



## Extraction of Vegetable Oil from Avocado Seeds for Production of Biodiesel

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**ABSTRACT:** The avocado seed was used as a raw material for the production of biodiesel as an alternative to petrodiesel. The oil was extracted using a soxhlet extractor with n-hexane as the solvent and transesterified with methanol (5:1 oil to methanol ratio) using potassium hydroxide as catalyst in 15 minutes reaction time. The percentage yield of the purified biodiesel was 78%. The physical and chemical properties of the biodiesel on comparison to standard biodiesel and petroleum diesel indicated that it was of good quality. It had a relative density of 0.86, cetane number of 62.2, kinematic viscosity of 3.94cst. The economies of this extraction turns avocado seed from bio-waste to wealth.

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Vegetable oils and fats are lipid materials derived from plants. Although many plant parts may yield oil, but in commercial practice, oil is extracted primarily from seeds. Therefore seed oils can also be referred to as vegetable oils. Worldwide, these oils are increasingly becoming important and quite a number of analysis have been carried out primarily because of extensive demands for oils both for human consumption and for industrial applications; Seed oils are important sources of nutritional oils, and are of industrial and pharmaceutical importance. The characteristics of oils from different sources depend mainly on their compositions and no oil from a single source can be suitable for all purposes (Mohammed & Jorf-Thomas, 2003). The analysis of the fruit constituents reveals that it abounds in various fatty acids, vitamins, carotenoids and other phytochemicals. It is hence a very nutritious food. Avocado (*persea americana*) is well known in Nigeria, and in all the tropical parts of the world. Consequently, with the increasing awareness of the importance of these seed oils, there is need to search for oils from non-conventional sources to augment the available ones and also to meet specific applications. Increase in human population plays an important role to the existence of energy supply, especially for unrenewable energy. In a long term, import of petroleum fuel will dominate national energy supply. So that government has to make a policy about alternative renewable fuel. The alternative fuel must be technically acceptable, economically competitive, environmentally acceptable, and easily available. Increasing

environmental concern, diminishing petroleum reserves, and agriculture based economy of our country are the driving forces to promote biodiesel as an alternative fuel. The supply of petroleum based fuels is decreasing and we will one day run out of it. The trend of the high demand, decreasing supply greatly affects the environment. One popular alternative fuel is biodiesel that is a diesel like product extracted from plant matter. Biodiesel is a liquid biofuel obtained by chemical processes from vegetable oils or animal fats and an alcohol that can be used in diesel engines, alone or blended with diesel oil. ASTM International (originally known as the American Society for Testing and Materials) defines biodiesel as a mixture of long-chain monoalkylic esters from fatty acids obtained from renewable resources, to be used in diesel engines.

Biodiesel is known as fuel derived from renewable resources for use in diesel engines. Biodiesel is environmentally friendly liquid fuel similar to petroleum diesel in combustion properties. As an alternative fuel, it has many advantages. It is derived from a renewable, domestic resource, thereby relieving reliance on petroleum fuel imports. It is biodegradable and non-toxic. Compared to petroleum fuel, biodiesel has a more favorable combustion emission profile, such as low emission of carbon monoxide, particulate matter and unburned hydrocarbons. Carbon dioxide produced by combustion of biodiesel can be recycled by photosynthesis, thereby minimizing the greenhouse

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effect. The most common way to produce biodiesel is by transesterification, which refers to a catalyzed chemical reaction involving vegetable oil and an alcohol to yield fatty acid alkyl esters (i.e., biodiesel) and glycerol.

## MATERIALS AND METHODS

**Materials:** The sample used for the experiment is the avocado seed sourced from a local market in Port Harcourt. The equipment used to carry out the experiment are Soxhlet extractor, Thermometer, Viscometer, Koenler Automatic Cloud and Pour point Analyser, Stirrer, Density bottle, Distilled water, Burette, Pipette, Refractometer, Separating Funnel, Conical flask, Measuring cylinder, Hot plate, Weighing balance, Calorimeter, Beaker, Retort stand and Filter paper. The reagents used for the extraction of the avocado seed oil and for the production of biodiesel are N-hexane, Methanol, Sodium hydroxide, Potassium hydroxide, Sulphuric acid, Sodium thiosulphate, Ethanol, Starch indicator, Sodium chloride, Potassium iodide, Phenolphthalein, Iodine crystal, Acetic acid, Chloroform and Sodium sulphate. All the reagents used in this work were of analytical grade and all solutions were prepared with distilled water.

**Preparation of Avocado Seeds:** The avocado fruits were washed with clean water to remove dust. The outer skin was peeled-off and the edible part of the ripe avocado fruits were eaten-off, then the inner seeds were removed. The avocado seeds were cut into pieces and dried in the sun for about one week to get rid of the moisture content. The dried seeds were grinded mechanically to a particulate size of 1.0mm size, or 120 mesh and stored for extraction of oil.

**Extraction of Avocado Seed Oil:** 250g of the grounded avocado seeds were weighed into a thimble (semi-permeable membrane) and placed into the soxhlet extractor with 250ml of hexane solvent for the first run. The solid particles were removed by filtration to get the extracted lipids. The apparatus was placed on the heater and was left to run for 2hrs. Extracted oil was poured into a test-tube and kept aside to be distilled and characterized. The percentage (%) yield of the avocado fruit seed oil was determined, scaled up, characterized and subsequently used for biodiesel production. This method of extraction of oil was clearly shown by Okene and Evbuomwan

**Production of Biodiesel:** 100ml of vegetable oil was measured and heated to about 60°F. 0.85g of potassium Hydroxide (catalyst) was weighed into 20ml of methanol and shake for about 5 minutes. The mixture was poured into the heated oil, sealed tightly

and shake for about 15 minutes. The mixture was poured into a separating funnel and allow to settle for 8-10 hours. After which, the biodiesel at the top was separated from the glycerol at the bottom. The extracted biodiesel was washed with distilled water and heated to de-water it (Bizimana et. al., 1993).

**Characterization of the Oil and Biodiesel:** The oil and biodiesel were characterized to obtain the following physical and chemical properties. The physical properties characterized were: Relative density, Kinematic viscosity, Refractive index, Flash point, Pour point, Cloud point and Cetane number while the chemical properties were; Acid value, Free fatty acid, Iodine value, Peroxide value, saponification value.

**Determination of Relative Density:** Relative density was determined using the method of Balamiet al. (2004). A clean empty specific gravity bottle was weighed on an electronic balance and the weight ( $W_1$ ) noted. It was then filled with distilled water and the weight ( $W_2$ ) noted. The water was removed and the specific gravity bottle dried and cooled. It was then filled with the sample (extracted biodiesel) and weighed ( $W_3$ ). All the determinations were at 25°C. The formula used to determine the Relative density was:

$$RD = \frac{W_3 - W_1}{W_2 - W_1} \quad (1)$$

where  $W_1$  = weight of empty bottle,  $W_2$  = weight of water + weight of empty bottle,  $W_3$  = weight of oil + weight of empty bottle.

**Kinematic Viscosity:** The sample was poured through one arm of the viscometer and drawn from the opposite arm to fill the bulb, a stopwatch was used to determine the time when the sample gets to the top mark. The time taken for the sample to flow from the top mark to the bottom mark was noted. The viscosity of the extracted biodiesel calculated by adopting the method of Barlamen and Sunil (2018) using equation (2)

$$\kappa v = t \times K \quad (2)$$

where,  $\kappa v$  = kinematic viscosity,  $t$  = time in seconds,  $K$  = viscometer constant = 0.00768

**Flash Point:** The sample (oil or biodiesels) was poured into a clean crucible and a thermometer was inserted in the middle of the sample (ASO or biodiesel). The heater was adjusted in such a way that there was 0.5°C rise in temperature per minute. Flame was intermittently passed over the sample until such a point when a flash was noticed on the sample. The

temperature at which this happened was the flash point.

**Refractive Index:** A drop of the extracted biodiesel oil was placed on the measuring prism and closed with the small cover plate. The refractive index was read through the aperture in % Brix (Ibanga 2014).

**Pour Point And Cloud Point:** The Cloud and Pour points of the extracted biodiesel were analysed using the Cloud and Pour point apparatus. A sample of the extracted biodiesel was put into the glass tube, which was enclosed in an air jacket filled with a freezing mixture of crushed ice and sodium chloride crystals. The glass tube containing the sample was taken out from the jacket at every 10°C interval as the temperature reduced, and was inspected by titling it horizontally. The point at which a haze was first seen at the bottom of the sample was taken as the cloud point. The temperature 10°C above the temperature at which no motion of fuel was observed for five seconds on tilting the tube horizontally was taken as the pour point (ASTM).

**Cetane Number:** The cetane number was calculated as reported by Mohibbe et al. (2005) as:

$$CN = 46.3 + \frac{5458}{SV} - 0.225 * IV \quad (3)$$

where: CN = Cetane Number, SV = Saponification value, IV = Iodine value

**Acid Value:** Acid value was determined using the method described by Association of Official and Analytical Chemists (AOAC, 1990). Two grams (2g) of the extracted biodiesel sample was weighed into a flask and 50 ml of neutralized ethanol was poured into the flask. The contents were mixed together and boiled. It was then titrated with 0.1N KOH to a faint pink colour that persisted for at least 15 seconds. The acid value was calculated as:

$$AV = \frac{56.1 * N * T * 100}{1000 * G} \quad (4)$$

where: AV = Acid Value, N = Normality of standard KOH used, T = Titration volume, G = weight of sample

**Free Fatty Acid:** Free Fatty acids was determined using the method described by Association of Official and Analytical Chemists (AOAC, 1990). Ethanol (100ml) was neutralized with few drops of NaOH solution (until color changed) using phenolphthalein as the indicator. About 5g of the sample (oil or biodiesel) was weighed into the neutralized ethanol

and then mixed thoroughly. The solution was titrated with standard NaOH solution (of known concentration) until color changed to pink. The percentage free fatty acid was calculated as:

$$\% \text{free fatty acid} = \frac{V * N * M}{10 * W} \quad (5)$$

where, V = Volume of titre, N = Normality of NaOH, M = Molar Mass of oil, W= Weight of sample.

**Iodine Value:** 0.4 g of the sample was weighed into a conical flask and 20ml of carbon tetra-chloride was added to dissolve the oil. Then 25ml of Dam reagent was added to the flask using a safety pipette influenced chamber. A stopper was inserted and the content of the flask vigorously swirled. The flask was placed in the dark for 2 hours and 30 min. At the end of this period, 20 ml of 10% aqueous potassium iodide and 125 ml of water was added using a measuring cylinder. The content was titrated with 0.1 M sodium-thiosulphate solution until the yellow colour almost disappeared. A few drops of 1% starch indicator was added and the titration continued by adding thiosulphate drop-wise until blue coloration disappeared after the vigorous shaking. The same procedure was repeated for the blank test and for subsequent samples. The iodine value (IV) was given by the expression (Laboratory handbook, 1997):

$$IV = 12.6 * C * (V_1 - V_2) * m \quad (6)$$

where, C = concentration of Sodium thiosulphate used, V<sub>1</sub> = volume of Sodium thiosulphate used for the blank, V<sub>2</sub> = volume of sodium thiosulphate used for determination, m = mass of the sample

**Peroxide Value:** Peroxide value was determined using the methods described by the Association of Official and Analytical Chemists (AOAC, 2000). 10g of the sample was weighed into a conical flask and 30ml of acetic acid chloroform solution was added. The flask was swirled to dissolve the sample, then 0.5ml of the KI solution was added. The flask was allowed to stand for exactly one minute and swirled occasionally. After one minute, 30ml of distilled water was added. It was titrated with standard thiosulphate solution using starch as indicator. A blank was conducted in the same way but without the sample. The Peroxide value was calculated as:

$$PV = \frac{(T-B) * N * 1000}{g} \quad (7)$$

where, T = titration volume for sample, B = titration volume for blank, N = normality of thiosulphate used, g = weight of sample

**Saponification Value:** Saponification value was determined using the method described by Balami *et al.* (2004). 2g of the sample was weighed into the flask, then 25ml of 0.5N alcoholic Potassium hydroxide was added to it and boiled under reflux for 1 hour. The excess alkali was determined by titration with 0.5N hydrochloric acid while the solution was still hot using 0.5ml of 1.0% alcoholic solution of phenolphthalein as indicator. A blank was determined under the same condition without using the sample. The saponification value was calculated as:

$$S.V = \frac{56.1 * BS * N}{W} \tag{8}$$

where, *S* = volume in ml of standard HCl required for the sample, *B* = volume in ml of standard HCl required for the blank, *N* = normality of HCl, *W* = weight of oil used.

**RESULTS AND DISCUSSION**

**Yield of Avocado Seed Oil:** Table 1 shows that the weight of the grounded avocado seeds used was 3950.25g and the weight of the oil extracted from the grounded seed was 120.12g. Hence, the percentage yield of the oil was calculated using the formular:

$$\% \text{ yield of oil} = \frac{\text{Weight of oil extracted (g)} * 100}{\text{Weight of seed (g)}} \tag{9}$$

**Table 1:** Average percentage (%) yield of avocado seed oil

Parameter	Values
Weight of ground avocado seed(g)	3950.25
Weight of oil extracted (g)	120.12
Amount of oil obtained (ml)	132
Oil yield (%)	3

From the percentage yield calculated (3%), avocado seed gave a very low oil content which implied that processing it for bio-oil production would not be economically viable.

**Characterization:** Table 2 depicts the results of the analysis of the physical properties of the Avocado seed oil. It highlights that the viscosity of Avocado Seed Oil (ASO) was 36.7 Centistokes (cst) and the relative density was (0.912g/ml), the refractive index was 1.46%, the flash point was found to be 99°C, its cloud point was 12°C, and the pour point was -17°C.

**Table 2:** Physical properties of the avocado seed oil

Parameter	Values
Relative Density (g/ml)	0.912
Kinematic Viscosity (cst)	36.7
Refractive index	1.46
Flash point (°C)	99
Cloud point (°C)	12
Pour point (°C)	-17

The pour point of the extracted biodiesel was very low. The refractive index (1.46%) showed that the oil was very pure. The high flash point implied that the bio-oil should be properly handled because of its volatility. Table 3 depicts the results of the analysis of the chemical properties of the Avocado seed oil. The peroxide value of the Avocado seed oil was 3.30, the free fatty acid value was 1.68%, acid value as 1.65mgKOH/g, while the iodine and the saponification values were 42.66 and 187.18 respectively.

**Table 3:** Chemical Properties of the Avocado Seed Oil

Parameters	Values
Acid value (mgkoh/g)	1.65
Free Fatty Acids (%)	1.68
Iodine value (mg iodine/g)	42.66
Peroxide value (meq/1000g)	3.30
Saponification value (mgKOH/g)	187.18

The results showed that avocado seed oil had a low acid value. Oil having low acidity is suitable for consumption which implied that avocado seed oil was not only used for biodiesel production but also as edible oil. The results also show that the oil had low peroxide values. This may be attributed to the high stability of the seeds during the extraction operations (Besbes *et al.*, 2004). The Iodine value of 46.66g/100g showed that the ASO oil had a high degree of unsaturation (Ikhuoria and Maliki (2007).

**Biodiesel Yield from the Avocado seed oil (Aso):** Table 4 shows the percentage yield of biodiesel (methyl esters) from the Avocado seed oil. The Avocado seed oil used in production of methyl esters (biodiesel) was 100ml (91.00g), the volume of methyl ester produced was 87ml (79.17g).

**Table 4:** Average (%) Yield of the Aso based-Biodiesel

Material	Quantity
Avocado seed oil (ASO) used (ml)	100
Methyl ester produced used (ml)	87
Biodiesel Yield (%) after purification	78

The (%) yield of the biodiesel was calculated as:

$$\% \text{ yield of oil} = \frac{\text{Mass of biodiesel (g)}}{\text{Mass of oil (g)}} * 100 \tag{10}$$

The ASO yielded 87% biodiesel and after purification, the yield became 78%.

**Characterization of the Aso Biodiesel:** The physical properties of the produced biodiesel (methyl ester) is shown in Table 5. The physical properties of the fuel indicated that it was biodiesel. The result showed that ASO based biodiesel had a high cetane number. The cetane number of 62.18 for the ASO based biodiesel

indicated that it had high auto ignition characteristics suitable for diesel engines.

**Table 5:** Physical Properties of the Aso based-Biodiesel

Parameters	Value
Cetane Number	62.18
Flash point (°C)	110
Cloud Point (°C)	10
Pour Point (°C)	-15
Relative density (g/ml)	0.86
Kinematic Viscosity (cst)	3.94
Refractive index (%)	1.45

The higher the cetane number of oil, the shorter the ignition delay or the more easily the fuel will combust in a compression setting (such as a diesel engine), while low cetane number causes ignition delay, starting difficulties and knock. The biodiesel was also shown to have a high relative density (0.86 ml/g). A kinematic viscosity of 3.94cst indicated that the produced biodiesel had a low viscosity, which means the biodiesel could flow easily. The result also showed that the biodiesel had high flash point of (110°C), cloud point of 10°C and pour point of -15°C. The result of the analysis of the chemical properties of the ASO based biodiesel is depicted in Table 6.

**Table 7:** Comparative Analysis of Diesel Fuel Properties

Fuel Parameters	Aso Biodiesel	Biodiesel (ASTM D6751)	Petrodiesel (ASTM D975)
Cetane Number	62.18	48 - 65	42
Pour Point (°C)	-15	-10 to -15	-15 to -35
Relative density	0.86	0.88	0.86
Kinematic Viscosity (cst)	3.94	1.9-6.0	1.3 - 4.1
Acid value (mgKOH/g)	0.89	0.8	-

The cetane number of the Aso based-biodiesel was higher than the range specified by the Standard biodiesel, while the Pour point was within the specified ranges. The relative density of the Aso based-biodiesel was the same with the petroleum diesel standard and within the range of standard biodiesel with a percentage deviation of 0.02%. The kinematic viscosity of ASO based biodiesel fell within the range of Standard biodiesel and Petroleum diesel, while its acid value was slightly below that of the biodiesel standard by 0.09mgKOH/g.

**Conclusion:** This work demonstrates that vegetable oil extracted from avocado seed can be used to produce biodiesel. The physicochemical properties of the ASO based biodiesel indicated reasonable agreement when compared with other bio and petro-based diesel obtained from literature. Thus, high dependency on petroleum fuel, which has led to high degradation and pollution of the environment, can be reduced by encouraging this alternative source of diesel fuel. The

**Table 6:** Chemical Properties of the Aso based-Biodiesel

Parameters	Values
Acid value (mgKOH/g)	0.89
Free Fatty Acids (%)	0.40
Iodine value (mg iodine/g)	38.2
Peroxide value (meq/1000g)	2.86
Saponification value (mgKOH/g)	223

The results show that Aso based-biodiesel had an acid value of 0.89, free fatty acid of 0.4 and iodine value was 38.2. The peroxide and saponification values of the Aso based-biodiesel were 2.86 and 223mgKOH/g, respectively. The results indicated that the biodiesel has a low free fatty acid.

*Comparative Analysis of the ASO based biodiesel with Petro-diesel and Biodiesel Standards:* Table 7 shows the comparison between the standard diesel, petrodiesel and that of the produced ASO based biodiesel indicating reasonable agreement with literature (Kulkarni *et al.*, 2008).

scale-up and industrial production of cost effective diesel for compression-ignition engines using agricultural seeds such as the avocado seed becomes very imperative for a cleaner and healthier environment.

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