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Characterization of Crude, Bleached Rubber (*Hevea brasiliensis*) Seed Oil and Synthesis of its Methyl Ester Using Soy-Bean (*Glycine max*) Lipase as Biocatalyst

Ozoemena E. Eje[1]*, Ebube N. Ibezim[1], Chimeremnma V. Ogbonna[2], Austin I. Ugoh[1], Ogbonnaya E. Ikwuagwu[1]

[1]*Lipid and Lipoprotein Research Unit, Department of Biochemistry, University of Nigeria, Nsukka, Enugu, Nigeria*
[2]*Department of Community Health Extension Worker, School of Public Health/Nursing Technology, Nsukka, Enugu, Nigeria*

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ABSTRACT

Biodiesel is one of the alternatives to fossil fuels derived from vegetables and plant sources. This paper reports biodiesel production from rubber seed oil (*Hevea brasiliensis*) via soybean lipase-catalyzed transesterification. 86.62% biodiesel and 27.21% glycerol yield were obtained at lipase activity of 24375 IU ascertained by the titrimetric method. 80% ammonium sulfate saturation was used to precipitate lipase. The pellet showed 376250 IU lipase loading, oil to methanol equivalent molar ratio of 5:1, and under 24 hours. The physicochemical properties of the crude, bleached oil, and biodiesel such as peroxide, acid, anisidine, saponification, and fatty acid values were analyzed. The flashpoints recorded were 80.5±1.5 °C, 97±5 °C, and 56±0 °C respectively; it is safer than fossil diesel since it is not ≤ 50ºC. The saponification values (SV) were 62.3, 61.7, and 103.8±2.4 mgKOH/g. Since little quantity of soap in biodiesel can minimize the friction occurring in moving parts and enhance fuel pump purity, it could be employed in running a compression engine. Enzymatic transesterification of rubber seed oil gives a high yield of biodiesel with robust properties suitable for automobile application.

Keywords: *Hevea brasiliensis*, Lipase, Biodiesel, *Glycine max*, Transesterification.

Introduction

The International Energy Agency (IEA) report showed that global demand for energy in 2030 is estimated to be 14,896 million tons of oil equivalent (Mtoe), rising to 18,048 Mtoe by 2035. From time immemorial, the limited supply and depletion of fossil energy reserves, coupled with rising energy consumption lead to increasing demand for energy.[1] To meet these demands, renewable energy technology which accounts for only 20% of global energy demand, (with the remaining 80% supplied by fossil fuels) was introduced as an alternative energy source. As a result, more research and development of manufacturing technology are required.[2] Biodiesel is characterized by its unique attributes as an environmentally friendly alternative energy, renewability, energy efficiency, and fossil fuel substitute that does not degrade engine performance, as led to the growing demand for it on an industrial scale. This is a result of fossil fuel's impact on the environment because of the emissions from combustion, and a decline in domestic oil production.[1,2] According to ASTM International, biodiesel is regarded as fuel that consists of long-chain monoalkyl esters of fatty acids obtained from renewable vegetable oils or/and animal fats that meet the required baseline of ASTM D675. Biodiesel is primarily obtained from consumable and inedible oils; it is typically generated through the catalytic breaking of the link and bond between glycerin and fatty acid which can be used as a single-player fuel (pure form – 100% or it can be mixed with petrodiesel.[3,4] The reaction mechanisms involved in the transesterification process are divided into chemical and enzymatic processes. Most biodiesel industrially available is synthesized using the chemical process in which acid/base is used as the catalyst. Wang et al.[5] reported that different acids, bases, and solid catalysts have been effectively employed for the manufacturing of biodiesel from a diversity of feedstocks. However, despite the cost-efficient, high yield, and highly reactive potentials of this process,[6] there exist some drawbacks as difficulties in downstream processes, which include glycerol recovery, elimination of inorganic salts, and molecules of water out of the biodiesel, also, the treatment of alkaline wastewater appeared difficult, hence it invites extra expenditures due to the formation of soap.[7,8] Another challenge accompanying the use of conventional catalysts apart from high energy consumption is the difficulty in the transesterification of triglycerides with high free fatty acid contents.[9] The aforementioned shortcomings associated with chemical catalysts have been overcome by the use of a lipase-catalyzed system that gives room for the synthesis of not just purer but specific forms of biodiesel with ease of glycerol recovery.[10] Rachmadona et al.[11] reported that the use of liquid rather than immobilized lipases is more effective in biodiesel synthesis with high water tolerance. Apart from these, edible materials like vegetable oils serving as a precursor for biodiesel synthesis could impose insecurity on food due to inflation on edible items.[12] Therefore, the rubber tree (*Hevea brasiliensis*) stands out as a potential source for biodiesel production since the oil is considered poisonous for human consumption. Hence this study aims to isolate lipase from *Glycine max* for refining RSO and comparatively characterize the physicochemical attributes of the crude, refined RSO and the possibility of producing efficient biodiesel from bleached RSO. The novelty of this research is proven by the effective utilization of RSO which is non-edible, and the enzyme which gives pure products that are easily separated from the chemical method of transesterification catalyzed process.

Materials and Methods

Chemicals and reagents

All chemicals used for this study were obtained from the Lipid Section of the Department of Biochemistry, University of Nigeria Nsukka. The reagents were bought from Sigma Chemical Co. located in St. Louis, MO, USA, (otherwise stated) and they include absolute ethanol, carbon tetrachloride, chloroform, petroleum ether, phenolphthalein, potassium

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*Corresponding author. E-mail: ozoemena.eje.pg01794@unn.edu.ng
Tel: +2348064714243

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hydroxide, potassium iodide, sodium thiosulphate, starch indicator, Wiji's solution, sulfuric acid, distilled water (Joechem Co), acetic acid, p-anisidine and ferric chloride. All methods adopted for this study were according to slightly modified American Oil Chemists' Society (AOCS) (1980) procedures or otherwise stated.

Collection of sample

Rubber seed oil (RSO) was acquired on February 2015 from the Rubber Research Institute of Nigeria Rubber Estate, located at Iyanomo, Benin City, Edo State, Nigeria, and fresh soybean seeds were purchased from Nsukka central market-Ogige Main Market in Nsukka Senatorial Zone of Enugu State, Nigeria on March 2015.

Lipase isolation and activity assay

A 200 g of *Glycine max* seeds were soaked in distilled water for 24 hours, it was further drained and spread on a flat surface, and covered with a wet cloth to sprout for 4 days. The seeds were homogenized in chilled acetone, then centrifuged to separate the filtrate. Enzyme activity assay was carried out by adding 2 mL of the substrate (5 g of olive oil and 2.5 g of gum Arabic in 100 mL of 0.1 M phosphate buffer of pH 7.0), and 0.1 mL of soybean lipase (SoyLp). The reaction mixture was then incubated at 37^oC until 15 minutes and 2 mL acetone-ethanol solution was added in a ratio of 1:1 to terminate the reaction. The enzyme solution was titrated against 0.05 M NaOH until a pink colouration appeared (using drops of phenolphthalein, as an indicator) and the titer values were recorded. This was carried out in triplicates and SoyLp activity was estimated using the formula below. The blank contained no enzyme.

$$\text{Enzyme activity} = \frac{(V2-V1) \times 10 \times 10^3\ [\mu/I],25^{o}C}{\mathbf{4}} \quad \dots\dots\dots\dots\ (1)$$

Where
V_1 is the titre value in mL of the blank**,**
V_2 is the titre value in mL of the sample

Ammonium sulfate precipitation and synthesis of biodiesel

The crude lipase was precipitated using 51.6 g of solid $(NH_4)_2SO_4$ in 100 ml of the crude enzyme solution making it 80 % saturation. Then it was gently stirred and kept in the fridge at 4°C for 24 h. The pellet showed higher activity than the supernatant after centrifugation at 400 rpm, hence it was used for biodiesel production. To produce the biodiesel, 50 mL of n-hexane was transferred into 100 g of the bleached RSO, swirled, then methanol and precipitated SoyLp was added in a volume equivalent of 5:1 respectively. The mix was placed on a magnetic stirrer and stirred for 24 h at 150 rpm. After it was transferred into a separatory funnel, 100 mL petroleum ether was added to the mixture and shaken for proper mixing. Two layers, the brown layer at the bottom showed glycerol while the upper layer showed biodiesel. 50 mL of 5.5 % Na_2CO_3 was added into the separatory funnel to the trapped solvent before filtering off the glycerol. The upper layer (FAME) was preceded by downstream processing with anhydrous sodium sulfate to eliminate any water molecules and the n-hexane was evaporated at 60 °C in an oven. The remaining product after evaporation was used for characterization. The estimated recovery of the produced biodiesel was 86.62% and 27. 21% glycerol.[12]

Characterization of the crude, refined rubber seed oil, and its methyl Ester

The physicochemical properties of the oil samples and biodiesel such as specific gravity (SG), peroxide value (PV), saponification value (SV), unsaponifiable matter, iodine value (IV), anisidine value (p-AnV), flash point, fire point, photometric color index (PI) and viscosity were estimated using standard procedures.[13]

Determination of acid value

Two grams of the oil samples were introduced in 20 mL of hot neutralized ethanol in a 250 mL conical flask and heated to boil in a water bath. The mixture was titrated with HCl to neutrality while still hot using 0.5 M NaOH and drops of phenolphthalein with consistent shaking until the appearance of persistent pink colouration for 30 seconds. The titre value was recorded by measuring the volume of 0.5 M NaOH used to reach the endpoint.[13,14] The acid value was estimated as shown:

$$\text{Acid value (AV)} = \frac{39.997 \text{ x Titre value x Normality of NaOH}}{\text{Weight of sample}} \quad \dots\dots\dots\ (2)$$

39.997 = equivalent weight of sodium hydroxide

Titre value = HCl for sample (mL) – HCl for blank (mL)

Peroxide value

A measured solution of acetic acid (60 mL) and chloroform (40 mL) in the ratio of 3:2 was added to 5g of the crude, refined oil, and FAME samples respectively in a beaker and mixed properly. Following this, 20 mL of 5% saturated KI was added with 30 mL of distilled water. This mixture was titrated gradually using 0.01 M sodium thiosulphate with constant vigorous shaking until the disappearance of the yellow colouration. 0.5 mL of 2% starch indicator was added to the mixture with strong shaking to eliminate iodine from the chloroform layer. Titration was persistent until the blue-black or brownish-blue colouration disappeared. Peroxide value was determined as described by AOCS,[13] and Ikwuagwu et al.[12] The peroxide value was estimated viz:

$$\text{Peroxide value (PV)} = \frac{\text{(Titre of sample – Titre of blank) x Normality of NaOH x 1000}}{\text{Oil sample weight}} \quad \dots\dots\dots\dots\ (3)$$

Determination of saponification value

To determine the saponification value (SV), 2 g of the oil samples and FAME were transferred separately into a round-bottom flask, and a few anti-bumping granules, 25 mL 0.5 M alcoholic KOH were added.[14] The container was fitted onto a condenser on a steam bath and refluxed for 30 minutes. After cooling, the sample was back-titrated against 0.5 N HCl with 5 drops of hot phenolphthalein as an indicator as described by.[15, 13] The saponification value was estimated using equation 4.

$$SV = \frac{56.1\ N\ (V_o - V_1)}{M} \quad \dots\dots\dots\dots\dots\dots\dots\ (4)$$

Where 56.1 is the equivalent weight of potassium hydroxide, Vo represents the volume of acid solution used for the blank, V_1 = volume of acid solution used for the sample, N = Normality of HCl, and M = Mass of the sample.

Unsaponifiable matter

The unsaponifiable matter was determined by taking 2 grams of oil sample into a 250 mL conical flask followed by the addition of a few anti-bumping granules and 25 mL of 0.5 M alcoholic KOH. The flask was fitted in a condenser on a steam bath and refluxed for 30 minutes. After cooling, 50 mL of distilled water was added and transferred into a separatory funnel. Different volumes of diethyl ether, 100 mL, 50 mL, and 50 mL were used to extract unsaponifiable matter. The combined diethyl ether extract was further washed thrice consecutively with 25 mL of distilled water and rinsed with 25 mL 0.5 M KOH until neutrality with phenolphthalein. The ether layer was treated with sodium sulfate for drying following the method of AOCS.[13] The flask containing the residue was weighed and the unsaponifiable matter was determined by the formula.

$$\text{\% Unsaponifiable matter} = \frac{\text{Weight of unsaponifiable matter} \times 100}{\text{Weight of the oil before saponification}} \quad \dots.\ (5)$$

Estimation of iodine value

A Wiji method was used for determining the IV of the samples. Briefly, 0.1 g of the oil samples and FAME were measured separately into a 250 mL iodine flask containing 15 mL of CCl_4 and 25 mL of Wiji's reagent (Dissolving 26.0 g of iodine (I_2) in 2 L of glacial acetic acid), stoppered placed in a dark cupboard for 2 h, and a 20 mL and 150 mL solution of 10% KI and distilled water respectively was added successfully. Using a 2% starch indicator which was added towards the disappearance of blue colouration, endpoint, after vigorous shaking, this mixture was titrated with 0.1 N sodium thiosulphate ($Na_2S_2O_3$) according to the AOCS method.[13] The iodine value was estimated as shown below:

$$\text{Iodine value (IV)} = \frac{12.69\ \text{M (V−V1)}}{\text{Weight of oil sample}} \quad \text{……………………. (6)}$$

Where,
V^1 = volume of $Na_2S_2O_3$ used for the blank in mL,
V = volume of $Na_2S_2O_3$ used for the oil sample in mL,
M= molarity of $Na_2S_2O_3$ which is 0.1 M.

Anisidine value (p-AnV)
To determine the secondary oxidation compounds, primarily 2-alkenals and 2, 4-alkadienals generated due to hydroperoxide decomposition, 0.3g of the sample was transferred into 25 mL of n-hexane in a conical flask and covered to make it airtight. 5 mL was drawn from the mixture and transferred into triplicate tubes and 1 mL of 0.25 % w/v p-anisidine in glacial acetic acid and incubated for 10 minutes at ambient condition, and absorbance was measured at 350 nm against the blank.[13]

$$\text{Anisidine value (p-AnV)} = \frac{25 \text{ x } 1.2 \text{ (Rp − Asb)}}{M} \quad \text{…….……… (7)}$$

Where
Rp represents sample absorbance
Asb represents blank absorbance
m represents the sample mass

Specific gravity estimation (density)
An empty density bottle was weighed and assigned M_0 and then 10 mL of the sample was introduced and the mass obtained was M_1. The oil sample was replaced with water and the procedure above was repeated and denoted M_2. These were carried out in triplicates and the mean value of M_0, M_1, and M_2 were recorded. The expression for specific gravity is as given below.[16]

$$\text{Specific gravity} = \frac{Sample\ mass}{Mass\ of\ water} = \frac{M1 - M0}{M2 - M0} \quad \text{………………. (8)}$$

Flash and fire point estimation
A crucible was filled with 15 mL of the oil sample and placed on a hot plate connected to a power source, using a thermometer as a stirrer for even distribution of heat and an ignition source was used to pass lightly over the liquid surface. Flashpoint was recorded when vapour caused an ignition spark of flame accompanied by an audible popping sound. The fire point showed a steady glow for 5 seconds. This experiment was replicated thrice.[16]

Photometric colour index (P.I)
The oil sample (1 mL) was measured into a test tube followed by 2 mL of n-hexane added. After mixing thoroughly, the absorbance was recorded at wavelengths of 460 nm, 550 nm, 620 nm, and 670 nm against the blank. The PI was determined by the AOCS formula.[13]

$$\text{The P.I} = 1.29\ A_{460} + 69.7A_{550} + 41.2\ A_{620} - 56.4\ A_{670} \quad \text{…….. (9)}$$

Where, A is absorbance at 460, 550, 620, and 670 nm wavelengths**.**

Determination of viscosity
Ten millilitres of the oil sample were transferred to a U-tube calibrated viscometer at a controlled temperature, and the flow rate (sec) was measured with a stopwatch under gravity.[13] The blank is replaced by 10 mL of water at 20°C. The flow properties were estimated via the formula below

$$\text{Viscosity (centistokes)} = \frac{Flow\ time\ of\ sample \times 1.0038}{The\ flow\ time\ of\ water} \quad \text{…………… (10)}$$

Where 1.0038 is the factor on the glass calibrated viscometer

Statistical Analysis
The results of the study were analysed using descriptive statistics (SPSS version 20)

Results and Discussion

The SoyLp showed maximum activity of 24375 IU on day four. The crude enzyme showed 53750 IU while the pellet at 80 % ammonium sulfate saturation showed 376250 IU and was used for the transesterification process. The physic-chemical features of the crude, bleached oils, and FAME are summarized as indicated in Table 1. The results indicate that bleaching of RSO leads to a slight decrease in the AV, unsaponifiable matter, viscosity, and photometric colour index. The fact that bleaching eliminates any impurities and contaminants such as pigments, soaps, non-hydratable lecithin, oxidized fats, and several other non-lipid materials present in the oil samples may be diagnostic to the explanation of this drop in these aforementioned parameters. The RSO refractive index obtained for the crude, bleached RSO and the FAME was 1.470. This is in tandem with that recorded by Ikwuagwu *et al*,[12] Nehdi *et al*,[17] and Pankaj *et al.*[18] but higher than Eze and Iko who reported 0.714.[19]

Table 1: Physico-chemical properties of crude, bleached rubber seed oil, and biodiesel

Analysis	**Crude RSO**	**Refined RSO**	**RSO methyl ester**
Index of Refraction	1.470 ± 0.0	1.470 ± 0.0	1.470 ± 0.0
Specific gravity	0.905 ± 0.0	0.912 ± 0.0	0.893 ± 0.0
Flash point (ºC)	80.500 ± 1.5	97.000 ± 5.0	56.000 ± 0.0
Fire point (ºC)	109.330 ± 0.8	106.000 ± 2.0	61.000 ± 1.0
Photometric index	47.301 ± 2.4	36.828 ± 5.7	33.766 ± 5.2
Acid value (mgKOH/g)	5.049 ± 0.6	0.580 ± 0.0	2.861 ± 0.3
Peroxide value (mg/g)	1.600 ± 0.1	10.000 ± 0.2	0.000 ± 0.0
Saponification Value (mgKOH/g)	62.300 ± 0.0	61.700 ± 0.0	103.785 ± 2.4
Unsaponifiable matter (%)	0.018 ± 0.0	0.014 ± 0.0	0.040 ± 0.0
Iodine value (gI/100g)	175.550 ± 22.3	29.530 ± 0.9	200.833 ± 2.2
Anisidine value (g^{-1})	16.330 ± 0.4	29.530 ± 0.9	14.167 ± 3.9
Viscosity (cSt)	49.000 ± 0.3	44.039 ± 0.1	*ND

ND means ‘Not Determined’, Values are presented as mean ± standard deviation of three determinations

The crude and refined oils showed a specific gravity (0.91) similar to that reported by Kant *et al.*[20] sorghum oil biodiesel and Rajesh *et al.*[21] and falls within the ASTM baseline, it decreased as transesterification occurred. This biodiesel's specific gravity conforms to results by Fukuda *et al.*,[22] and Rajesh *et al.*[21] on biodiesel produced from *Jatropha* oil (JO), and *Karajan* oil (KO) who recorded 0.876, 0.789, and 0.717 respectively. Flashpoints of oil enable safe monitoring, handling, and storage of fuel, the higher the flash point the safer. The flashpoints of the crude and refined oil (80.5/97.0 ºC) are lower than those reported by Ramadhas *et al.*,[23] 198 ºC; Ikwuagwu *et al.*[12] 294/290 ºC for crude and refined RSO respectively; Rajesh *et al.*[21] reported 232ºC for *Jatropha* and 205 ºC for *Karajan*; Kant *et al.*[20] also reported 225 ºC and 167 ºC for *Jatropha* and *Karajan* oil and Iko and Eze,[19] reported 340 ºC from *Q. undulata* seed oil. However, the flashpoints of the crude and refined oils were high, making them appropriate for biodiesel making and conferring safety during storage and transportation. The flashpoint of the biodiesel is lower than those obtained by Ikwuagwu *et al.*[12] Adebayo *et al.*[24] Ramadhas *et al.*[23] and Rajesh *et al.*[21] who reported 235, 170, 130, and 98 ºC respectively.

The fire points of the crude RSO decreased to 106.0 and 61.0 ºC after bleaching and transesterification respectively. This temperature was lower than Njoku and Ononogbu's,[25] and Ikwuagwu's[12] reports. The fire point of the diesel was lower than those of the crude and partially refined oil. This property made lipase-catalyzed biodiesel safe; even though it has high nitric oxide emission, and lesser quality compared to fossil diesel and base-catalyzed biodiesel, the process of its production is 'greener'. Therefore it can be used alone or in blends with petro-diesel to run compression ignition engines. The photometric colour indexes of the crude and bleached RSO were 47.301 and 36.828 respectively. The kinematic viscosity of the crude and refined RSO oil of this present study showed 49.0 cSt & 44.04 cSt respectively and higher than that reported by Ikwuagwu [12] who obtained 41.24 cSt and 37.85 cSt for crude and bleached RSO respectively. Pankaj *et al.*[13] reported 30 cSt; Eze and Iko[19] reported 7.401 cSt from *Q. undulata* oil. Hence, since viscosity increases with an increase in molecular weight but decreases with increasing unsaturation level and temperature, it is important in determining optimum handling and storage. The photometric color index obtained for the RSO biodiesel is 33.766.

With regard to the acid number, a certain quantity of basic solution is needed to titrate the oil samples and FAME to the exact endpoint in which the value is recorded. This acid number also referred to AV is a measure of fatty acid (FA) liberated, hence a marker for the estimated quantity of FA found in the oil samples or biodiesel. In this study, AV from the crude and bleached RSO and its biodiesel conform to the ASTM standard and those reported by other studies.[12,20,21], however, Ulfah et al.[23], Iko and Eze[19], and Reshad et al. [18] reported higher values. Enzyme-based transesterification of oil reduces free fatty acid formation. However, an excessive FFA in oil or biodiesel enhances corrosiveness, and emulsions formation in the product resulting to difficulties in separating FAME from glycerin, hence, consuming the catalyst during the base-catalyzed transesterification, a rise in soap formation. This has the ability to dwindle the transesterification process, or cause partial transesterification, and increased oxidative degradation which could result in the poor yield of the biodiesel and may signify the presence of water in the biodiesel. The high peroxide value (PV) shows that the oil sample or biodiesel possesses a self-ignition feature. This indicates that rancidity and fuel oxidation characteristics of the oil/biodiesel increases with PV. In this study, PV for the crude and refined RSO showed 1.6 and 10.0 mg/g respectively which agrees with Ebewele *et al.*[26] reports on soybean oil and sunflower oil and RSO. Ikwuagwu *et al.*[12] reported PV of 2.5 and 1.0 mg/g for the crude and bleached RSO respectively and Iko and Eze[19] reported a 6.758. The FAME of this study showed zero value on peroxide. The saponification value (SV) indicates the non-fatty impurity and it reveals the amount of alkali that would be needed by the fat/oil for its conversion to soap. In the present study, the saponification value of the crude RSO decreased with bleaching to 61.7 mgKOH/g showing its suitability for soap and shampoo making, this value is lower compared to the one reported by Ikwuagwu *et al.*[12] which reported 185 mgKOH/g for bleached RSO and[18] which reported 235.28 mgKOH/g with seed oil of *Phoentx carnatensis.*

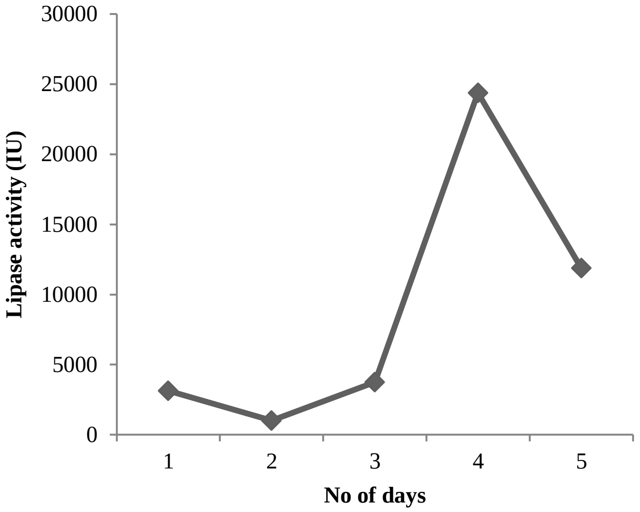

Figure 1: Optimization of lipase activity for isolation

This rubber oil biodiesel showed an SV of 103.785 mgKOH/g which is moderate. As earlier stated, since a reasonable quantity of soap present in biodiesel lowers the friction between moving parts and also enhances the purity of the fuel pump of the vehicle, it can be used in a compression engine. The saponification value (SV) obtained for the crude and refined RSO is lower than given as mgKOH/g for crude and refined respectively. The values are also not in agreement with, Nehdi *et al.*[17] who reported 191.28 and 93.266 mgKOH/g respectively. The iodine value (IV) is a measure of the quantity of iodine absorbed totally by the double bond molecules of methyl ester in 100g of oil. It relates the number of double bonds in the oil sample or biodiesel and increases with an increase in unsaturated FAME. The higher the iodine number, therefore more iodine is required to break the double bonds, and it indicates the oil's efficiency as biodiesel. Meanwhile, IV of biodiesel imitates the degradation and oxidation stability (OS) of diesel. Certain properties of diesel: viscosity, low-temperature flow, and combustion performance are also affected by the value of iodine.[31] However, the report that biodiesel with a lower IV is a more combustible, and efficient fuel compared to biodiesel with a higher IV value is still a debate.[27] In this present study, the iodine values of the rubber seed oil increased with bleaching but the biodiesel showed IV of 200.833 gI/100g which is higher than the values reported by Ikwuagwu *et al*,[12] Adebayo and Abass,[24] and Rajesh *et al*,[21] who recorded 144, 1.64, and 89.91 gI/100g from *Hevea brasiliensis*, *Jatropha curcas* and *Jatropha Karajan* respectively. The Iodine value of fuel indicates the degree of unsaturation and drying quality. The iodine value measures the chemical stability of biodiesel against oxidation and increases with an increase in the number of the double bonds conferring lesser stability. Although, the double bonds in biodiesel help to attract oxygen to the compound and aid the burning of biodiesel over fossil diesel. ASTM D6751 and Brazilian baseline have no threshold of requirements for IV. On the other hand, EN 14214 has a maximum IV limit specified at 120 gI/100g. Nevertheless, the maximum and accepted limit of IV is not obeyed uniformly across all countries of the world due to the variety of biodiesel IV. A proposed baseline of 130 gI2/100g was reported by the international fuel charter.[28] It is important to know that there is a need to reduce IV which could lead to coking and gumming issues in the injector line and combustion cylinder. These issues occur as a result of a high degree of unsaturation (DU) of the oil, hence reducing the lubrication of biodiesel because on heating, polymerization of glycerides from higher unsaturated fatty acids occurs.[29, 30] Hence, fuel polymerization contributes to excessive deposits formation found in automobile engine and combustion chamber resulting in storage stability problems. Anisidine value (p-AnV) represents the content of secondary oxidation products; it is used instead of, or together with, PV to evaluate lipids which is a more reliable indicator of oil rancidity due to oxidation than PV in a long-term storage stability study of oil.[31] In this study, the p-anisidine value for the crude and refined oil obtained were 16.33 and 29.53g-1 respectively which is higher compared to [17] who reported 3.67g-¹ from *Phoentx carnatensis* oil. In this study, the RSO biodiesel anisidine value (14.167 ± 3.9 g-¹) showed that the diesel has long shelf life and storage stability.

Conclusion

The present analysis reveals that biodiesel from rubber seed oil is quite suitable as an alternative to diesel. From the work conducted, the biodiesel produced conforms to America Standard Testing and Material (ASTM) and European (EN) standard values and will be very suitable for use in power combustion engines alone or blends with fossil diesel. The process of its production is 'greener' although high nitric oxide emission is possible. Blending biodiesel with diesel fuel can be used to fuel diesel engines, providing comparable performance, lower emissions, less wear on engine components, and neutral lubricating oil effects. Having seen the fuel properties of biodiesel that it is the best alternative to fossil diesel, it is recommended that research and development on additional fuel property measures, long-term run, how to increase its yields and stability, and wear analysis of biodiesel-fueled engine should be encouraged.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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