

Extraction and Optimization of *Azadirachta Indica* Seed Oil for Biodiesel Production

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ABSTRACT

Energy happens to be among the most important needs required to sustain and improve technological development and quality of life in any society with the major part being obtained from fossil fuels such as coal and petroleum and renewable resources such as water, wind and Solar. The threat imposed on the environment by the continuous use of fossil fuel products has called for the development of an alternative energy sources and an improvement in science and technology has led to the development of biodiesel which is been produced from renewable resources in this study, "Extraction and optimization of Azadirachta indica seed oil for biodiesel production". Oil was extracted by varying some of the parameters that affect the yield of oil extracted from plant seeds. The parameters varied include seed moisture content, extraction temperature and extraction time keeping the particle size of 1.18mm constant. Response surface methodology was used to design the experimental matrix by using central composite design. The oil yield was observed to be 43.72% at a moisture content, extraction temperature and extraction time of 6%, 60°C and 4h respectively. The result obtained was subjected to statistical analysis and it was observed that all the process parameters varied has effect on the percentage oil yield of Azadirachta indica seed. The Physiochemical properties of the optimum yield was determined and this shows a density of 0.97g/ml, specific gravity of 0.87, kinematic viscosity of 37.42, cloud point, pour point, smoke point, flash point and fire point of 11.7°C, 7.0°C, 157°C, 171°C and 190°C respectively. Saponification to value of 240mgKOH/g, acid value of 11.03mgKOH/g, free fatty acid of 5.52(%w/w) and peroxide value of 9.43(neqO₂/Kg). The extracted oil was subjected to biodiesel production and a yield of 85% was obtained. The produced biodiesel was analyzed and found to confound with ASTM standard.

KEY WORDS: Biodiesel, Extraction, Yield, Response surface, Central composite, Optimization, Renewable

1.0 Introduction

Biodiesel is not a biotechnological product, it is manufactured with any suitable vegetable oil from crops with no history of plant biotechnology and even from animal fats by an entirely chemical procedure. Biodiesel has number of merits over fossil diesel fuels. The major one is that it can be obtained from renewable domestic resources (e.g.

vegetable oil) and has the capacity to reduce carbon dioxide emissions by 78% when compare to diesel fuels on a life cycle basis (Luque, 2010).

Even though biodiesel has gained the interest of the world for the need for an alternative source rather than fossil fuels, not lesser than 95% of the renewable bioresources used for its production are edible oil (Gui, 2008), which in the future may affect food availability to the rapid growing world population and high cost of biodiesel than fossil diesel therefore, attention has been draw to the use of on non-edible resources to tackle the problems mentioned above. There are numerous non edible oil plants used in the production of biodiesel. These include; *Ficuselastica*, *Nicotianarustica*, *jatrophacurcas*, *Azardirachta indica*, *delonix ragia* (Adejumo *et al.*, 2019).

Azardirachta indica oil is a non-edible oil obtained from *Azardirachta indica* plant seeds which is common to the Northern parts of Nigerian in places like Sokototo, Kebbi, Kastina, Borno and other states at the border. The *Azardirachta indica* oil plant is fast growing plant with a productive life span of 150 to 200years, its ability to survive on drought and less fertile soils at a high temperature of 44°C and a low temperature of up to 4°C (Karmarker, 2011), and its fat content ranges from 39.7 to 60% (Martin, 2010). A fully grown *Azardirachta indica* produces 30 to 50kg fruits every year (Karmarker, 2011). It has high percentage of monounsaturated fatty acids (C16:1, C18:1), low proportion of unsaturated acids (C18:2, C18:3) and a controlled amount of saturated acids (C16:0, C18:0), (Wang, 2011). The above characteristics of *Azardirachta indica* seed oil and its fatty acid composition makes it suitable for biodiesel production (Aransiola, 2018). Adequate attention must be given to the optimization of oil from non-edible oil plant for the production of biodiesel. This research work therefore focuses on the optimization of process parameters has it affect the quantity of oil obtained from *Azardirachta indica* oil seed to increase biodiesel production from non-edible oil plants.

2.0 MATERIALS AND METHODS

2.1 Sample Preparation

The *Azardirachta indica* seed that was used in this research work was collected from Bosso campus of Federal University of Technology Minna, Niger state, Nigeria. The seeds were collected manually by hand and de-pulped. The seed was washed thoroughly to remove particles of the pulp from the seed and then milled to a particle size of 1.18mm before variation of moisture content.

2.2 Determination of Moisture Content of the oil Seed

The milled seeds prior to drying was weighed and recorded as W_1 . The milled seed were placed in an electric oven to dry at a temperature of 105°C . The sample was removed after every 24hrs and the weight was determined until a constant weight was achieved. The final weight was recorded as W_2 . The sample was sealed in an air tight container to discourage moisture absorption by seed before further experiment.

The percentage moisture content present in the seed was calculated using the formula below;

$$\%MC = \frac{(W_1 - W_2)}{W_1} * 100 \quad (\text{AOAC, 1990}) \quad (i)$$

Where;

%MC is the moisture content in percentage

W_1 (grams) is the initial weight of the sample before drying

W_2 (grams) is the final weight of the sample after drying.

2.3 Design of Experiment (DOE)

The experiment carried out was designed with the aid of Minitab 18 software using central composite design of the response surface methodology. In designing the experiment, the factors considered were the moisture content, extraction temperature and extraction time while the response of the experiment was chosen to be the yield of the oil obtained from the seed. The maximum and minimum level used for the factors is given in Table 1 and alpha value of the design is 1.6879. A total of twenty experimental runs were obtained and carried out using soxhlet apparatus.

Table 1 Experimental Matrix Design for *Azadirachta indica* seed oil extraction

Variables	Symbol	Levels	
		Minimum	Maximum
Moisture content (%)	A	6	18
Extraction temperature ($^{\circ}\text{C}$)	B	40	60
Extraction time(hr.)	C	2	6

2.4 Variation of Moisture Content

The required moisture content level below seed moisture content was achieved by the addition of calculated amount of distilled water to the seed. The water added was thoroughly homogenize with the milled sample and was sealed and refrigerated at a temperature of 5°C for seven days. The samples were brought out of the refrigerator 24hours before use to allow equilibrium with the room temperature.

The mass of the water to be added was calculated using the equation below;

$$Q = \frac{W_i(M_f - M_i)}{100 - M_f} \quad (\text{ASABE, 2005}) \quad (ii)$$

Where;

Q (grams) is the mass of water to be added.

M_i (grams) is the initial moisture content of the sample.

M_f (grams) is the desired moisture content.

2.5 **Extraction of Oil from Azadirachta Indica Seed Using Soxhlet Method.**

15g of the conditioned seeds at various moisture content levels as shown in Table 2 was poured and wrapped inside a filter paper of determined weight. The samples were carefully inserted inside the extraction chamber which is being suspended above a round bottom flask containing 300ml of petroleum ether solvent and below a condenser (plate I). Heat was being applied to the flask with the aid of a heating mantle at the desired extraction temperature. Solvent evaporates moves to the condenser where it is cooled and converted into liquid and falls back into the extraction chamber containing the sample. (Juliet, 2017). The flask containing the solvent and oil was removed at the end of the extraction process after completing the stated time of extraction in the experimental run and oven at a temperature of 50°C to evaporate the solvent. The residue of the sample after extraction was oven dried.



Plate 1. Soxhlet apparatus (Getty Laboratory Glassware.Co)

2.6 **Determination of Percentage Oil Yield**

The percentage oil yield of each of the sample was obtained using the calculation as follows

$$\% \text{ oil yield} = \frac{W_i - W_r}{W_i} \times 100 \quad (\text{iii})$$

Where;

$W_i(g)$ is the weight of sample before extraction.

$W_r(g)$ is the weight of the sample after extraction.

2.7 Analysis of Data and Response Equation

Regression models were developed for the oil yield and each of the factors considered. Minitab 18 software was used to analyze the extraction data for developing response equation for analysis of variance (ANOVA), generate surface plots and determining the optimum extraction conditions using its optimization tool.

2.8 Determination of Physicochemical Properties of the Oil.

The following physicochemical analyses (density, viscosity, saponification value, iodine value, acid value, peroxide value, and free fatty acid) of the *Azadirachta indica* oil were carried out using the association of Official Analytical Chemists methods (AOAC 1990)

2.9 Production and Characterization of Biodiesel

The production and characterization of biodiesel from *Azadirachta indica* oil was achieved using methods described by (Aransiola, *et al.* 2012, and Adejumo *et al.*, 2019).

3.0 Results

3.1 The Extraction Process of Azadirachta Indica Seed Oil

The result of the experimental runs with oil yield as the response is presented in Table 2. It was observed that the highest percentage of oil yield was 43.72% which was obtained at an extraction temperature of 60°C, extraction time of 6hrs and moisture content of 6%. This is higher than the reported percentage oil yield from seed ranges from 20%-42% (kyari, 2008). While the least percentage yield of 22.34% was obtained at the moisture content of 12%, extraction temperature of 50°C and extraction time of 0.64hrs.

Table 2: Central composite experimental design arrangement and response

StdOrder	RunOrder	PtType	Blocks	Moisture content (%)	extraction temperature(°C)	extraction time	%yield
6	1	1	1	18	40	6	34.62
16	2	0	1	12	50	4	40.18

20	3	0	1	12	50	4	40.26
17	4	0	1	12	50	4	40.11
5	5	1	1	6	40	6	41.40
8	6	1	1	18	60	6	36.65
13	7	-1	1	12	50	0.64	24.34
2	8	1	1	18	40	2	22.93
18	9	0	1	12	50	4	39.60
14	10	-1	1	12	50	7.36	43.45
11	11	-1	1	12	33.18	4	29.87
3	12	1	1	6	60	2	34.94
4	13	1	1	18	60	2	30.62
9	14	-1	1	1.91	50	4	35.83
1	15	1	1	6	40	2	38.38
7	16	1	1	6	60	6	43.72
12	17	-1	1	12	66.82	4	40.52
10	18	-1	1	22.09	50	4	25.42
19	19	0	1	12	50	4	39.75
15	20	0	1	12	50	4	40.21

3.2 Statistical Analysis

Table 3 Analysis of variance

Source	Sum of Squares	Df	Mean Square	F Value	p-value Prob> F
Model	671.19	9	74.58	4.23	0.02
A-Moisture Content	67.13	1	67.13	3.81	0.08
B-Extraction Temperature	87.88	1	87.88	4.98	0.05
C-Extraction Time	315.47	1	315.47	17.88	0.00
AB	0.04	1	0.04	0.00	0.96
AC	2.73	1	2.73	0.16	0.70
BC	2.53	1	2.53	0.14	0.71
A ²	175.12	1	175.12	9.93	0.01
B ²	19.46	1	19.46	1.10	0.32
C ²	23.23	1	23.23	1.32	0.28
Residual	176.41	10	17.64		
Lack of Fit	167.69	5	33.54	19.22	0.00
Pure Error	8.72	5	1.75		
Correction Total	847.60	19			
Std. Dev.	4.20		R-Squared	0.79	
Mean	35.47		Adj R-Squared	0.61	
C.V. %	11.84		PredRSquared	-0.52	
PRESS	1283.86		Adeq Precision	6.69	

Regression Equation in coded Units

$$\begin{aligned} \text{Yield (\%)} = & -35.1 + 2.21A + 1.54B + 6.93C - 0.0968A^2 - 0.0116B^2 - 0.317C^2 - 0.0012AB - 0.049AC - \\ & 0.0281BC \end{aligned} \quad (iv)$$

ANOVA result in Table 3 and the model variables in equation (iv) show that B, C, AB and A² were all significant, while the model was significant, which was validated by the P-value of 0.02. In regression equation, when an independent variable has a positive sign, it means that an increase in the variable will cause an increase in the response while a negative sign will result in a decrease in the response. The individual variables (moisture content, extraction temperature and extraction time) examined in this study have an increasing effect on the percentage oil yield of *Azardirachta indica* seed as shown in equation (iv).

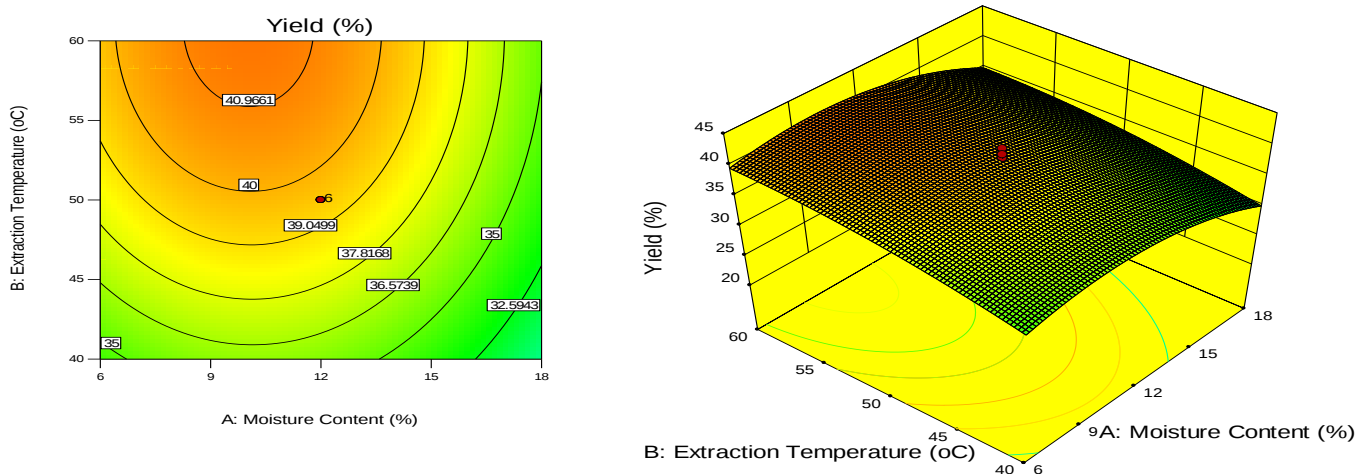


Figure 1: Surface plot of the effect of moisture content and extraction temperature on the percentage oil yield

The effect of moisture content and extraction temperature was studied using both 3D surface response and contour plot. It was discovered that the variation of moisture contents with temperature affected oil yield as reported by Juliet et al. (2017). The higher the moisture content, the lower the percentage oil yield and also further decrease in the moisture content, led to decrease in the oil yield which may be as a result of excessive drying of the milled *Azardirachta indica* plant seed. It could be seen that at the temperature above 60°C, decrease in percentage oil yield was discovered from the *Azardirachta indica* seed. The higher the temperature, the higher the extraction rate, until the temperature rises above the boiling temperature of the solvent, when further increment of the temperature led to a

decrease in the oil yield. It can be deduced that the combined effects of moisture content and extraction temperature have a significant effect on the oil yield from *Azadirachta indica* seed.

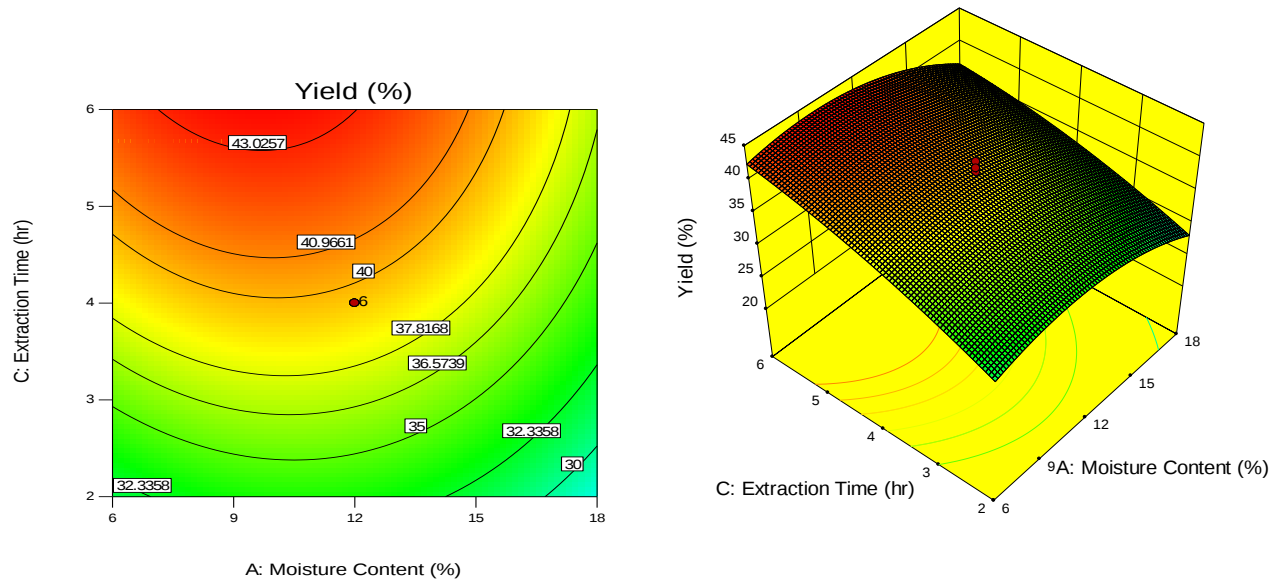


Figure 2: Surface plot of the effect of moisture content and extraction time on the percentage oil yield

The 3D surface plot interaction in Figure 2 represents the effects of seed moisture contents and extraction time. Their interactions on the oil yield of *Azadirachta indica* seeds for extraction were studied. It was observed that increase in extraction time favors the percentage oil yield of *Azadirachta indica* seeds as much time is needed for extraction until the boiling temperature of the solvent is reached, while the oil yield decreases gradually as moisture content increases

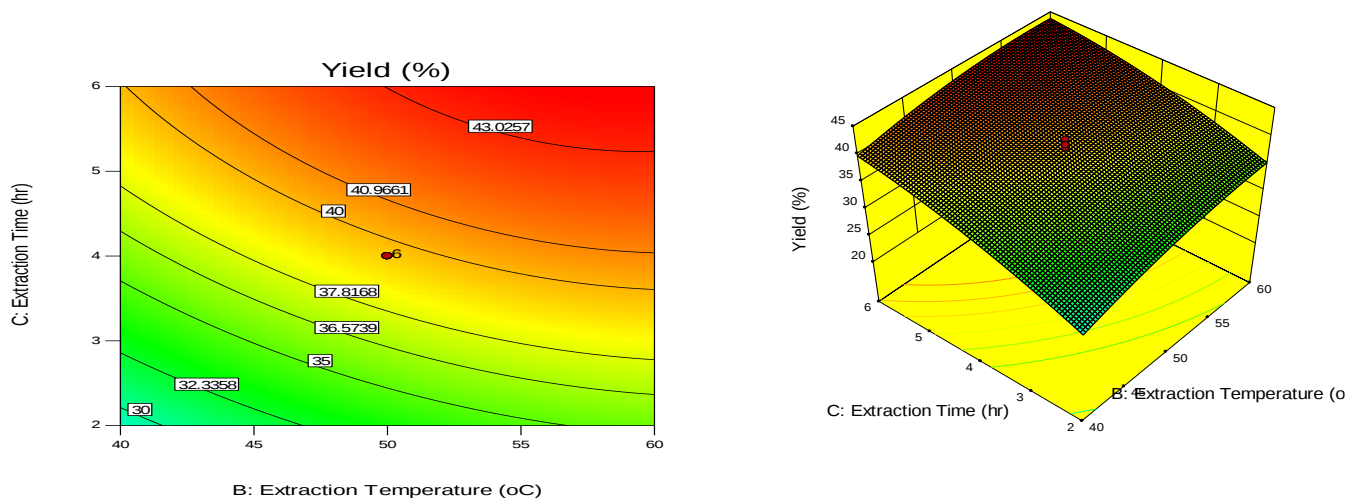


Figure 3: Surface plot of the effect of extraction temperature and extraction time on the percentage oil yield

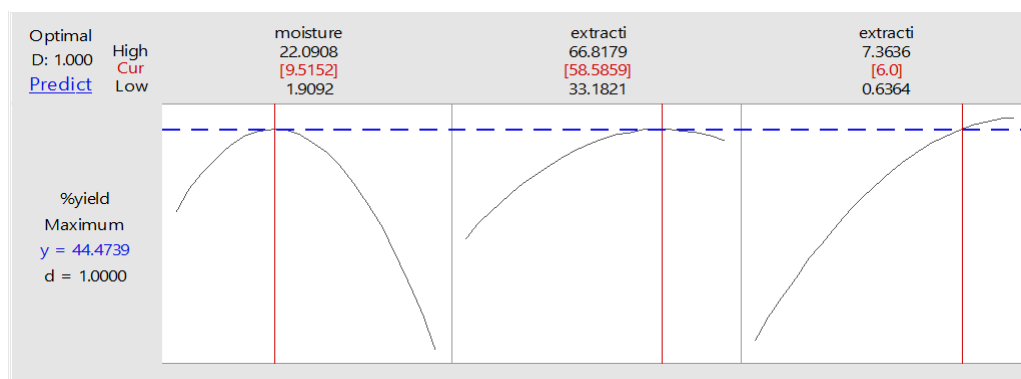
The nature of the plots indicated that interaction between the extraction temperature and time affected the percentage oil yield. It was observed that increase in time favored the extraction of oil until it got close to the moderate reaction time. Increased reaction time above this level tends to produce lower oil yields. The oil yield was observed to decrease with higher temperature particular at the temperature higher than the boiling point of the solvent. This is because at this temperature higher than the boiling temperature of the solvent, evaporation of the solvent will take place as a result of the increased temperature. This was in line with the report by Oniya (2017) for the extraction of oil from sand box seed. The combined effect of these two reaction variables at high-level experimental process will obviously result in a decrease in oil yield as recorded in Table 2.

3.3 Response Optimization

Table 4 Response Optimization and validation

Solution	moisture content(%)	extraction temperature(⁰ C)	extraction time(hrs)	%yield Fit	Composite Desirability
1	9.51515	58.5859	6	44.4739	1

Figure 4: Optimization plot of *Azardirachta indica* seed oil



Mintab 18 software was used to optimize the oil yield with the response set to maximum level. It was revealed that the combination of 9.5152% moisture content, 58.5859°C extraction temperature and extraction time of 6hr as predicted gave an optimum percentage oil yield of 44.4739% as shown in Table 4 and Figure 4. The predicted variables obtained were subjected to experimental validation. Oil was extracted using the combined variables predicted and a percentage oil yield of 44.4521 was obtained. This shows a close value with the predicted value.

3.4 Properties of *Azardirachta Indica* Oil

Table 5: Physiochemical properties of *Azardirachta indica* oil

s/no	Properties	Result
1	Density	0.97g/ml
2	Specific gravity	0.87
3	Kinematic viscosity	@40°C 37.42
4	Cloud point	11°C
5	Pour point	7.0°C
6	Smoke point	157°C
7	Flash point	171°C
8	Fire point	190°C
9	Saponification value	204mgKOH/g
10	Acid value	11.03MKOH/g
11	Free fatty acid	5.52(%w/w)
12	Peroxide value	9.43(neqO ₂ /kg)

The density of *Azadirachta indica* seed oil obtained was 0.97g/ml, this was found to confound with the result obtained by Ramning *et al.*, (2013) and was higher than the value of 0.92g/ml reported by (Aransiola, 2012). The specific gravity of the oil was found to be 0.87 which was lower than 0.91 reported by Oniya (2017) for sand box kernel oil. The kinematic viscosity at 40°C was found to be 37.42cSt which is higher than the value reported by Ramning *et al.*, (2013) and Ragit *et al.*, (2010). The cloud point, pour point, smoke point, flash point and fire point were found to be 11°C, 7.0°C, 157°C, 171°C and 190°C. The cloud point was low than what was reported by Aransiola *et al.*, (2012) and Ragit (2011) by 2°C and 8°C respectively. The pour point was found to be 7.0°C which was agreement with what was obtained by Aransiola *et al.*, (2012) and within the range of the study reported by Ramning *et al.*, (2013). The saponification value of the oil obtained was 204mgKOH/g which is higher than the value of 199.86mgKOH/g and 202mgKOH/g reported by Aransiola *et al.*, (2012) and Ragit *et al.*, (2012) but lower than the value of 210mgKOH/g reported by Awolu *et al.*, (2012). Bilal *et al.*, (2013) also reported that high saponification value may result to formation of soap when the oil is used for biodiesel production. The acid value of the oil was obtained to be 11.03mgKOH/g and free fatty acid of 5.52mgKOH/g this is close to the value obtained by Aremu *et al.*, (2006) of 11.60mgKOH/g and 5.83mgKOH/g acid value and free fatty acid respectively for palm kernel oil. The acid value obtained was also found to be close 10.92MGKOH/g Reported by Ramning *et al.*, (2013) and Awolu *et al.*, (2013). The peroxide value was found to be 9.43(neqO₂/Kg) which falls with range recommended for fresh oils.

3.5 Properties of *Azadirachta Indica* Oil Biodiesel

The biodiesel produced was found to have a percentage yield of 85%. The specific gravity of the biodiesel was found to be 0.87 which was in line with the ASTM standards. Therefore, the specific gravity gives a good ignition property by Clark, 1988. The flash point, pour point and cloud point was obtained to be 157°C, 4.2°C and 6.7°C respectively. The value obtained for flash point, pour point and cloud point of 150°C, 4.2°C and 6.7°C reported by Aransiola *et al.*, (2013) were found to be lower than the values obtained in this study. The kinematic viscosity @40°C of 4.3 mm²/s was obtained for the biodiesel which falls within the ASTM standard. The cetane number of 51.1 was obtained which was found to be within the range of ASTM standard and (Jamil *et al.*, 2016) reported the value 58.23 lower the value obtained in the study. The biodiesel acid value was found to be 0.31 mgKOH/g and was within the range of ASTM standard. Also confound with the report by Ramasamy and Nagarajan (2016) value of 0.31 mgKOH/g for *Datura* biodiesel. The moisture content of the biodiesel was found to be 0.17% which was within the set limit by ASTM standard.

4.0 Conclusion

Azadirachta indica seed oil was extracted using solvent extraction method. The optimization of the extraction process was investigated by varying the seed moisture content, extraction temperature and extraction time and maintaining a constant particle size of 1.18 mm. The optimum oil yield of 43.72% was obtained at seed moisture, extraction temperature and extraction time of 6% (wet basis), 6 hrs, and 60°C respectively.

The extracted optimum oil yield has a moisture content of 11.37% (wet basis), density of 0.97 g/ml, specific gravity of 0.87, cloud point of 11°C, pour point of 7°C, smoke point of 157°C, flash point of 171°C, fire point of 190°C, saponification value of 204 MKOH/g, acid value of 11.03 MKO/g, free fatty acid of 5.52 (%W/W) and peroxide value of 9.43 (meqO₂/Kg).

The optimum yield of the extracted oil was used for biodiesel production and a yield of 85% was achieved. The biodiesel was characterized and compared with standards of American standards for testing machine (ASTM).

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