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TRANSESTERIFICATION OF NEEM (*Azadirachta indica*) SEED OIL

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ABSTRACT

Azadirachta indica seed oils were extracted, characterized and transesterified using methanol in the presence of a heterogeneous catalyst (clinker). Effects of particle size, temperature, extraction time and volume of solvent as the extraction yield parameters were studied. Results showed that the optimum oil yield was 38.50% at 65 °C and 0.150 mm particle size. Physicochemical characterization of the purified oil revealed relatively low acid values 15.02 mgKOH/g, low iodine values 83.03, low peroxide values 2.38 meq/g, low ash content 1.04, but relatively high specific gravity 0.92, kinematic viscosities 48.78 mm^2/s and saponification value 198.11 mgKOH/g. The Neem biodiesel properties obtained were viscosity (6.71 mm^2/s), flash point (175 °C), density (890 kg/m^3), pour point (12 °C), cloud point (16 °C). Fourier transform infra-red (FTIR) spectroscopic analysis confirmed the presence of major functional groups in the oil and fatty acid methyl ester (FAME), notably hydroxyl, carbonyl, olefinic and methylenic. The fatty acid composition of the oil and their biodiesel were determined by gas chromatography-mass spectrometry (GC-MS). Although the oil samples extracted is good feedstock for biodiesel production and other industries, the Neem oil methyl ester (NOME) is considered suitable for use in diesel engine when compared with standards of ASTM D6751 and EN 14214.

Key words: *Azadirachta indica*, non-edible oil, characterization, clinker, transesterification, biodiesel.

INTRODUCTION

The present desire towards the synthesis of biodiesel is as a result of the conservation of the fossil fuels as well as concerns over environmental problems. The fast growing demand for energy and industrial raw materials from crude oil and the resultant depletion of crude oil with adverse environmental problems and very fluctuating nature of its prices make necessary the need to explore alternative sources of fuel and industrial raw materials. Concerted effort has been made in producing alternative renewable energy like biodiesel which is also known as Fatty Acid Methyl Ester. Fatty Acid Methyl Ester is an alternative biofuel produced through transesterification of triglycerides (TGs) or the esterification of Free Fatty Acids (FFAs) with methanol [1]. Also, biodiesel possesses all the favourable characteristics of diesel and it is renewable, abundant, biodegradable, non-toxic and carbon neutral since no net amount of carbon is released to the atmosphere. Biodiesel, biogas and Bio-ethanol are all examples of biofuels. One important feature is that they can be blended at any proportion with any fuel obtained from petroleum whose properties conform to the end use. For example, biodiesel with diesel fuel, bioethanol with gasoline can all be blended [2]. According to Ramadhas et al [3], biodiesel has no sulphur content, no storage difficulties and have good lubricating properties.

Vegetable oils and fats are the main feedstock for biodiesel and an economical supply that is sustainable is an important factor [4]. Many researchers used edible oils for example melon and palm oil to produce biodiesel but the fluctuating prices of edible oils and increasing demands for nutritional needs have made Neem Seed oil to be the raw material of choice. In order to overcome these issues, there is a dire need to produce biodiesel using second generation vegetable oil and the use of catalyst that reduces energy requirement in transesterification as well as cost effective.

Neem plant is an evergreen tree which is endemic to the Indian sub-continent and has been introduced to many other areas in the tropics like Nigeria.

MATERIALS AND METHOD

The raw materials used to carry out this research are the neem seed. The seeds were collected from Auchi Polytechnic in Edo State, Nigeria and subsequently sorted into good and bad ones. All chemicals used in this study were from Sigma – Aldrich Limited, Germany and are of analytical grade.

Neem Seed Oil Extraction

The good Neem seeds were washed and sun dried for three days, later they were deshelled, sundried for two days. The kernels, crushed mechanically, were stored in a polythene container. Later oils were extracted from known weights of crushed seed samples using soxhlet extractor and hexane as solvents after which the oil was filtered and oil yield calculated using equation 1:

$$Oil\ yield(y) = \frac{\text{weight of oil extracted X 100}}{\text{Weight of seed}} \qquad (1)$$

Oil Extraction Procedure and Factors that Affect Oil Yield

The oil extraction, transesterification and characterizations were carried out in Auchi polytechnic; Springboard Research Laboratories, Awka; and at Ahmadu Bello University, Zaria, Nigeria. The crushed seeds were extracted using soxhlet extractors with n–hexane as the solvent. The extraction was carried out considering four factors that affect the yield of oil extracted. These factors are namely particle size, temperature, extraction time and volume of solvent used. The extracted oil for each factor was then collected and weighed.

The oil was then removed from the evaporator, dried in the oven, cooled in the desiccators and weighed again to determine the yield of oil extracted. Further extractions were carried out at different extraction process variables and the oils obtained were subjected to pre-treatment procedures like: degumming, neutralization and dehydration.

Determination of physico-chemical properties of the oil extracted

The properties determined were acid value, saponification value, kinematic viscosity, peroxide value, unsaponifiable matter, refractive index, Flash plant, Phenolic content, Colour, pH value, Ester value, Iodine value, Moisture content, specific gravity, and odour; using standard methods.

Preparation of Limestone based (Clinker) Catalyst

Cement Clinker was collected from Dangote Cement Plant, Obajana, Kogi State, Nigeria. The cement clinker was washed with 1% aqueous solution of sulphuric acid to remove dirt and stains on the surface of the shell. The cement clinker was crushed and ground using grinding machine to reduce the particle size to around 150 μm to ensure a large surface area per unit mass. Chemical composition of the clinker was obtained from X-ray florescence with in-built XRD

(ARL 8660S, Germany). The catalyst activation was performed by soaking with methanol followed by calcination at 700 °C for 6 hours in the furnace.

FTIR Test for the Oils and FAMEs (FTIR spectroscopic analysis)

FTIR was used to characterize the structural functionalities in the oil and FAME. FTIR spectrum of oil (in KBr pellets) was recorded with a Shimadzu 8400S FTIR spectrophotometer over the range 4500-350 cm^{-1}.

GC-MS Test for the Oils and FAMEs (Fatty Acid Profile Analysis)

The oil was also characterized in terms of its fatty acid profile. Fatty acid composition was determined after converting the fatty acids in the oil to fatty acid methyl esters. The FAMEs were prepared according to the modified ISO method [5]. The separation of the FAMEs was performed on a GC-MS (QP2010 plus Shimadzu, Japan) instrument, comprising an AOC-20i liquid auto sampler and a GC equipped with fused silica capillary column (30 x 0.25mm ID x 1 µm df) coated with 5% phenyl-, 95% dimethylsiloxane polymer.

Transesterification (Biodiesel Production)

About 1 Litre of the oil sample was measured out into a three mouth flask having a cooling system to circulate the methanol. The oil samples were heated up to 65 ^oC while undergoing stirring by a combination of hot plate and magnetic stirrer. Previously prepared alcohol-catalyst solution was added to the oil in the flask at the same temperature, while stirring the mixture. The alcohol catalyst solution was added while the oil was being stirred because of the fact that the reaction is very slow at the beginning of the reaction due to mixing and dispersion of alcohol into feedstock. Hence the mixing of the reactants enhances the reaction rate and increases the ester conversion. When the reaction was completed, the catalyst was filtered out and the end product was poured into a separating funnel and was left for 12 hours to ensure total separation.

The transesterification reaction was performed at 6:1 molar ratio of methanol/oil while the temperature and reaction time was maintained at 65 ^oC and stirred for 2 hours, optimum catalyst concentration of 5% (w/w). During the transeterification of the oil for methyl ester (biodiesel) production, effect of temperature, contact time, catalyst particle sizes and oil to methanol ratio were investigated. The standard procedures as reported by Meda et al [6] were employed.

RESULTS AND DISCUSSION

N-hexane was used in the extraction of the oil from neem seed oil and this gave a maximum yield of 38.5 wt%. This shows that Neem is a good source of oil compared to other seeds like cotton seed, water melon seed etc which contains about (35-40)%, (25-35)%, respectively and can easily serve as a source of raw materials in process industries. A two-step acid–base catalysed transesterification reaction has been shown to be appropriate for biodiesel production from neem oil due to its high FFA (7.51 mg KOH/g), the pre-treatment of the oil is critical before the alkaline transesterification. There are many factors that affect the yield of oils under this study.

The Effect of Extraction Temperature on the Extraction Yield

Figure 1 shows linear relationships between temperature and the oil yield, the higher the temperature the higher the oil yield. It was observed that oil yield increased initially with increasing temperature and then subsequently decreased with further increase in temperature. The optimum oil yield neem seed sample was obtained at 65 °C. The reason for the increase in the yield is that increase in temperature is believed to facilitate the rupturing of oil cell walls, creating a void which serves as migratory space for the contents of the oil bearing cells. Increase in temperature also lowers the viscosity of the oil and coagulates protein, thus facilitating the release of the oil out of the cells into the inter-kernel void. However, at higher temperature of extraction, there is substantial loss of moisture leading to a hardening of samples and oil degradation. It was observed that at 90 °C the colour of the oil turned dark brown.

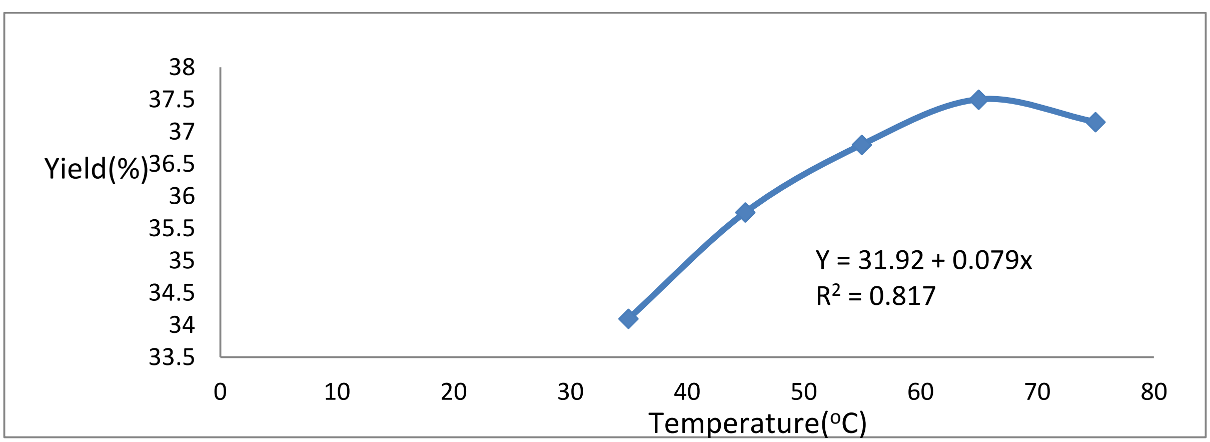

Figure 1: Effect of temperature on extraction yield of neem seed oil (NSO) when particle size =0.355 mm, Time = 60 min and volume of solvent = 250 ml

The Effect of Particle sizes on the Extraction Yield

Figure 2 show the variation of neem seed particle sizes on the oil yield. From the figure, it could be seen that decrease in particle sizes brought about increase in oil yield and this made the optimum size of 0.150 mm to give the maximum oil yield of 38.5% . This agrees with the fact that size reduction increases the surface area and increases number of ruptured cells resulting in a high oil concentration at the particle surface which in turns increases the yield of the seeds. Diffusion into the particles surface takes place. Therefore, the amount of oil available for extraction is proportional to the surface area of solid particles. Sayyar et al. [7] while investigating the extraction of oil from Jatropha seed posited also that larger particles present smaller contact surface areas and are more resistant to solvent entrance and oil diffusion. So, less amount of oil will be transferred from inside the larger particles to the surrounding solution compared to the smaller one. However, linear relationship exists between the two variables. Increase in the particle size leads to decrease in yield and vice - versa. The regression analysis of the result in Table 2 gives equation y = 39.15-6.308x and correlated (R^2 = 0.955).

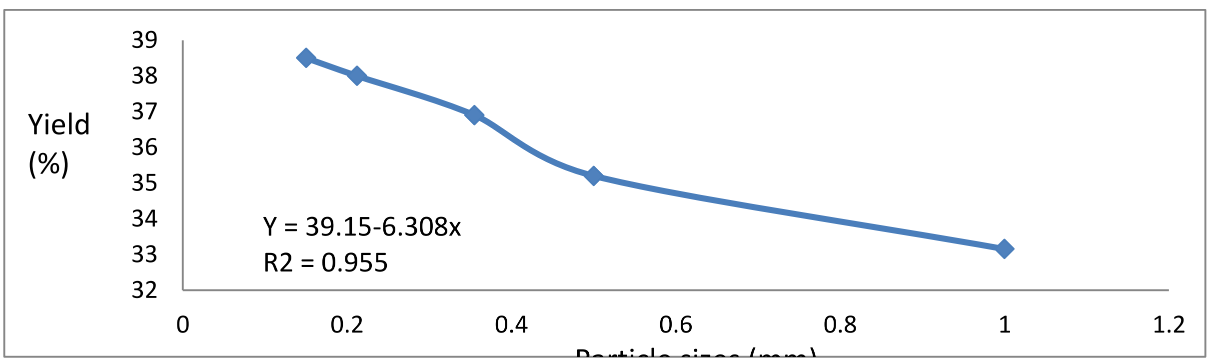

Figure 2: Effect of particle sizes on extraction yield of neem seed oil (NSO) when temperature = 68 °C, Time = 60 min and volume of solvent = 250 ml

The Effect of Volume of Solvent on the Extraction Yield

Figure 3 below explain the effect of volume of solvent on oil yield of neem seed samples. These figure shows that the optimum volume of solvent to give the maximum oil yield for 100 g of the ground sample is 350ml. The oil yield at this point is 37.10%, but as the volume of solvent reduces below this point, the oil yield decreases and when the volume increases beyond this point, the yield remains unchanged. It was also observed that when the ratio of weight of sample to volume of solvent increased beyond 1:5, the yield began to decrease. This is due to higher energy and longer time requirements to heat the solvent to boiling point.

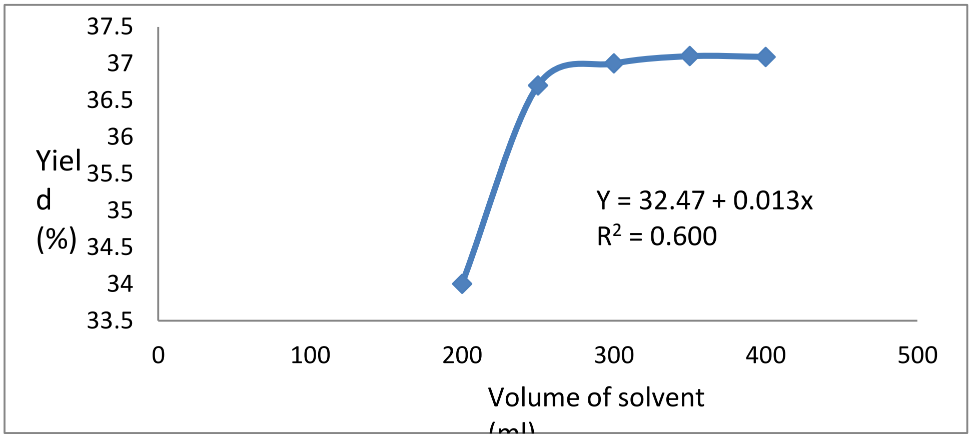

Figure 3: Effect of volume of solvent on extraction yield of neem seed oil (NSO) when temperature = 68 °C, Time = 60 min and Particle sizes = 0.355 mm

The Effect of Time on the Extraction Yield

Figure 4 shows that at extraction time of 70 minutes, the maximum oil yield of 37.85% at optimum time was obtained. It is important to note that below and above this time the oil yield reduces.

Consequently, the regression analysis carried out show significant relationships between the variables and the R^2 values obtained in each case is good enough to describe the degree of variability between the variables, high correlation also exist between the variables in each case

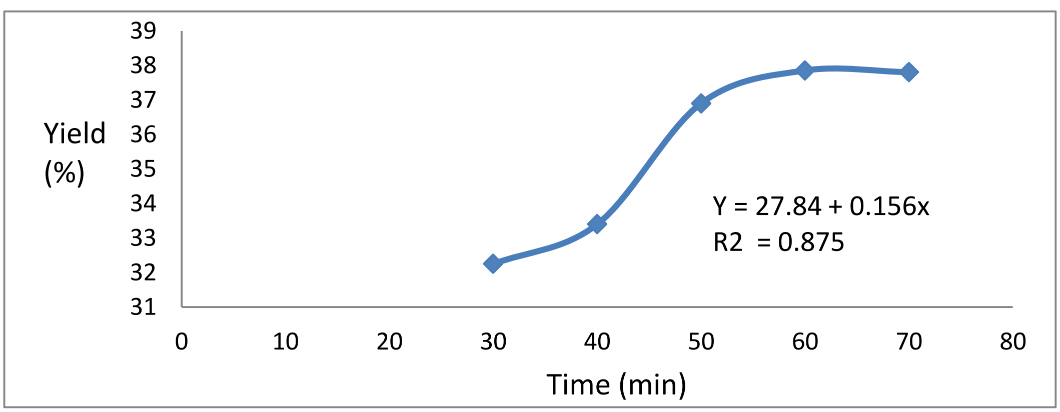

Figure 4: Effect Time on extraction yield of neem seed oil (NSO) when temperature = 68 ^oC, volume of solvent = 250 ml and Particle sizes = 0.355 mm

The regression equations developed are good enough to predict the values of Y when the value of X is known (Table 2)

Table 2: Regression equation for neem seed oil

S/NO	Parameters	Regression Equation	R^2
1.	Particle Size Versus Yield	y=39.15-6.308x	0.955
2.	Temperature Versus Yield	y=31.92+0.079x	0.817
3.	Time Versus Yield	y=27.84+0.156x	0.875
4.	Volume of Solvent Versus Yield	y=32.47+0.013x	0.600

Characterization of calcined clinker

The elemental composition analysis of the calcined clinker sample with XRF spectroscopy was tabulated as shown in Table 3 below. XRF analysis showed that the sample was composed mainly of calcium oxide with 66.81wt.%. These results indicated that all calcium carbonate ($CaCO_3$) in the samples was completely transformed to CaO. So, large quantity of Calcium (Ca) and Oxygen (O_2) were observed in the sample.

Table 3: Clinker analysis with XRF-XRD

Element	CaO	SiO_2	Al_2O_3	Fe_2O_3	MgO	SO_3	K_2O	Na_2O	P_2O_5	TiO_2
Wt. %	66.14	22.02	5.96	3.50	0.68	0.44	0.79	0.12	0.03	0.32

Characterization of Neem seed oil, and the Biodiesel

From Table 4, the acid value of neem oil is 15.02. The acid value gives an indication of the amount of FFA present in the oil at the time of the test. Low acid value is an indication of good non-degraded state of the oil and is within limits for industrial useful oils. The FFA values of the oils were as low as 7.51 for NSO. The low FFA also suggests low levels of hydrolytic and lipolytic activities in the oils. The acid value also gives a measure of the extent to which the constituent glyceride has been decomposed by lipase action. The maximum limit of acid value specified in the biodiesel fuel standards such as ASTM D6751 and EN 14214 is 0.50 mg of KOH/g. The acid values of biodiesel obtained from NSO oils is 0.27 mg of KOH/g which are far below the specified maximum limit and the free fatty acid values is 0.135 mg of KOH/g.

The saponification value provides information on the amount of KOH that will saponify the oils. Table 4 also shows that the saponification value of neem is 198.11. This implies that the triglycerides of neem oil have higher molecular weight of fatty acids (saturated and unsaturated). The results obtained compared favorably with the saponification value of palm oil (196-205), olive oil(185-196), soy oil (193) [8] Also, the biodiesel standards such as ASTM D6751 and EN

14214 have no specification for the saponification number. The biodiesels obtained from soybean, sunflower, palm, canola, and Jatropha curcas oils have saponification numbers 201, 200, 207, 182 and 202 mg KOH/g respectively [9]. In the present study, saponification numbers of biodiesels prepared from NSO seed oil was calculated and found to be 182.3 and 237.2 mg KOH/g respectively which are comparable to the values of biodiesels obtained from soybean, sunflower, palm, canola and Jatropha curcas oils.

In Table 4, the physical analysis of NSO shows the specific gravity to be 0.92 and 0.89 for NOME. This shows that neem biodiesel and it's oil is a dense biodiesel and it's oil whereas the oils extracted and the methyl esters produced are less dense than water. Also, the value seems to indicate that no heavy element is present in the oil.

At 28 ^{o}C refraction index value of 1.47 for NSO and 1.44 for NOME. The refractive indices obtained falls within the range (1.447-1.490) reported for some other seed oils (1.480 for Telfairiaoccidentalis, 1.468 for Jatropha curcas, 1.47 for soybean oil and 1.47 for corn oil) which have myriad industrial applications.

The iodine value provides information on the measure of unsaturation of oils. In general, the greater the iodine value, the higher the degree of unsaturation and the higher the tendency of the oil to undergo oxidative rancidity Table 4 shows the iodine value to be 83.03. The low iodine values of the sample indicate that the oil is non-drying type of oil. Although the iodine values is low, the oil have proven to be good source of raw materials for biodiesel production even against that, the higher the iodine value the more the number of unsaturated double bonds present in the molecular structure and the less the viscosity of the oil .

The peroxide value of 2.38meq/g was obtained (Table 4). Peroxide value depends on a number of factors like oxidation by oxygen, extraction methods and storage. The low peroxide values suggest that neem seed oils are stable to oxidative degradation caused by over exposure to oxygen, heating and improper storage .

The cetane numbers of NOME (54.0) is within ASTM D6751 standard while NSO (47.0) fall outside the range (Table 4). Cetane number (CN) is a dimensionless indicator that characterizes ignition quality of fuels for compression ignition engines (CIE). Since in the CIE burning of the fuel air mixture is initiated by compression ignition of the fuel; the number is a primary indicator of fuel quality as it describes the ease of self- ignition.

Table 4: Physiochemical properties of NSO, NOME and Biodiesels, ASTM D6751

S/N	PARAMETERS	UNIT	BIODIESEL ASTM D6751	NSO	NOME
	Acid Values	mgkOH/g	<0.50	15.02	0.27
	pH	-		6.4	6.8
	Iodine Value	-		83.03	78.14
	Saponification Value	mgkoH/g		198.11	237.2
	Refractive Index at 28^0C	-		1.47	1.44
	Free Fatty Acid	mgkoH/g		7.51	0.135
	Colour	-		Dark Yellow	Greenish Yellow
	Specific Gravity	-	0.88	0.92	0.89
	Kinematic Viscosity (a 40^0C)	mm^2/s	1.9-6.0	43.78	6.71
	Cetane Index	-	48-65	54.0	47.0
	Ash Content	%		1.04	0.38
	Flash Point	°C	100-170	167.80	175
	Peroxide Value	Meq/kg		2.38	-
	Moisture Content	%	0.05	2.24	Nil
	Fire Point	^{0}C		3720	
	Yield	%		38.9	89
	Physical State at room temp.	-	Liquid	Liquid	Liquid
	Pour Point (^{0}C)	^{0}C	-15 to 10	12.0	10.0
	Cloud Point (^{0}C)	^{0}C	-3 to 12	16.0	8.0

Fourier-transform infrared spectroscopy analysis

FT-IR spectra (4000-400cm^{-1}) of the neem seed oil (NSO) and its biodiesel (NOME) in Figure.5 (a) and (b) respectively, reveal major functional group absorption bands, notably for hydroxyl, carbonyl, olefinic, methylenicetc groups**.** The NSO shows two small peaks at 3483.21 cm^{-1} and 1625.94cm^{-1} corresponding to the stretching and bending vibration of O-H bonds of water molecule of the unsaturated fatty acid in the oil. The peaks centred at 2977 cm^{-1} and 2766 cm^{-1} are attributed to the anti-symmetric and symmetric stretching vibrations respectively, of aliphatic C-H in CH_2 and terminal CH_3 groups. The strong single peak appeared at 1625.943 cm^{-1} is attributed to the C=O stretching vibration of carbonyl groups of the triglycerides due its acidic content. Alkene group (CH=CH) is attributed to the band of 3212cm^{-1}. The peaks in the 1400-864 cm^{-1} region are mainly attributed to the bending vibrations of CH_2 and CH_3 aliphatic groups like symmetric HCH bending at 1391.93 cm^{-1}. The peak at 864.47 cm^{-1} is due to the overlapping of the $(CH_2)n$ rocking vibration. The stretching vibrations of CH_3,CH_2, and CH groups appeared at 2977-2950, 2950-2850 and 3050-3000 cm^{-1}, whereas the bending vibrations of these groups appeared at 1391-1350, 1350- 1150 and846cm^{-1}respectively. The FT-IR spectra of the NSO and its biodiesel are very similar to each other as a result of the similarities in the chemical natures of the triglycerides and methyl esters.

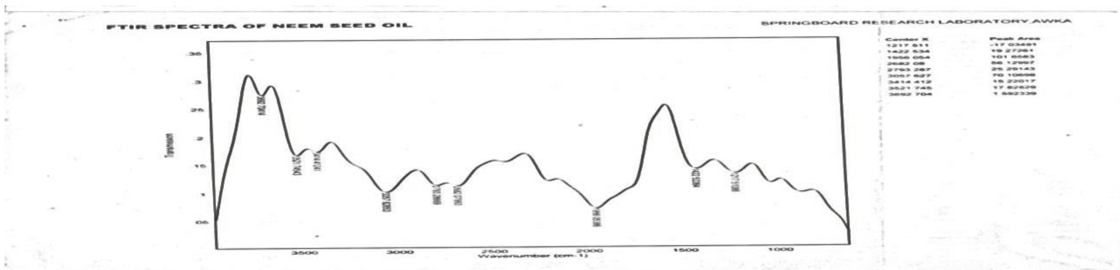

Figure 5a: FTIR Spectra of Neem Seed Oil

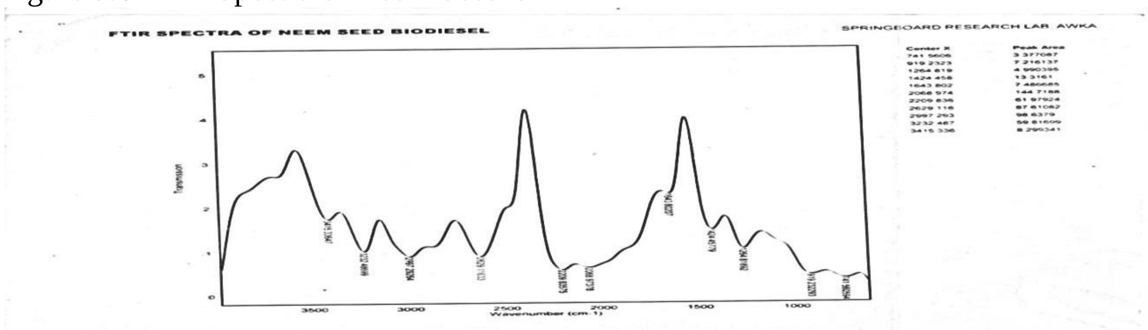

Fig. 5b: FTIR Spectra of Neem Seed Biodiesel

Fatty acid profile of NSO, and NOME

Figure 6 shows eight peaks representing major constituents of fatty acid in NSO which are (peak 2) Lauric acid (35.616%), (peak 5) Palmitic acid (31.838%),(peak 6) Oleic acid (10.532%), (peak 7), Palmitoleic acid (10.368%), (peak 4) Linoleic acid (5.565%), (peak 1) palmitoleate (3.634%), (peak 3) Myristic acid (1.798%) and (peak 8) Arachidic acid (0.649%). Saturated fatty acid (SFA) for NSO was 69.901%, while the unsaturated fatty acid (UFA) is 30.099% 9 (Table 5). These compares well with reported values for fatty acid of edible vegetable oils like sunflower, safflower, linseed and soybean oils [10] Neem oil methyl ester (NOME) has about 46.024 % of unsaturated and 53.975 % of saturated fatty acids

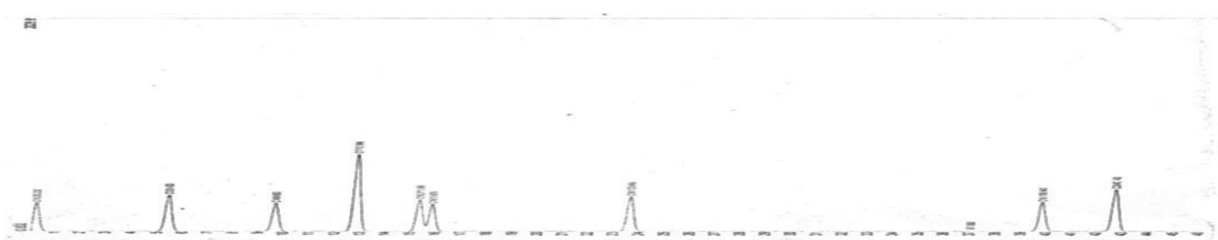

Figure 5: Neem seed oil Fatty acid profile

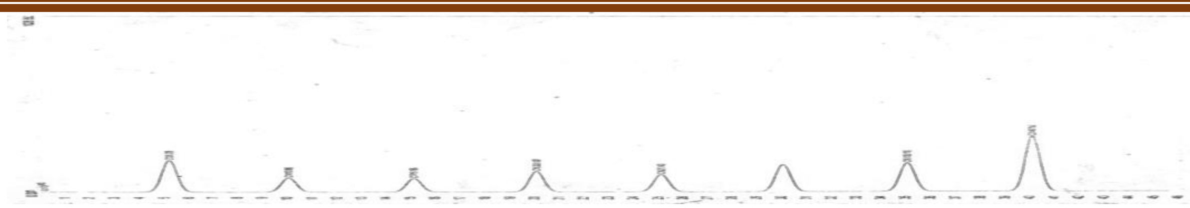

Figure 6: Neem biodiesel fatty acid profile

Table 5: Neem seed oil fatty acid profile

Component	Name	Concentration PPM	% Concentration
C16.2	Palmitoleate	2.0032	3.634
C12	Lauric Acid	19.6337	35.616
C14	Myristic Acid	0.9913	1.798
C18:2	Linoleic Acid	3.0677	5.565
C16	Palmitic Acid	17.5510	31.838
C18:1	Oleic Acid	5.8058	10.532
C16:1	Palmitoleic Acid	5.7155	10.368
C20	Arachidic Acid	0.3850	0.649
	Saturated fatty acid		69.900
	Unsaturated fatty acid		30.100
Total		148.5451	

Table 6: Fatty acid profile for neem seed biodiesel

Component	Name	Concentration PPM	% Concentration
C18	Methyl Stearate	2.0818	1.616
C12	Methyl Laurate	38.6822	30.034
C14	Methyl Myristate	0.7533	0.585
C17	Methyl Margarate	0.3352	0.260
C18:2	Methyl Linoleate	30.4350	23.630
C16	Methyl Palmitate	16.7180	12.980
C16:1	Methyl Palmitoleate	39.0829	30.345
C20	Methyl Arachidate	0.7072	0.549
	Saturated fatty acid		46.030
	Unsaturated fatty acid		53.960
Total		128.7956	

CONCLUSION

The result of the analysis of the factors that affect oil yield of the neem seeds shows that the smaller the particle size, the higher the oil yield. Also increase in temperature, time volume of solvent all increases the yields of the oils. The optimum yield of neem is 38.5 wt%.

From the oil yield, it can be concluded that the heterogeneous catalyst, clinker, showed a good conversion (94.32%) of high FFA neem seed oil to biodiesel. The results revealed that the catalyst is not negatively affected by moisture and free fatty acids and can be recycled very easily without a significant loss in its activity. The catalyst can be recovered by filtering out the catalyst and washing with acetone for reuse.

The characterization results of the oil seed and biodiesel produced compare favourable to ASTM D6751 standard, hence, the fatty acid methyl ester of neem seed oil can be used in diesel engine.

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