

# FATTY ACID COMPOSITION OF SEED OIL FROM *PACHIRA AQUATICA* GROWN IN NIGERIA

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## Abstract

The relative composition of the fatty acids in *Pachira aquatica* seed oil were determined using GC-MS and <sup>1</sup>H NMR. The results obtained from GC-MS and <sup>1</sup>H NMR show that the oil contained saturated fatty acids (>67 %), oleic acid (>18 %), linoleic (>3%) and linolenic acid (≤0.01 %). <sup>1</sup>H NMR gave more reliable and reproducible results.

Keywords: *Pachira aquatica*, seed oil, Fatty acids, GC-MS, NMR, Nigeria.

## 1. Introduction

There is presently a global interest in the nutritional composition of underutilized tropical fruits and seeds. *Pachira aquatica*, a member of the Bombacaceae family is one of such underutilized fruits in Nigeria. It is commonly known as Malabar chestnut, Brazil nut, Brown nut, Wild cocoa, French peanut, Guinea peanut, Money tree, Lucky tree and Epa igi (Yoruba). The plant is believed to originate from Brazil but has extended to almost all parts of the tropics and subtropics while in the temperate regions it is a component urban forests [1-6]. It can be found frequently in marshy lands and riparian forests, however, it easily adapts to diverse soils and climatic conditions [7,8].

The plant is adaptable and produces a large quantity of fruits and seeds with desirable organoleptic properties and they are eaten by some Amazonian populations [9], however, it is of

little use in other parts of Brazil and Nigeria where it is only cultivated and grown as an ornamental plant.

The plant has a wide range of uses, being valued especially for its edible seeds, use in traditional medicine [10, 11], as fibre and dyestuff, wood etc. The seeds are delicious and the roast seed flavor is similar to that of peanuts. Roast seeds can also be ground to make a hot drink similar to chocolate. The young leaves and flowers are also edible. The seeds are reported to contain 16% protein, 40-50% fat and 25% carbohydrate. The bark is used to treat stomach problems and headaches, and also taken to “purify blood” [12, 13]. Even though the seeds are rich in oil, they are extracted only in a few communities as cooking oil [9, 12-15]. Edible oils are made up of triacylglycerols (TG) and defining the triglyceride composition of an oil is a very challenging task [16]. The triglyceride composition determines the quality, nature, physicochemical and nutritional properties of the oil. Gas chromatography (GC) is used commonly to determine the fatty acid composition [17-20]. This method involves conversion of the lipids into methyl esters before analysis. This is however a destructive method because it involves hydrolysis of the triacylglycerols and methylation of the free fatty acids before analysis [21]. It is a labor intensive and time consuming. Nuclear magnetic resonance (NMR) on the other hand, allows determination of compounds without derivatization [22,23].  $^1\text{H}$  nuclear magnetic resonance ( $^1\text{H}$ -NMR) offers many advantages over methods in the study edible oils because it allows a rapid, simultaneous, noninvasive, and nondestructive study of oils and also provides information about the acyl distribution of the triglycerides [24-27]. This study describes the use of  $^1\text{H}$ -NMR spectroscopy to quantify the fatty acids in *Pachira aquatica* seed oil.

## **2. Experimental**

### **2.1. Sample collection**

*Pachira aquatica* seeds were collected from Ilorin, Kwara State Nigeria. The pods dried and the seeds removed. Seeds were then dried and ground into powder using an analytical mill (IKA<sup>®</sup> A 10 basic Analytical mill).

### **2.2 Extraction procedure**

The seed powder (100g) was extracted with 500 mL of hexane for seven hours using a Soxhlet extractor. The extract was filtered and the solvent removed using a rotary evaporator at 40°C to yield a golden yellow oil. [28]

### **2.3 GC-MS analysis**

The method of Thoss *et al.*, 2012 [29] was used with modifications. The fatty acid methyl esters (FAME) of the seed oil were determined with a gas chromatograph (6890N, Agilent technologies network) coupled to an Agilent technologies inert XL EI/CI Mass Selective Detector (MSD) (5975B, Agilent technologies Inc., Palo Alto, CA) at the Central Analytical facilities Stellenbosh University, South Africa. The GC-MS system was coupled to a CTC Analytics PAL autosampler. A non-polar ZB-Semivolatiles (30 m, 0.25 mm ID, 0.25 µm film thickness) capillary column was used for the GC. Helium was used as the carrier gas at a flow rate of 1 ml/min. The injector temperature was maintained at 250°C. 1µL of the sample was injected undiluted in 100:1 split ratio. FAME content of the oil sample were identified against a standard Supelco 37 Component FAME Mix.

### **2.5. NMR analysis**

$^1\text{H}$  and  $^{13}\text{C}$ -NMR spectra were recorded on a Bruker AV400 spectrophotometer with  $\text{CDCl}_3$  as solvent and TMS as internal standard. The spectra were processed using MNova software.

### 3. Results and Discussion

The GC spectrum of the seed oil and FAME standard is given in Fig.1 while the percentage composition of the fatty acids from GC analysis is given in Table 1.

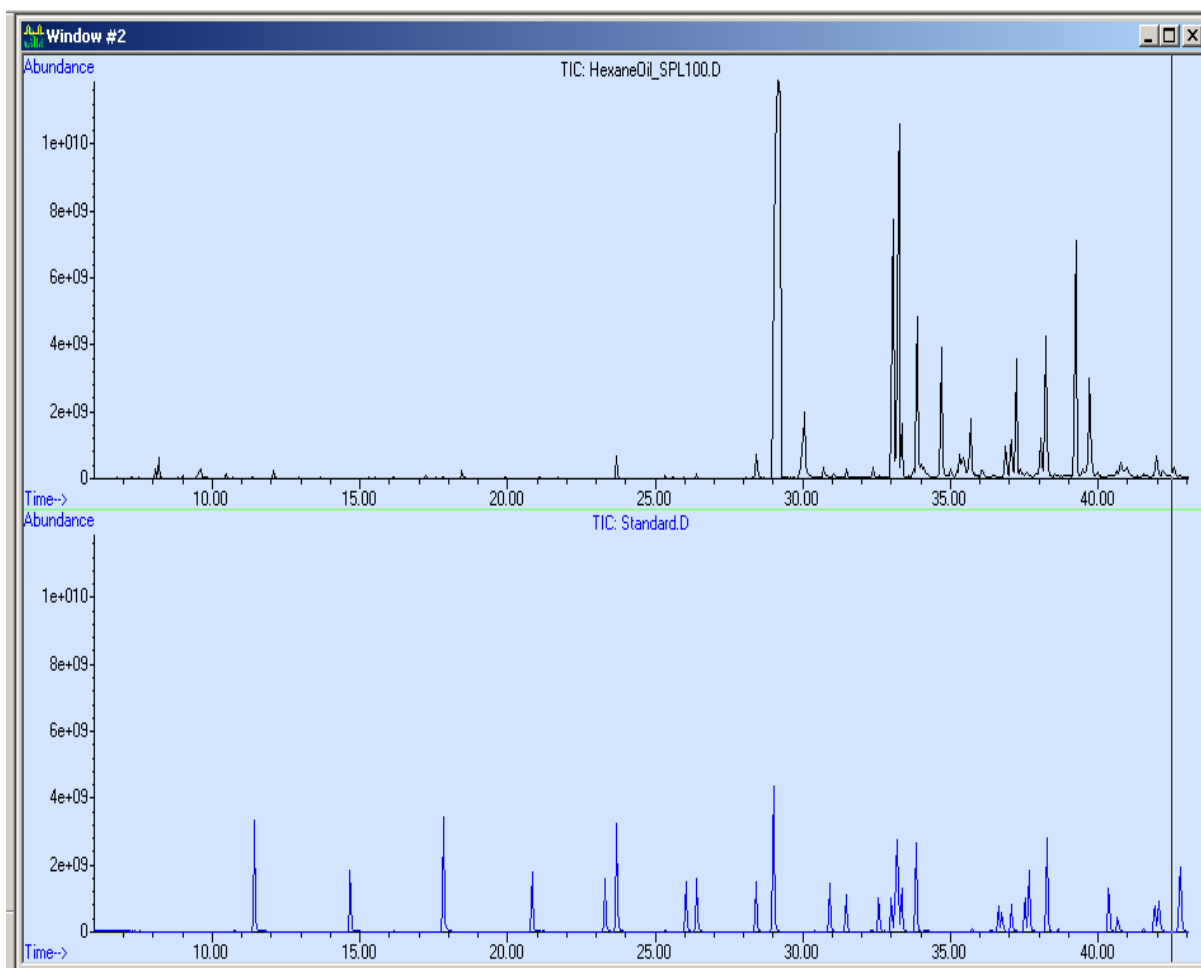


Fig. 1: Fatty acid methyl ester chromatogram against FAME standard

**Table 1: Fatty acid methyl ester composition of *Pachira aquatica* seed oil**

Lipid number	Systematic Name	Trivial name	Relative amount (%)
C – 12:0	Dodecanoic acid	Lauric acid	0.02
C – 14:0	Tetradecanoic acid	Myristic acid	0.79
C – 15:0	Pentadecanoic acid	Pentadecylic acid	0.17
C – 16:0	Hexadecanoic acid	Palmitic acid	49.30
C – 16:1	Cis-9-hexadecenenoic acid	Palmitoleic acid	1.04
C – 17:0	Heptadecanoic acid	Margaric acid	0.39
C – 18:0	Octadecanoic acid	Stearic acid	8.68
C – 18:1	Cis-9-octadecenoic acid	Oleic acid	18.17
C – 18:1	Tr-9-octadecenoic acid	Elaidic acid	2.17
C – 18:2	9,12-Octadecadienoic acid	Linoleic acid	11.19
C – 20:0	Eicosanoic acid	Arachidic acid	8.08
MUFA	Monounsaturated fatty acids		21.38
PUFA	Polyunsaturated fatty acids		11.19
SFA	Saturated fatty acids		67.43

The fatty acid composition of the seed oil indicated that the fatty acid range was from 12:0 to 20:0 (Table 1). Overall, 11 fatty acids were identified, including saturated, monounsaturated and polyunsaturated fatty acids. Saturated acids were lauric (12:0), myristic (14:0), pentadecanoic (15:0), palmitic (16:0), margaric (17:0), stearic (18:0) and arachidic (20:0) acids and they accounted for 67.43 % with palmitic acid being the highest (49.03 %). The monounsaturated acids were palmitoleic (16:1), oleic (18:1) and elaidic (18:1) acids, accounting for 21.4 % with oleic acid as the highest among these (18.17 %). The only polyunsaturated fatty acid identified, linoleic acid (18:2) accounted for 11.19%.

The percentage saturated fatty acids (67.43 %) recorded in this study was higher than the value 57.44 %, reported by Chaves *et al.* [30] for *Bombacopsis glabra* (*Pachira glabra*), but similar to 63.70 % reported by Bohannon and Kleiman, [31] for baobab seed. The palmitic acid content (49.03 %) was lower (53.06 %) to that of *Bombacopsis glabra* (*Pachira glabra*) as reported by Chaves *et al.* [30], but higher than 10.42 % reported by Mukhtar [32] for peanut (SP-96) seed.

For the unsaturated fatty acids, the value 32.57 % obtained in this study was lower than the 51.08 % reported by Jorge and Luzia [33] for *Pachira aquatica* seeds. The monounsaturated fatty acid value (21.38 %) recorded in this study was also, lower (39.27 %) to that reported by Jorge and Luzia [33]. These differences in yield of the fatty acids might be due to locations, agronomic practices and environmental factors.

The  $^1\text{H}$  NMR spectrum is shown in Figure 2. The proton resonances of the major triacylglycerols (TG) present in the oil were assigned according to literature [24-26, 34-38] and are given in Table 2.

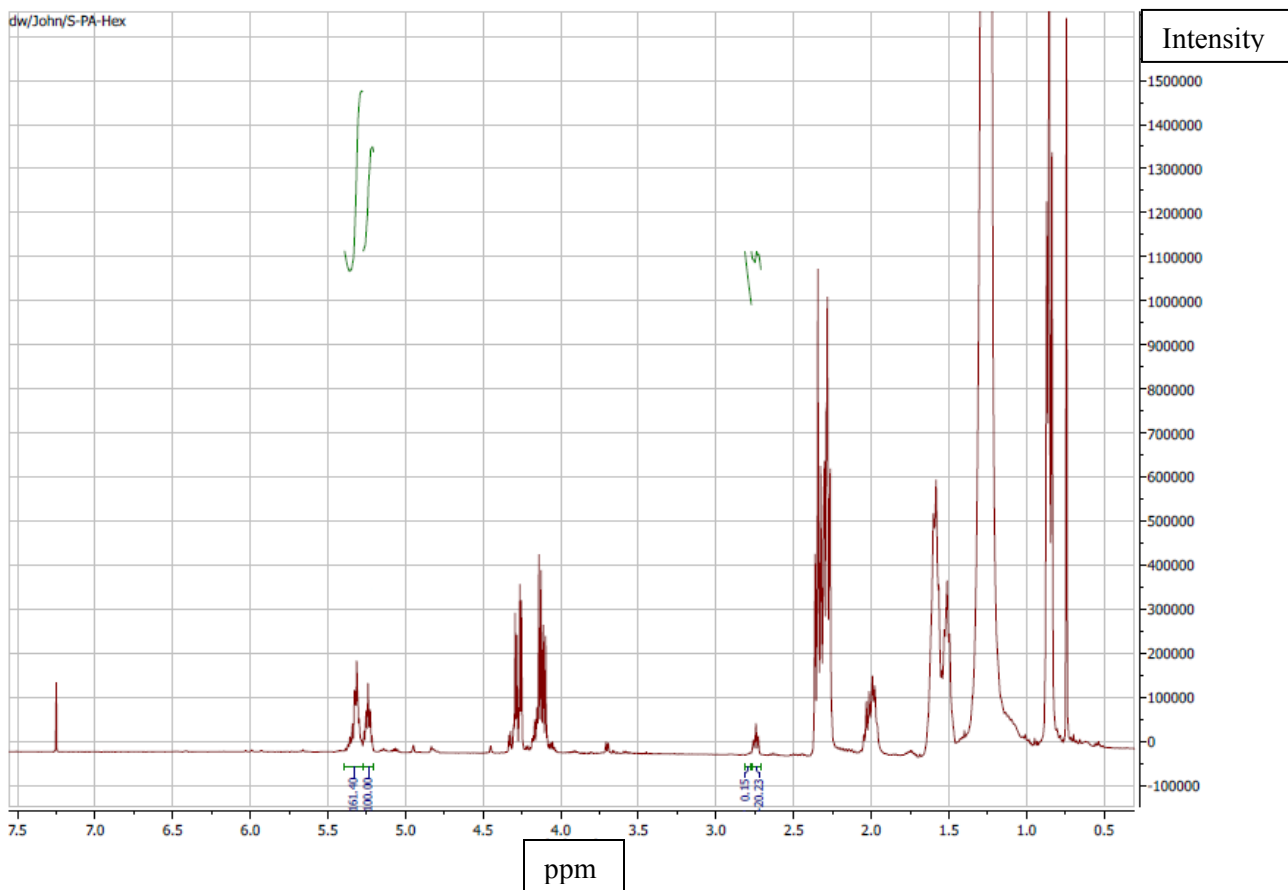


Figure 2:  $^1\text{H}$  NMR spectrum of *Pachira aquatica* seed oil.

Table 2: Assignment of Chemical shifts for the  $^1\text{H}$ -NMR spectrum of *Pachira aquatica* seed oil

Signal	Structural unit	Remark	Multiplicity	Chemical shift (ppm)
1	$-\text{CH}_3$	Terminal methyl chain	T	0.86-0.88
2	$-\text{CH}_2-$	Acyl chain	M	1.21-1.29
3	$-\text{CH}_2-\text{C}-\text{CO}_2$	Acyl chain	M	1.51-1.61
4	$-\text{CH}_2-\text{CO}_2-$	Methylene carboxylic acid	M	1.97-2.06

<b>5</b>	$-\text{C}-\underline{\text{CH}_2}-\text{C}=\text{C}-$	Allylic methylene hydrogen signal	M	2.25-2.38
<b>6</b>	$-\text{C}=\text{C}-\underline{\text{CH}_2}-\text{C}=\text{C}-$	Bisallylic methylene hydrogen signal	T	2.74-2.78
<b>7</b>	$-\text{C}=\text{C}-\underline{\text{CH}_2}-\text{C}=\text{C}-\underline{\text{CH}_2}-\text{C}=\text{C}$	Bisallylic methylene hydrogen signal	T	2.78-2.80
<b>8</b>	$-\text{C}-\underline{\text{CH}_2}-\text{O}-\text{CO}-\text{C}$	Glycerol hydrogen	Dd	4.09-4.14
<b>9</b>	$-\text{C}-\underline{\text{CH}_2}-\text{O}-\text{CO}-\text{C}$	Glycerol hydrogen	Dd	4.24-4.34
<b>10</b>	$\underline{\text{CH}}(-\text{C}-\text{O}-\text{CO}-\text{C}-)_2$	Glycerol hydrogen	M	5.21-5.26
<b>11</b>	$\text{C}-\underline{\text{HC}}=\underline{\text{CH}}-\text{C}$	Olefinic hydrogen signal	M	5.30-5.36

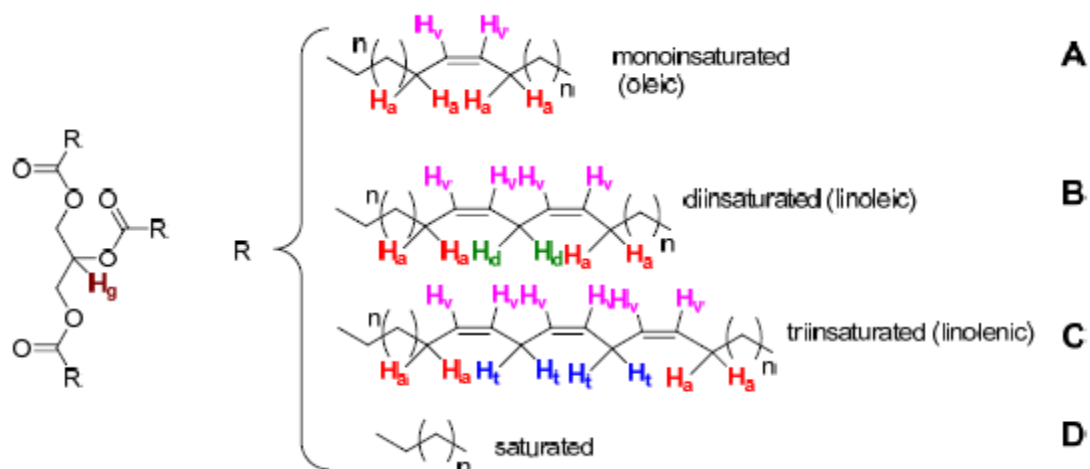
Signal multiplicity: s, singlet; d, doublet; t, triplet; m, multiplet; dt, doublet of triplets; dd, doublet of doublets.

The qualitative analysis as presented in Table 2 showed functional groups that indicate the presence of oleic, linoleic, linolenic and saturated fatty acid in the oil sample. To determine the quantity of these fatty acids in the oil sample, some selected peaks were measured and integrated as shown in Figure 2.

The vinylic protons ( $\text{H}_v$ ) have a characteristic chemical shift, and were used to determine the ratio of saturated to unsaturated fatty acids. The bisallylic protons ( $\text{H}_d$ ,  $\text{H}_t$ ) were used to differentiate the nature of the polyunsaturated fatty acids while the tertiary proton in the glycerol moiety ( $\text{H}_g$ ).

Figure 3: Chemical structure of the main triglycerols in oils.





However, the enhancement of resolution allowed setting a relationship among the integral values of these protons.

$$\text{Vynilic hydrogens integral (H}_v\text{)} = 2A + 4B + 6C$$

$$\text{Linoleic bisallylic integral (H}_d\text{)} = 2B$$

$$\text{Linolenic bisallylic integral (H}_t\text{)} = 4C$$

$$A + B + C + D = 3 \quad [36]$$

Where A = amount of oleic acid present in triglycerol

B = amount of linoleic acid present in triglycerol

C = amount of linolenic acid present in triglycerol

D = amount of saturated acid present in triglycerol

Based on the proton spectrum (Figure 2), the peak of the tertiary proton of the glycerol moiety ( $\delta$  5.26 - 5.21 ppm) was integrated as 100, leading to an integral value for vinyl proton ( $\delta$  5.36 - 5.30 ppm) of 161.40 and for the bisallylic proton ( $\delta$  2.78 - 2.74 ppm) of 20.23 for linoleic and for the bisallylic proton ( $\delta$  2.80 - 2.78 ppm) of 0.15 for linolenic acids.

A, B, C and D as the proportion of each kind of fatty acid involved in the triglyceride the oil (oleic, linoleic, linolenic and saturated respectively), the following equations were [vinyllic  $H_v = 161.40 = 2A + 4B + 6C$ ]; [bisallylic  $H_t = 4C = 0.15$ ] and [bisallylic  $H_d = 2B = 20.23$ ]. Therefore, the values for the acids in the seed oil are: A 20.12 %, B 3.37 %, C 0.01 %, and D 76.50 %, for oleic, linoleic, linolenic and saturated fatty acids respectively.

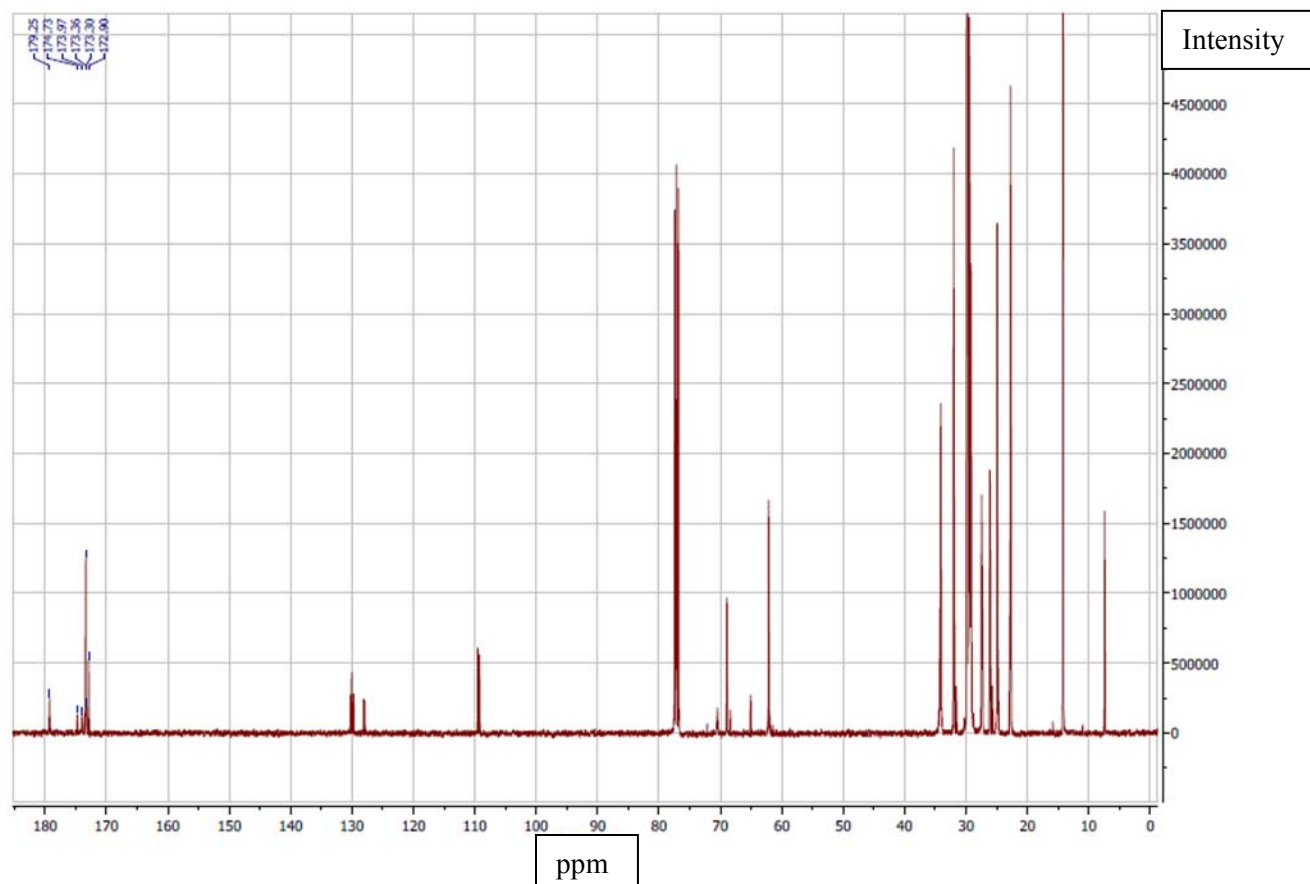


Figure 4:  $^{13}\text{C}$ -NMR spectrum of *Pachira aquatica* seed oil.

The  $^{13}\text{C}$ -NMR chemical shifts were assigned according to literature data [36, 38] and Figure 4 shows the spectrum obtained. As observed in the spectrum of other vegetable oils, the  $^{13}\text{C}$  NMR spectrum of *Pachira aquatica* seed oil showed signals in the carbonyl (172 – 174 ppm), olefinic (124 – 134 ppm), glycerol (60 -72 ppm), methylene and methyl (10 – 35 ppm) regions. The

signals at 173.3 and 172.9 ppm with chemical shift difference of 0.4 ppm are those for saturated 1,3 TG and 2- positions of oleyl, linoleyl esters. The signals at the olefinic carbon regions confirm the presence of unsaturation in the oil. Linoleyl and linolenyl chains can easily be detected only in this region. The signals at 130.1 ppm and 129.9 ppm indicate the presence of oleic esters, together with 130.0 ppm, 128.2 ppm and 128.0 ppm from linoleic esters, but no peaks corresponding to linolenic acid were detected. These results confirm the well-known limitation of  $^{13}\text{C}$  for the analysis of TG due to its low gyromagnetic ratio and its very low natural abundance. The glycerol carbons of mono-, di-, and triglycerols resonate in the spectral region 60 – 72 ppm. The signals at 68.9 and 62.2 ppm indicated those of triacylglycerol in the seed oil. Three signals at 34.0 ppm assigned to Carbon -2 atom of 1,3- diacylglycerol in the oleyl, linoleyl and linolenyl and all acyl chains. The C-16 carbon appear at chemical shifts 31.9 and 31.7 ppm with saturated, oleyl, linoleyl chains resonating in this region while C-15 – C-8 resonates between 29.8 ppm – 25.2 ppm, within each set of signals, saturated, oleyl, linoleyl and linolenyl resonating from higher to lower frequencies. Also, G2 and G1 carbon regions were found at 22.9 ppm – 14.1 ppm. G2 of all acyl chains resonate at 22.9 ppm and G1 at 14.1 ppm for all acyl chains. These signals were assigned according to literature [36, 38]. The qualitative analysis of *Pachira aquatica* seed oil using  $^{13}\text{C}$  NMR spectroscopy showed the presence of saturated and unsaturated fatty acid in the seed oil. Table 3 shows a comparison of the fatty acid composition of *Pachira aquatica* seed oil using GC-MS and NMR.

Table 3: Comparative fatty acid composition of *Pachira aquatica* seed oil by GC-MS and  $^1\text{H}$  NMR

Fatty acid	GC-MS (%)	$^1\text{H}$ NMR (%)
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<b>Oleic</b>	18.17	20.12
<b>Linoleic</b>	11.19	3.37
<b>Linolenic</b>	0.0	0.01
<b>Saturated</b>	67.43	76.50

The results obtained from  $^1\text{H}$ -NMR of crude and GC-MS analysis of the FAMES agree with each other.

#### 4. Conclusion

The results from this study indicate that the seed oil from *Pachira aquatica* grown in Nigeria is highly saturated (>67.43 %) and it contains more than 18 % of oleic acid methyl esters (C18:1) and little or no amount of linolenic acid methyl ester ( $\leq 0.01$  %). However *Pachira aquatica* seed oil should be stable to oxidation on account of its high saturation content and therefore should be suitable for deep frying purposes, food purposes and industrial purposes.

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