its T uniform. A small fluorescence tube was brought near the apparatus's glass slide, and observed through the aperture (i.e. the eye piece of the refractometer). The circle's center was adjusted (shrunk) to where it corresponded to the graduated scale that pointed to the RI. Hence, RI of NSO and Bd sample were determined.

Determination of PV

1 g each of NSO and Bd was added to a clean dry boiling tube containing 1 g powdered Kl and 20 mL solvent mixture (2 vol glacial acetic acid + 1 vol chloroform). CO_2 was gently bubbled through the mixture of each sample and the solvent. The tube was placed in boiling water, for 30 sec. Each of the contents was quickly poured into a flask containing 20 mL 5% Kl solution, and the tubes were washed out twice with 10 mL water, then titrated with a 0.002 M Na₂S₃O₃ solution, using a starch indicator. The titration proceeded until the yellow color disappeared. A blank determination (without NSO or Bd) was carried out at the same times and conditions. PV was calculated by the expression below:

$$PV = \frac{100 \times N(V_1 - V_2)}{W}$$
(9)

where N is Na₂S₃O₃ normality and 100 represents PV constant.

OS determination

Bd oxidative stability was determined by Rancimat method. During Rancimat test, the Bd sample was heated to 110 °C, with the supply of oxygen. With oxygen at high T, the oxidation reaction took place, and its derivatives were transferred to the measuring chamber containing Millipore water. The increase in hydric conductivity was detected when the oxidation derivatives were transferred into water. Induction time is defined as the time required for the water conductivity to rapidly increase, being used as an indicator of Bd oxidative stability.

Instrumental characterization

Gas chromatography analysis

FAME was analyzed using GC-MS. The analysis was carried out with an Agilent 7890A GC-MS equipped with a FID fitted with a DB-S column (30 m x 0.25 mm ID and film thickness of 0.25 μ m), to qualitatively and quantitatively analyze the conversion of triglycerides into Bd. 1 μ L diluted Bd sample was injected into the column. The initial

oven T was set at 185 °C, and held for 1 min. Then, T was increased to 190 °C. The injector and detector were maintained at 25 and 250 °C, respectively. The detector made up gas was He. It was served at an inlet pressure of 26.708 psi. H was used as a carrier gas for the FID, which had a T of 380 °C, and its flow rate was kept at 2 m³/min.

FTIR

FTIR analysis was carried out using a FTIR spectrophotometer for the determination of functional groups present in Bd.

DOE

The optimization of Bd yield from transesterification of NSO into FAME was conducted by applying CCD of RSM version of DOE available on the Design-Expert software. CCD technique is a full or partial factorial design method with a center point that is amplified with a gathering of the axial point, which grants non-linearity is evaluated in the predicted model [12]. The process parametric factors investigated for the optimization of NSO transesterification process are shown in Table 1.

Table 1:	Independent	t variables and	levels used	for RSM of	design and	their coded	levels
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Independent variable	Units	-2	-1	0	1	2
Ct of catalyst (A)	Wt%	2(-2)	3(-1)	4	5(+1)	6(+2)
MeOH (B)	Mol/mol	4(-2)	6(-1)	8	10(+1)	12(+2)
T (C)	°C	35(-2)	45(-1)	55	65(+1)	75(+2)
RT (D)	h	2(-2)	3(-1)	4	5(+1)	6(+2)
SS (E)	Rpm	200(-2)	300(-1)	400	500(+1)	600(+2)

The catalyst weight (%wt), MeOH:oil ratio (mol/mol), reaction T (°C), RT (min), and SS (rpm) were selected on the basis of related literature research and preliminary experiments in the laboratory [7], and considered as independent variables affecting the conversion rate of NSO to Bd.

The data collected from 32 experimental runs were analyzed by RSM CCD by Design Expert Software version 10. The Ct of the catalyst (A), MeOH/oil molar ratio (B), reaction T (C), RT (D) and SS (E) were selected as independent factors for the optimization study. The chosen response was FAME yields obtained from refined NSO transesterification. The experiments were performed in random order, to avoid systematic errors.

The regression analysis was performed to estimate the response function as a second-order polynomial:

$$Y = \beta_0 + \sum_{i=1}^k \beta_i X_i + \sum_{i=1}^k \beta_{ii} X_i^2 + \sum_{i=1}^k \sum_{j=i+1}^k \beta_{ij} X_i X_j$$
(10)

where Y is FAME yield%, xi and xj are independent study factors (coded variables), β_0 , β_i , β_{ii} and β_{ij} are constant coefficient, linear, quadratic and interaction terms regression coefficient, respectively, and k is the number of factors studied and optimized in the experiment (independent variables). The levels selection for each

factor was based on the experiments performed to study the effects of process variables on NSO transesterification reaction (Table 2).

Run	Ct of catalyst	MeOH/NSO	\mathbf{T}	Time	SS	Experimental	Predicted
order	(Wt%)	molar ratio	(C)	(n)	(rpm)	vield(%)	vield(%)
	A	B	<u> </u>	D	E	,	
1	3.0	6:1	45	3	500.0	50.00	45.97
2	5.0	6:1	45	3	300.0	60.00	53.55
3	3.0	10:1	45	3	300.0	70.00	62.72
4	5.0	10:1	45	3	500.0	86.00	78.80
5	3.0	6:1	65	3	300.0	63.00	55.72
6	5.0	6:1	65	3	500.0	57.00	49.80
7	3.0	10:1	65	3	500.0	53.00	44.97
8	5.0	10:1	65	3	300.0	73.00	63.55
9	3.0	6:1	45	5	300.0	60.00	60.72
10	5.0	6:1	45	5	500.0	47.00	47.80
11	3.0	10:1	45	5	500.0	40.00	39.97
12	5.0	10:1	45	5	300.0	33.00	31.55
13	3.0	6:1	65	5	500.0	50.00	49.97
14	5.0	6:1	65	5	300.0	57.00	55.55
15	3.0	10:1	65	5	300.0	83.00	79.72
16	5.0	10:1	65	5	500.0	60.00	57.80
17	2.0	8:1	55	4	400.0	53.00	58.14
18	6.0	8:1	55	4	400.0	50.00	57.81
19	4.0	4:1	55	4	400.0	47.00	49.48
20	4.0	12:1	55	4	400.0	50.00	59.48
21	4.0	8:1	35	4	400.0	56.00	58.48
22	4.0	8:1	75	4	400.0	58.00	67.48
23	4.0	8:1	55	2	400	60.00	78.48
24	4.0	8:1	55	6	400	77.00	70.48
25	4.0	8:1	55	4	200	57.00	65.98
26	4.0	8:1	55	4	600	50.00	53.98
27	4.0	8:1	55	4	400	86.00	90.34
28	4.0	8:1	55	4	400	88.00	90.34
29	4.0	8:1	55	4	400	94.00	90.34
30	4.0	8:1	55	4	400	93.00	90.34
31	4.0	8:1	55	4	400	91.00	90.34
32	4.0	8:1	55	4	400	91.00	90.34

Table 2: Experimental set up for 2-level-5-factor RSM design, experimental and predicted values for Bd production from refined NSO.

Results and discussion

Characterization of results

Physicochemical characteristics

The results of NSO and Bd physiochemical characteristics are shown in Table 3. The major characteristics (KV, AV and FFA) are in good agreement with the standard. Table 3 indicates that SG of NSO was reduced from 0.948 to 0.843, after transesterification, being within the acceptable limit. SV of NSO and Bd were 212.3 and 185.3 mg/g, respectively, which implies that the oil's triglycerides have FA higher molecular weight A. Saponification is paramount when checking adulteration. PV is less suitable for monitoring oxidation; although it is not specified in Bd standards, it influences cetane number, a parameter that is stated in fuel standard. Since low PP and CP values show that NSO will hardly solidify at room T,

it can be stored for a long time. Obtained NOS was within ASTM D6751 range (minimum of 3 h and maximum of 6 h).

S/N	Property	Units	NSO	Bd from NSO
1	SG	-	0.948	0.893
2	KV at 30 °C	cSt	29.91	5.2
3	FFA	%	0.15	0.11
4	MC	%wt.	0.001	0.001
5	Flash point	°C	150	150
6	SV	(mg/g oil)	212.3	185.3
7	EV	(mg/g)	211.7	191.34
8	PV	(mg/kg)	40.0	32
9	IV	$(g I_2/100g)$	66.21	122.45
10	PP	°C	3	4.8
11	СР	°C	13	6.2
12	AV	KOH mg/g	0.28	0.21
13	RI	at 25 °C	1.476	1.3
14	pH value	-	3.39	2.8
15	ÔS	At 110 °C	5.0 h	4.50

Table 3: Characterization of refined Bd from NSO.

GC-MS result of FAME

In this study, quantification of FAME present in Bd from NSO was categorized using GC-MS, as shown in Fig. 1.



Figure 1: GC-MS of FAME from NSO.

The analysis shown in Table 4 revealed the presence of twenty major FA from C8:0 to C22:0, at a retention time of 5.329 to 22.523 min. The total percentage of FA identified was 100%, whereby the results were represented as the relative percentage area from the sum of all identified peaks. From the FA analysis, its highest acids contents were: oleic (C18:1n9t), elaidic (C18:1n9c), lauric (C12:0), margaric (C17:0) and lauric (C12:0), with 16.89, 30.30, 31.25, 22.98 and 16.68% wt., and retention times of 16.622, 16.530, 10.572, 14.916 and 10.497 min, respectively. Its lowest acids contents were: arachidic (C20:0), stearic (C18:0), palmitoleic (C16:1) and undecyclic

(C11:0), with 0.20, 0.22, 0.53 and 0.30% wt., and retention times of 15.578, 21.05 and 4.940 min. The difference in FA and its composition may be due to the transesterification process.

S/no	Retention time	Ester name (FAME)	FA name	Corresponding acid	FA molecular structure	%wt.
1	5.329	Octanoic	Caprylic	C8:0		3.90
2	7.934	Decanoic	Capric	C10:0		4.49
3	10.497	Dodecanoic	Lauric	C12:0	~~~~·l~	16.68
4	11.237	Eicosanoic	Arachidic	C20:0	\sim	0.20
5	12.759	Pentadecanoic	Pentadecyclic	C15:0	γ	9.67
6	14.540	Tetracosanoic	Lignoceric	C24:0	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	1.18
7	14.916	Heptadecanoic	Margaric	C17:0	$\sim \sim $	22.98
8	15.452	Tetradecanoic	Myristic	C14:0	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	0.40
9	15.578	Octadecanoic	Stearic	C18:0	~~~~~	0.22
10	15.774	Docosanoic	Behenic	C22:0		0.44
11	16.622	9-Octadecenoic	Oleic	C18:1	~~~~~ <	16.89
12	16.745	n-Octadecanoic	Linoleic	C18:2	~~~~~Ľ~	5.97
13	18.277	Undecanoic	Undecyclic	C11:0		5.13
14	18.433	Heneicosanoic	Heneicosylic	C21:0	~~~~~ů	2.04
15	19.060	Tridecanoic	Tridecyclic	C13:0	<u></u>	0.93
16	19.748	Hexadecanoic	Palmitic	C16:0	2	4.40
17	19.899	Tricosanoic	Tricosanoic	C23:0	5	1.25
18	20.032	Eicosenoic	Gonodoic	C20:1	~~~~····	1.67
19	21.536	Pentadecanoic	Pentadecyclic	C15:0	\sim	1.04
20	22.523	Linolenic	Linolenic	C18:3	γ	0.54
						100.00

Table 4: FA profile of FAME from NSO and their corresponding retention times, percentage weight of the various peaks in GCMS spectrum and molecular structure.

FTIR spectra

FTIR spectrum of FAME from NSO is shown in Fig. 2, which confirms the presence of volatile compounds at varying bending vibrations.



Figure 2: FTIR spectra of FAME from NSO.

Complete inferences of FTIR spectrum are shown in Table 5. Weak-medium peak signals at 721.43 to 1744.4 cm⁻¹ showed the presence of an aromatic alkane/alkyne group. Ethers known to be an impressive component for Bd contribute to the major stretching areas, from 1237.5 to 1461.1 cm⁻¹ and the signal at 1744.4 cm⁻¹, with a continuous, broad and strong-medium signal. The presence of a carboxylic derivative is identified by the inconsistent strong signals obtained at peaks from 2855.1 to 2922.2 cm⁻¹. The medium signals found at 2355.7 and 1744.4 cm⁻¹ showed the presence of phenols, aldehydes and ketone groups. FTIR spectra analysis of FAME from NSO is shown in Table 5.

Frequency wave number/peak (cm ⁻¹)	Transmittance%	Functional group
3008.8	95.002	O-H strong stretch
2922.2	51.628	C-H weak stretch
2855.1	64.113	C-H stretching(weak), phenols
1744.4	52.882	C=O stretching, aldehydes/ketones
1461.1	79.697	O-H weak double bending, alkynes
1375.4	87.810	O-H double bending/alkynes alcohol, phenols
1237.5	81.394	C-O stretching, acid, alcohol
1159.2	64.361	C-O stretching, acid, ether, alcohol
723.1	79.828	C-H weak/bending, alkanes

Table 5: FTIR analysis of FAME from NSO.

Modeling and optimization

Table 2 presents the results of NSO transesterification to FAME using RSM via CCD experimental design. The experimental (actual) FAME yield varied from 69.45 to 90.55%. The actual yields were evaluated to produce a suitable and workable regression model. An appropriate model was selected from categories such as mean, linear, quadratic, cubic and quartic. A quadratic regression model was generated and employed to predict optimal parameters for NSO transesterification to Bd. The best-fit model for FAME yield is shown in eq. (11).

 $Y = 90.34 - 0.083 \times A + 2.50 \times B + 2.25 \times C - 2.0 \times D - 3.0 \times E + 0.63 \times AB - 0.73 \times AC - 4.62 \times AD + 6.75 \times AE + 1.88 \times BC - 3.12 \times BD + 1.0 \times BE + 5.63 \times CD - 3.50 \times CE - 1.0 \times DE - 8.09 \times A^{2} - 8.97 \times B^{2} - 6.84 \times C^{2} - 3.97 \times D^{2} - 7.59 \times E^{2}$ (11)

where "Y" is the response, i.e., conversion to Bd, and A, B, C D and E show the test variables values, catalyst Ct, MeOH to oil molar ratio, T, RT and SS, respectively. The above equation represents the factors' quantitative effect (A, B, C, D, and E) upon the response (Y). Parameters with positive linear, interaction or quadratic coefficients have a desirable effect on FAME yield, while those negative adversely affected FAME yield. As shown in Table 2, the highest FAME yield (90.34%), as predicted by RSM regression model, was obtained with a catalyst Ct of 4% w/w, MeOH to oil ratio of 8:1, RT of 4 h, reaction T of 55 °C and SS of 400 rpm. The model equation was appraised for statistical importance using ANOVA test, R² and R_{adj}² shown in Table 6.

Source	Coefficient	Degree of	Sum of	Mean	F-value	P-value
	estimate	freedom	square	squares		(prob >F)
Model	90.34	20	8608.08	430.40	94.83	< 0.0001
A	-0.083	1	0.17	0.17	0.037	0.8515
В	2.50	1	150.00	150.00	33.05	0.0001
С	2.25	1	121.50	121.50	26.77	0.0003
D	-2.00	1	96.00	96.00	21.15	0.0008
E	-3.00	1	216.00	216.00	47.59	< 0.0001
AB	0.63	1	6.25	6.25	1.38	0.2654
AC	-0.37	1	2.25	2.25	0.50	0.4960
AD	-4.62	1	342.25	342.25	75.41	< 0.0001
AE	6.75	1	729.00	729.00	160.62	< 0.0001
BC	1.88	1	56.25	56.25	12.39	0.0048
BD	-3.12	1	156.25	156.25	34.43	0.0001
BE	1.00	1	16.00	16.00	3.53	0.0872
CD	5.63	1	506.25	506.25	111.54	< 0.0001
CE	-3.50	1	196.00	196.00	43.19	< 0.0001
DE	-1.00	1	16.00	16.00	3.53	0.0872
A^2	-8.09	1	1920.24	1920.24	423.09	< 0.0001
B^2	-8.97	1	2358.03	2358.03	519.55	< 0.0001
C^2	-6.84	1	1372.74	1372.74	302.46	< 0.0001
D^2	-3.97	1	461.37	461.37	101.65	< 0.0001
E^2	-7.59	1	1690.24	1690.24	372.42	< 0.0001
Residual		11	49.92	4.54		
Lack of fit		6	4.42	0.74	0.081	0.9959
Pure error		5	45.50	9.10		
Cor total		31	8658.00			

Table 6: ANOVA for FAME yield.

The model's F- and p-values were estimated as 94.83 and < 0.0001, respectively, indicating that it was statistically significant, at 95% CI level (p < 0.05) [13]. In that scenario, B, C, D, E, AB, AD, AE, CD, CE, A², B², C², D² and E² are statistically significant model terms. However, A, AB, AC, BE and DE had less effect (Prob>F more than 0.05) on Bd yield. F-value of 0.58 for the lack of fit denotes that it was less significant than the pure error. In addition, it was discovered that there was an 80.58% probability that a Lack of fit for F-value of 0.081 could be instigated by S/N. It is advantageous to have a non-significant Lack of fit (Table 6). The insignificant lack of fit indicates that the model is logical and valid [11]. Table 7 depicts the model summary statistics and Table 8 shows depicts ANOVA of R² equation.

Source	Std. dev.	R ²	Adjusted R ²	Predicted R ²	Press
Linear	17.62	0.0674	-0.1119	-0.2697	10992.72
2FI	19.44	0.3015	-0.3534	-7.1261	70356.09
Quadratic	2.13	0.9942	0.9837	0.9804	169.76 suggested
Cubic	2.85	0.9944	0.9709	0.5737	3690.56 aliased

Table 7: Model summary statistics

Table 8: ANOVA of regression equation.

Parameters	Values
Standard deviation	2.13
Mean	63.75
C.V.%	3.34
Press	169.76
\mathbb{R}^2	0.9942
Adj R ²	0.9837
Pred R ²	0.9804
Adeq precision	34.0

The model's measure of accuracy and precision was ascertained by R^2 and R_{adj}^2 values. An R^2 value of 0.9942 shows that 99.42% of the entire data was consistent with the predicted data. R_{adj}^2 variability of 0.9837 indicates the model's acceptable fitness. An adequate precision (S/N ratio) above 4 is desirable and advantageous. The difference between the adjusted and predicted R^2 is <0.2 [14]. S/N ratio of 34.0 indicates that the model is suitability to cross the design space, further proving that it is statistically significant. A standard deviation of 2.13 was recorded, and obtained CV low value of 3.34% is a sign of the good accuracy and dependability of this model, as indicated in Table 6 [14]. Also, the adequate correlation between the experimental values of the independent variable and predicted values further showed the model adequacy. The predicted and experimental values (Fig. 3) were in reasonable agreement (R^2 value close to unity), which means that the data fit well within the model.



Figure 3: Predicted vs. actual FAME yield.

The analysis was deepened using the residuals' normal probability plot (Fig. 4), which indicates that the errors are normally distributed in a straight line.



Figure 4: Normal probability plot of residual.

Process parameters interactions

RSM plots show the interactions, relationships and correlations of two different variables in the study, while maintaining the other factors at a fixed value. RSM curves were plotted to reveal the variables interaction and to determine the optimum level of each one, for maximum response. 3D curves of the response from the interactions between the variables are shown in Figs. 5-7. The curves obtained in this study showed that there is a relative significant interaction between all variables. Optimum conditions were also obtained from RSM plots. Optimum predicted yield is indicated by the surface confined in the smallest curve of the contour diagram. The variables optimal values were: catalyst Ct of 4% wt, MeOH to oil ratio of 8:1, RT of 60 min, reaction T of 55 °C and SS of 400 rpm. The predicted response value at these optimum values was 90.34%. Experiments were performed at these optimal values, to confirm them, and the experimental response value was 94.0%. This showed that the model correctly explained the influence of the process variables on FAME production from NSO.

Influence of MeOH/oil ratio (B) and T (C) on FAME yield

The interaction between MeOH:oil ratio and reaction T on FAME yield is shown in Fig. 5. Catalyst weight, RT and speed were kept constant at 4% wt, 60 min and 400 rpm, respectively, to adequately show the interaction between those two variables. As shown in the plot, the highest FAME yield was attained at a MeOH:oil ratio of 8:1 and reaction

T of 55 °C. An increase in reaction T from 45 to 65 °C resulted in FAME yield slight reduction from 94 to 83%. An increase in MeOH:oil ratio from 4:1 to 8:1 led to an increment in FAME yield from 47 to 94%. However, a further increase in MeOH:oil ratio to 10:1 caused a reduction in FAME yield to 33%. The effect of MeOH:oil ratio was more pronounced than that of reaction T.



Figure 5: Surface plot between reaction T (C) and MeOH:oil ratio (B) on Bd yield.

Influence of reaction T (C) and RT (D) and on FAME yield

Fig. 6 shows RSM plot for the interaction effect between RT and reaction T on Bd yield. The plot revealed that an increase in reaction T from 45 to 55 °C led to higher Bd yield under decreased RT. It was seen that an increase in RT did not improve catalytic activity at low reaction T.



Figure 6: Surface plot between RT (D) and reaction T (C) on Bd yield.

Influence of catalyst weight (A) and RT (D) on FAME yield

Fig. 7 shows that increased RT and catalyst weight led to higher yield. The increase in the catalyst Ct caused a significant increment in Bd yield, at low RT. Also, when RT was considerably, it was seen a significant increase in Bd yield (32 to 94%). Low Bd content at low RT might be due to the mass transfer effect of the threephase system(oil-MeOH-catalyst). However, Bd yield was slightly influenced by the rise in the catalyst at higher RT. The yield became steady when these parameters were increased further than the points indicated (beyond 4 h and 4 wt%). This might indicate that the transesterification reaction has reached equilibrium condition, and further increase may lead to a reverse reaction, thus reducing Bd content.



Figure 7: Surface plot between catalyst Ct (A) and RT (D) on Bd yield.

Conclusions

This research was conducted to study the effects of key process parameters on the yield of Bd from NSO. Optimization of Bd transesterification process from NSO was enabled by a five-factor CCD, using RSM in 32 experimental runs. A second-order quadratic model was developed, which was capable of predicting Bd yield from NSO, based on process variables. Process parameters for the transesterification reaction were investigated, such as catalyst weight, MeOH:oil molar ratio, reaction T, RT and SS. From ANOVA for FAME yield, both R² and Radj² showed that the models were validated and consistent with the predicted value, confirming their acceptability and adequacy. Experimental results indicated the following optimal conditions: catalyst Ct of 4 wt%, MeOH:oil molar ratio of 8:1; T of 55 °C and RT of 60 min. Statistical models developed for yield prediction showed good agreement between experimental and calculated values, demonstrating the utility of regression analysis as a tool for optimization purposes. Not only did the experimental values closely match those estimated by the model equation, but also the characteristics of the Bd produced from NSO reasonably met relevant quality standards.

Thus, the optimization of Bd synthesis from NSO and its characterization has provided valuable insights and advancements in the field of renewable energy. However, there are several future directions that can be explored to further enhance the process and broaden its applications: catalyst optimization: Further research can focus on optimizing the catalyst used in the transesterification reaction. Exploring different catalyst types, Ct, and their effects on reaction efficiency and Bd quality can lead to improved yields and properties of the Bd; process intensification: Investigating process intensification techniques such as ultrasound-assisted transesterification or microwave-assisted transesterification can potentially enhance reaction rates and reduce RT. These techniques can also contribute to energy and cost savings in the Bd production process; feedstock diversification: while NSO has shown promise as a feedstock for Bd production, exploring other non-edible oil sources can expand the availability and sustainability of Bd. Investigating the feasibility of using waste oils or algae-based oils can contribute to a more diverse and environmentally friendly Bd industry; characterization techniques: Advancements in characterization techniques can provide a deeper understanding of the Bd's properties and its impact on engine performance and emissions. Further research can focus on developing more efficient and accurate methods for analyzing Bd quality, stability, and compatibility with existing diesel engines; environmental impact assessment: conducting comprehensive life cycle assessments to evaluate the environmental impact of Bd production from NSO can provide valuable insights into its sustainability. Assessing factors such as greenhouse gas emissions, land use, and water consumption can help in making informed decisions regarding the overall environmental benefits of Bd. By exploring these future directions, optimization of Bd synthesis from NSO can contribute to the development of a more sustainable and efficient renewable energy source.

Authors' contributions

W. C. Ulakpa: conceptualization; experimental methodology; investigation; data curation and visualization; wrote the original draft. U. Godwin: performed some experimental work; reviewed and edited the manuscript. O. F. Chidiebere: reviewed and edited the manuscript. I. Omenogor: editing/result analysis. O. E. Etim: reviewed and edited the manuscript. I. C. Otuya: investigation; data curation and visualization.

Abbreviations

ANOVA: analysis of variance ASTM: American Society for Testing Materials AV: acid value Bd: biodiesel CaCl: calcium chloride CaO: calcium oxide CCD: central composite design CFPP: cold filter plugging point CP: cloud point Ct: concentration CV: coefficient of variation DOE: design of experiment EV: ester value FA: fatty acid

FAME: fatty acid methyl ester FFA: free fatty acid FID: flame ionization detector FTIR: Fourier transform infrared **GC-MS**: gas chromatography mass spectroscopy IV: iodine value Kl: potassium iodide **KOH**: potassium hydroxide **KV**: kinematic viscosity MC: moisture content MeOH: methanol Na₂S₃O₃: sodium thiosulphate NSO: neem seed oil **OS**: oxidation stability **PP**: plugging point **PV**: peroxide value \mathbf{R}^2 : coefficient of determination **RI**: refractive index **Rpm**: rotation per minute **RSM**: response surface methodology **RT**: reaction time **SG**: specific gravity S/N: signal to noise ratio SS: stirring speed SV: Saponification value T: temperature

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