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Nuclear Magnetic Resonance Spectroscopy Guided Biodiesel Production from Atoulfo Mango Seeds Oil and the Evaluation of Some of its Properties

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ABSTRACT
Ground Atoulfo mango seeds kernel was extracted using soxhlet apparatus with hexane to give an oil yield of 22.71%. The physicochemical properties of the oil reveals; acid value (15.32±0.20 mgKOH/g), free fatty acid (7.66±0.10 mg/g), iodine value (61.31±3.70 I_2g/100g) and saponification value (171.99±3.10 mgKOH/g). The production of biodiesel from the oil by transesterification reaction was monitored by proton nuclear magnetic resonance (^{1}H NMR) spectroscopy. The percentage triacylglycerol conversion in the oil to its corresponding methyl esters (biodiesel) was calculated to be 94%. The important properties of the biodiesel studied were density (0.88 g/cm^3), acid value (0.39 mg/KOH/g), kinematic viscosity (4.60 °C mm^2/sec), flash point (150 °C), cetane number (65), pour point (2.1 °C), sulphur content (0.011 %) and ash content (trace) respectively. The fuel properties studied significantly agreed with the specifications of American Society for Testing and Materials (ASTM D6751), thus qualifying the seed oil as a potential ingredient for the production of biodiesel.

Keywords: Acid value, Cetane number, Fuel properties, Transesterification, Rancidity

INTRODUCTION
Human activities largely depend on the use of fossil fuels such as petroleum, coal and natural gas which are non-renewable energy resources. These fuels contribute mostly to the world energy supply and their production and consumption have caused many problems such as human health problem, environmental degradation, global climatic change and emission of greenhouse gases (Atadashi *et al.*, 2011). Large scale utilization of fossil fuels in mechanical power generation in various sectors, like agriculture, commercial, domestic, and transport sectors have increased due to population explosion. The fear of depletion of global fuel reserves, market unpredictability and negative environmental impact has led to search for an alternative fuel in both developing and developed nations (Mamudu and Olukanmi, 2019; Naik *et al.*, 2010; Olatunji *et al.*, 2019). As a result, increased attention has been given to biofuels, particularly biodiesel which is renewable and environmentally friendly (Demirbas, 2008).

The use of raw vegetable oils to run diesel engines was first demonstrated in Paris Exposition by Sir Rudolph Diesel in 1900 (Zahan and Kano, 2018). The disadvantage of using vegetable oil to run diesel engine are its high density, high viscosity, acid composition and free fatty acid content. To solve this problem, transesterification reaction (Scheme 1) was employed as the most favourable for decreasing oil's density and viscosity and thus producing the so-called 'biodiesel fuel' (Hidayat *et al.*, 2018; Meher *et al.*, 2006). Transesterification reaction is a process in which triglycerides in the lipids are reacted with alcohol, usually methanol, in the presence of a catalyst usually potassium hydroxide or sodium hydroxide to produce alkyl esters or biodiesel and glycerol as by product (Aworanti *et al.*, 2019). Biodiesel comprised of monoalkyl esters of long-chain fatty acids derived from animal fats or edible and non-edible oil such as vegetable oils, palm oil, canola oil, soybean oil, sun flower oil, jatropha oil, castor seed oil and waste frying oil (Dhar *et al.*, 2012). Biodiesel can be used either pure or blended with petro-diesel fuel in diesel compression–ignition engines without requiring engine modifications, because it has similar characteristics to petroleum-based diesel fuels (Freedman *et al.*, 1984). Biodiesel have many advantages over petroleum-based diesel fuels; it is a clean renewable fuel with higher biodegradable and combustion efficiency (Thushari and Babel, 2018). It also has higher cetane number, high flash point, inherent lubricity, lower sulphur and aromatic content (Evangelos, 2013 and Knothe*et al.*, 2005). Therefore, this study examined the properties of oil obtained from Atoulfo mango seed kernel oil for their potential application in the production of biodiesel fuel, and proton nuclear magnetic resonance spectroscopy was employed to monitor the transesterification processes of the crude oil.

$$\begin{array}{llcll}
CH_2-OOC-R_1 & & & R_1-COO-R' & CH_2-OH \\
CH\ -OOC-R_2 & +\quad 3R'OH & \underset{\longleftarrow}{\xrightarrow{\text{Catalyst}}} & R_2-COO-R' \quad + & CH\ -OH \\
CH_2-OOC-R_3 & & & R_1-COO-R' & CH_2-OH \\
\text{Glyceride} & \text{Alcohol} & & \text{Esters} & \text{Glycerol}
\end{array}$$

Scheme 1: Transesterification reaction of triglycerides with alcohol

MATERIALS AND METHODS

Chemicals used

All solvents and reagents used in the preparation of solutions were of analytical grade purchased from Sigma-Aldrich (United Kingdom) and Qualikems (India).

Sample collection and Sample Treatment

Mango (*Mangifera indica*) fruits (Figure 1) were harvested between the months of April and July, 2018 at various locations within Keffi town, Nasarawa State, Nigeria, during the mango fruiting season. The fleshy part of the fruit was carefully removed using a laboratory knife and the seeds were sun- dried for 10 days. The dried seeds were cracked using a hammer to obtain the kernels, which were cut into small pieces and then ground in a mechanical grinder to reduce the particle size to a maximum diameter of 500 mm as measured by a sieve, sealed in a plastic container and stored in a refrigerator until extraction.

Figure 1. Atoulfo mango fruits.

Extraction of the oil

Oil was extracted from the sample according to the method described by Kittiphoom and Sutasinee, (2013). Ground sample (100 g) was weighed and soxhlet-extracted (RE-52A, Shanghai Ya Rong Biochemistry Instrument Factory, Shanghai) with n-hexane for 6 hours at 50°C. The extract was concentrated using rotary evaporator at 40 °C to give a golden yellowish oil and the solvent used recovered. The obtained extract was weighed, labelled and store in air-tight container at room temperature for further analyses.

Transesterification of *M. indica* kernel seed oil

The method of Thoss *et al.*, (2012) and Momoh *et al.*, (2014) were used with modification. The *M. indica* kernel seeds oil (25 g) was dissolved in sodium methoxide (0.5M, 100 ml) and heated under reflux at 60 °C for one hour to produce fatty acid methyl esters (FAMEs). Deionised water (200 ml) was then added, and the solution extracted with hexane (200 ml × 3). The upper phase (hexane fractions) were combined, dried over $MgSO_4$, filtered under gravity and the solvent removed using rotary evaporator at 40°C while the lower phase (aqueous fractions) was discarded.

Preliminary study of the crude mango seed kernel oil and biodiesel:

The acid value, percentage free fatty acid, iodine value and saponification value of the crude mango seed kernel oil were determined using standard procedures described in AOAC, (1995). The fuel properties of the biodiesel produced were evaluated using methods described in ASTM D6751, (2008).

^{1}H Nuclear Magnetic Resonance

The ^{1}H NMR spectra were recorded on a Bruker Avace 400 MHz NMR spectrometer operating at 400 MHz. The oil or biodiesel sample, weighing 5 mg was mixed with 500 µl of deuterated chloroform and this mixture was introduced into a 5 mm diameter tube. The acquisition parameters were: spectral width 5000 Hz, relaxation delay 2 s, number of scans 32, acquisition time 2.744 s and pulse width 45°, with a total acquisition time of 2.37 min. The experiment was carried out at 298K. The signal of the non-deuterated chloroform from Eurisotop (Saint-Aubin, France), which is always present in the deuterated chloroform at 7.26 ppm was used as a reference for chemical shift and coupling constant, J, is in Hertz. The expanded ^{1}H NMR spectrum, peak picking and integration were processed by the MestReNova program (Mestrelab Research, Santiago de Compostela, Spain).

RESULT AND DISCUSSION

The physicochemical properties of crude seed kernel oil are as presented in Table 1. The percentage yield of the oil extracted from the samples is 22.71% dry weight which is higher than 8.46, 14.0 and 14.7% reported for different varieties of mango seed kernel oil by Kittiphoom and Sutasinee, (2013); Nzikou *et al*., (2010) and Ogunsuyi, (2012) respectively, but lower than 38.5% reported by Agbede *et al*., (2012). The variation in the oil yield as reported by Nziko u*et al*., (2010); Khammuang and Sarnthima, (2011) could be attributed to the extracting solvent polarity, interaction between solvent and solute, their boiling temperature, sample variety as well as climatic conditions.

The major parameters used for characterization of oil quality are acidic, iodine, peroxide and saponification values (Kittiphoom and Sutasinee, 2013). The acid value measures the constituents of different fatty acids that make up the triglyceride molecule of the lipid (Ekpa and Ekpe, 1995). The acid value (15.32 mgKOH/g) obtained in this study is higher than 6.60 mgKOH/g reported by Ogunsuyi, (2012) and lower than 35.90 mgKOH/g for alphonso mango seed kernel oil reported by Momoh *et al*., (2014). The result reported in this study for this variety of mango kernel oil is favourable for its trials in biodiesel production, because oil seeds with acid value less than 3 mgKOH/g before petreatment have been reported to be good for human consumption and food processing industries (Predojevic, 2008).

Peroxide value measures the concentration of peroxides and hydro peroxides produced during the initial stage of lipid oxidation and are responsible for oxidative rancidity in oil. This study report 10.01 mg/g for peroxide value which is comparable to 10.08 mg/g reported for mango seed kernel oil reported by Agbede *et al*., (2012). Studies have shown that fresh oil with less than 10 mg/g of peroxide value could be stored for a longer period without deterioration (Nwobi *et al*., 2006). The iodine value determines the degree of unsaturation of the lipids and the larger the index value, the greater the adsorption on the double bonds present in the oil (Alam *et al*., 2014). The iodine value obtained in this research is higher than 38.50 I_2g/100g but lower than 76.4 I_2g/100g reported by Nzikou *et al*., (2010) and Agbede *et al*., (2012) for different varieties of mango seed oils. The saponification value of an oil indicates the amount of potassium hydroxide required to saponify one gram of oil (Aworanti *et al*., 2019). The value reported in this work is 171.99 mgKOH/g which is lower than 207.5 and 210.38 mgKOH/g reported by Kittiphoom and Sutasinee, (2013) and Ogunsuyi, (2012). Agbede *et al*., (2012) reported that oils with saponification value greater than 300 mgKOH/g are generally suitable for soap production.

Table 1. Physico-chemical properties of mango seed kernel oils (Mean ±S.D.).

Property	Test result
Total lipid yield (%)	22.71±0.17
Acid value (mgKOH/g)	15.32±0.20
Free fatty acid (mg/g)	7.66±0.10
Iodine value (I_2g/100g)	61.31±3.70
Saponification value (mgKOH/g)	171.99±3.10

The result of the acid value of biodiesel production from Mango kernel seeds oil was 0.39 mg/KOH/g (Table 2). According to the data of ASTMD-6751, the maximum allowed acid value in biodiesel is 0.5 mg/KOH/g. The result obtained is in accordance with the standard. The acid value is one of the most important parameters used to check biodiesel quality. High acid value can have a strong solvency effect on rubber seals and hoses in the engine, thereby causing a premature failure (Aworanti*et al*., 2019). It can also cause sediment deposit, which can clog the fuel filter or drop fuel pressure of the diesel engine. The lower the acid value of a biodiesel the higher will be the quality of biodiesel produced (Okta *et al*., 2017; Pinyaphong *et al*., 2011).

The density value (0.879 g/cm^3), kinematic viscosity (4.60 mm^2/sec.) and cetane number (65) are important properties of fuel that determines the fluidity of oils in automobile engines, and are responsible for the ignition delay as well as the ratio of premixed combustion to diffusion combustion in a diesel engine (Giakoumis and Sarakatsanis, 2019, Ishola *et al*., 2020). The values of these parameters obtained in this study are within the ranges of ASTM D6751 acceptable standard for biodiesel. Flash point measure the lowest temperature at which biodiesel burns when ignited or when in contact with ignition source (Kaisan *et al*., 2017). The value of flash point of the biodiesel produced from this sample was 150ºC and the value is above the minimum requirement of biodiesel flash point standard (ASTM D6751). Research has shown that transesterification of oils or fats reduces flash point of methyl ester or biodiesel produced and the cetane number is subsequently improved (Gashaw *et al*., 2015). The pour point of biodiesel is the lowest temperature at which oil will flow under gravity (Alamu *et al*., 2007), the value obtained for this sample is 2.1°C. This value fall within the range of biodiesel pour point standard (ASTM D6751). The values reported for other parameters such as sulphur and ash content were comparable to ASTM D6751 standard.

Table 2. Properties of biodiesel produced from Atoulfo mango compared with standard value.

Parameter	Test method	USA ASTM D6751	Biodiesel
Density (g/cm^3)	D4052	0.875-0.900	0.88
Acid value (mgKOH/g)	D974	0.50, max.	0.39
Kinematic viscosity at 40 °C mm^2/sec.	D445	1.9-6.0	4.60
Flash point, °C	D93	130, min	150
Cetane number	D976	47, min.	65
Pour point (°C)	D97	-15 -10	2.1
Sulphur content, % mass	D4294	0.05 max.	0.011
Ash content, % mass	D482	0.01, Max.	Trace

The ^{1}H-NMR-based procedures is fast and a simple suitable method used to monitor a chemical reaction, and only small amount of the sample is required to obtain a quantitative spectrum with significant information related to the substances of interest in the reaction media(Ghesti*et al*., 2007).

The terminal methyl group signal found in the spectra of both crude and transesterified oil appears as a triplet at 0.89 ppm (Figures 2 & 3). The signal of the methylene chain (-CH_{2n}-) resonated at δ_H1.30 ppm, while other signals at δ_H 2.01, 2.31 and 2.31 ppm are related to allylic protons. Resonances of the glycerol, a main component of the triacylglycerol fraction which served as a backbone to which fatty acid chains are attached appeared between δ_H4.15 – 4.27 and 5.26 ppm (Thoss*et al*., 2011). Other very significant resonance in the spectrum is that found at δ_H5.34 (Scheme 2, Table 3) which is assigned to ethylene -(C_2H_2)$_n$ – and is found within all unsaturated fatty acid (Usman *et al*., 2016).

$$\begin{array}{l} CH_3-(CH_2)a-CH_2-\ (CH=CH-CH_2)b-(CH_2)c-CH_2\ -CO-\ O-CH_2 \\ \qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\quad | \\ CH_3-(CH_2)a-CH_2-\ (CH=CH-CH_2)b-(CH_2)c-CH_2\ -CO-\ O-CH_1 \\ \qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\quad | \\ CH_3-(CH_2)a-CH_2-\ (CH=CH-CH_2)b-(CH_2)c-CH_2\ -CO-\ O-CH_2 \\ 0.89 \qquad\qquad\quad 2.01 \qquad\quad 5.34 \qquad 2.01 \qquad\qquad\qquad\qquad 2.31 \qquad\qquad 4.15\text{-}4.27 \end{array}$$

$$\begin{array}{l} CH_3-(CH_2)a-CH_2-\ (CH=CH-CH_2)b-(CH_2)c-(CH_2)x-CO-\ O-(CH_3)z \\ 0.89 \qquad\qquad\quad 2.01 \qquad\quad 5.34 \qquad 2.01 \qquad\qquad\qquad\qquad 2.31 \qquad\qquad 3.69 \end{array}$$

Scheme 2. The chemical shifts assignments of ^{1}H NMR signals for glycerides (up) and for methyl esters (down) of*M. indica* seeds kernel oil

The ^{1}H nuclear magnetic resonance (NMR) spectroscopy was used to monitor the conversion of glycerides to methyl esters and the yield of the reaction product. The relevant signals used to monitor and quantify this conversion is that described by Gilbard *et al*., (1995) (Figure 3). The signals used for the conversion of the transesterification process is the integration value of methoxy groups in the methyl esters at 3.69 ppm (singlet) designated Z and that of the α-carbonyl methylene groups present in all fatty esters derivatives at 2.31 ppm (triplet)labelled X according to equation 1;

$$\text{Percentage yield} = \frac{3X}{2Z} \times 100 \quad (1)$$

The percentage yield is 94% as checked by the NMR and the glyceryl-related signals found at 4.15-4.27 ppm were not visible as shown in Figure 3.

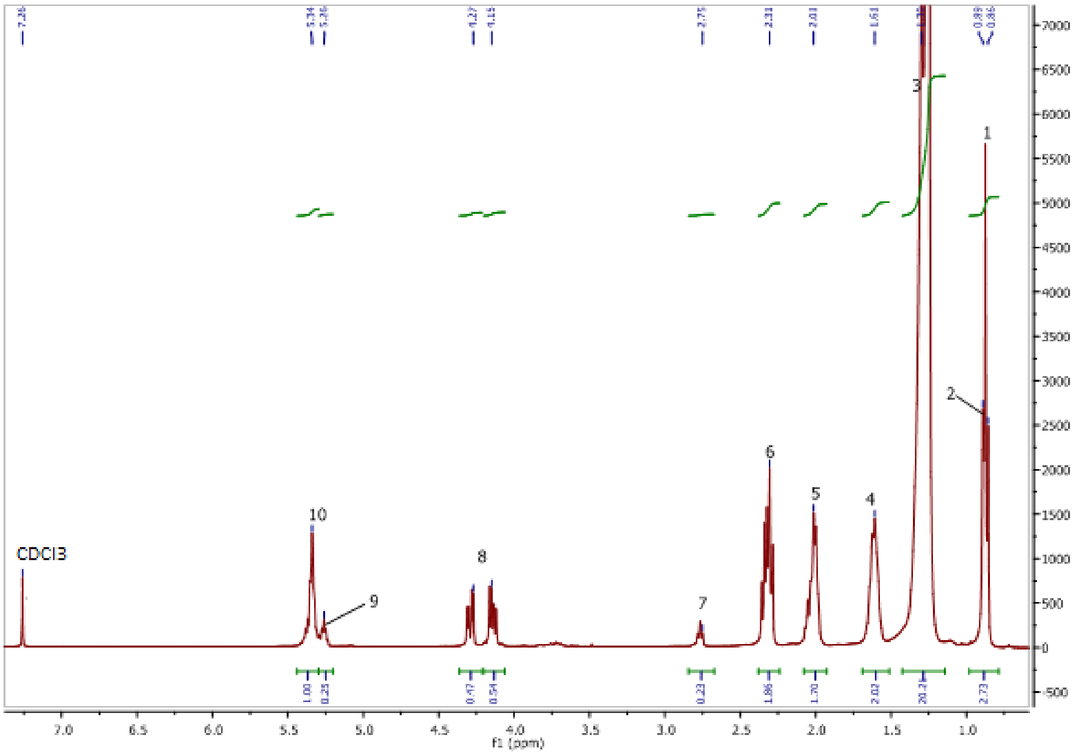

Figure 2: ^{1}H NMR spectrum of seed kernel oil of Atoulfo mango

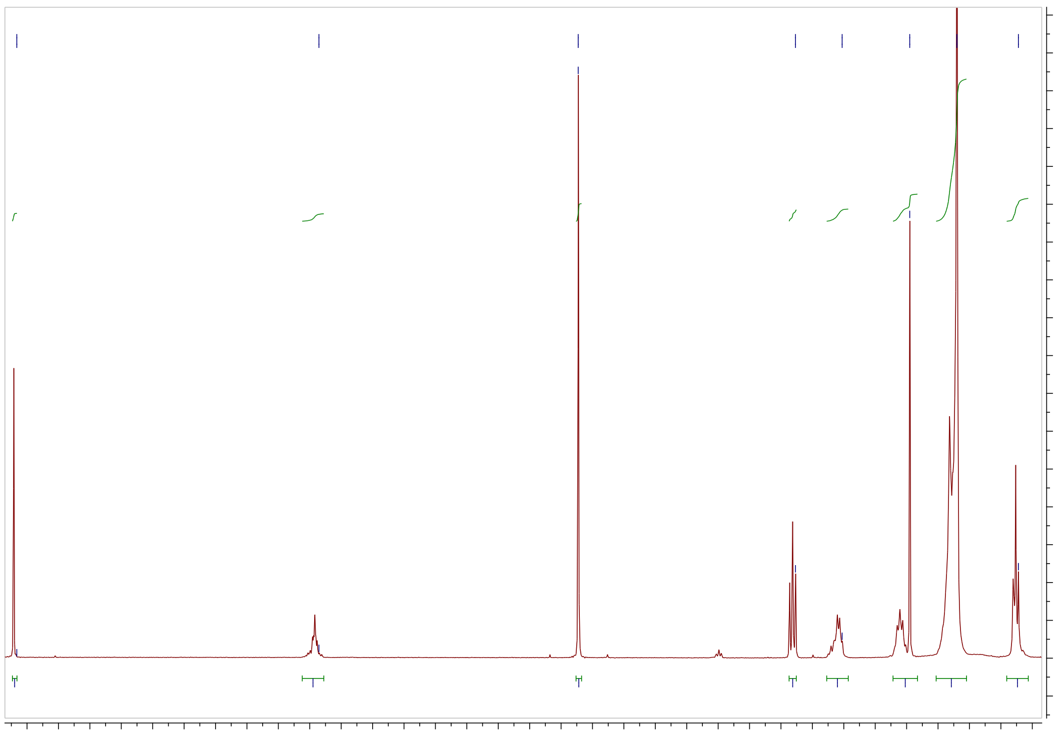

Figure 3: ^{1}H NMR spectrum of trans-esterified seed kernel oil of Atoulfo mango

Table 3. Assignment of the ^{1}H NMR signals of crude *M. indica* seed kernel oil

Signal	Chemical shift (ppm)	Functional group
1	0.86	$-CH_3$ (saturated acyl groups)
2	0.89	$-CH_3$ (Linoleic acyl groups)
3	1.30	- $(CH_2)n$- (Acyl groups)
4	1.61	$-OCO-CH_2-CH_2$ - (Acyl groups)
5	2.01	-CH2-CH=CH- (Acyl groups)
6	2.31	-OCO-CH2- (Acyl groups)
7	2.79	=HC-CH2-CH= (Acyl groups)
8	4.15-4.27	$-CH_2OCOR$ (Glyceryl groups)
9	5.26	=CHOCOR (Glyceryl groups)
10	5.34	-CH=CH- (Acyl groups)

CONCLUSION

Biodiesel was produced by a base-catalyzed transesterification reaction of Atoulfo mango seed kernel oil using methanol as confirmed by ^{1}H NMR analysis. The oil was found to be a suitable feed stock for the production of biodiesel fuel from the conformity of the various tested parameters to ASTM D6751 standard. The production of biodiesel from Atoulfo mango seed kernel oil can help to relieve the environment of the discarded seeds of the fruits which is always an eye sow during the fruiting season, thus reducing the environmental pollution. However, further research is needed on other fuel properties not studied herein, before its suitability for biodiesel production can be ascertain.

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