Trop J Nat Prod Res, August 2022; 6(8):1223-1227

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Spectroscopic Evaluation of Unsaturation in Some Medicinal Plant Seed Oils

Eman Santali[1], Nahandoo Ichoron[2], John V. Anyam[2], John O. Igoli[3]*

[1]*Department of Pharmaceutical Chemistry, College of Pharmacy, Taif University, Saudi Arabia*
[2]*Department of Chemistry, Joseph Sarwuan Tarka University, Makurdi Nigeria*
[3]*Department of Chemical Sciences, Pen Resource University, Gombe, Nigeria*

ARTICLE INFO

Article history:
Received 06 June 2022
Revised 16 August 2022
Accepted 27 August 2022
Published online 02 September 2022

ABSTRACT

Plant oils form a major component of our nutrition and also have various applications in medicine and industry. In Benue state, many plant seed oils are used for food and as components of traditional medicine. However, the levels of unsaturation of many of these oils has not been determined. A study of twenty (20) medicinal plants (*Abrus precatorius, Albizia lebbeck, Anacardium occidentale, Annona muricata, Canarium schweinfurthii, Chrysophylium albidum, Citrullus lanatus, Citrullus colocynthis, Citrus senensis, Cucumeropsis mannii, Cyperus esculentus, Dacryodes edulis, Delonia regia, Elaeis guineensis, Irvingia gabonensis, Moringa oleifera, Parkia biglobosa, Phoenix dectylifera, Sphenostylis stenocarpa and Terminalia catappa*.) seed oils was conducted to determine the levels of unsaturation and percentage composition of saturated fatty acids, mono unsaturated fatty acids and poly unsaturated fatty acids in the oils. The medicinal plant seeds were extracted in organic solvent (hexane) at room temperature by maceration. 1D and 2D Nuclear Magnetic Resonance (NMR) spectroscopy was conducted on the extracted oils. The NMR spectra obtained were processed and interpreted. The results were used to classify the oils based on their saturated, monounsaturated and polyunsaturated fatty acids content. Most of the oils were unsaturated and triglycerides while oils from *Irvingia gabonensis, Phoenix dactylifera* and *Elaeis guineensis* contained mainly saturated fatty acids.

Keywords: Seed oils, Fatty acids, Triglycerides, Medicinal plants, Maceration, Spectroscopy.

Introduction

The hexane extracts of medicinal plants are known to yield either in the crude or upon fractionation, fats and oils hence extraction with hexane and other non-polar solvents is commonly described as defatting. These oils or oil fractions are usually non-volatile or fixed oils which are often difficult to purify to single components. However, they are also found in certain amounts in polar solvent extracts especially whole extracts. They also form part of traditional or medicinal alcoholic or aqueous extracts. Chromatographic separation and purification of plant extracts usually yield these oil constituents as mixtures of triglycerides and fatty acids or as impurities in other fractions and compounds. Several of these medicinal plant oils have medicinal properties such as chaulmoogra oil active against *Mycobacterium leprae* and effective in treating early cases of leprosy,[1] castor seed oil and its industrial applications [2] and olive oil with its medicinal uses.[3,9] There is presently a demand for oils as renewable bioresources for industrial and other purposes apart from food use. This has necessitated the reassessment of plant species for the provision of rare or novel fatty acids. Some common fatty acids identified in plant seed oils include stearic acid **1** which is saturated, oleic acid **2**, monounsaturated, linoleic acid **3** and linolenic acid **4** which are polyunsaturated (Figure 1).[4] Compounds or products and oils with different properties could be useful especially as biodiesel, nutritional supplements or production of cosmetics, paints and other industrial materials.

*Corresponding author. E mail: j.o.igoli@uam.edu.ng
Tel: +2348130991308

Citation: Santali E, Ichoron N, Anyam JV, Igoli JO. Spectroscopic Evaluation of Unsaturation in Some Medicinal Plant Seed Oils. Trop J Nat Prod Res, 2022; 6(8):1223-1227. doi.org/10.26538/tjnpr/v6i8.11

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

To this effect, seed crops are being assessed and emphasis is placed on species that do not compete with food crops and with high yield of fatty acids with unusual fatty acids.[5] It is therefore pertinent to analyse the oils from seeds, stem or bark of these common medicinal plants to know their fatty acid composition as much as possible. One established method for the determination of fatty acid composition of oils is the acid value. The level of unsaturation is usually determined by the iodine value. However, being titrimetric methods, these techniques depend on colour changes at an endpoint, hence it may be difficult to obtain accurate results for certain or strongly coloured samples. The techniques are relatively slow, requires large amount of samples and use reagents that could be toxic. NMR spectroscopy is now emerging as one of the analytical alternatives to the classical techniques for the analysis of lipids, especially for the determination of relative composition of saturated, monounsaturated and polyunsaturated fatty acids.[6] NMR spectroscopy has the advantage of being non-destructive and non-invasive. It is quick, straightforward and produces information on the composition of a mixture even in a single spectrum, without the need for derivatization or pre-treatment of the sample. It uses very small amounts of sample and organic solvents or reagents. And because the integral of a signal is proportional to the relative number of the corresponding nuclei, it is possible to directly determine molar concentrations from an NMR spectrum.[7,8] In this study, ^{1}H NMR was used to investigate and classify twenty (20) seed oils on the basis of their saturated, monounsaturated and polyunsaturated fatty acid content.

Materials and Methods

Sample collection and preparation

The seeds of the following plants were collected between the month of January and May 2021 from around Makurdi, Benue State, Nigeria: *Colocasia esculenta, Annona muricata, Albizia lebbeck, Phoenix dactylifera, Delonix regia, Abrus precatorius, Terminalia catappa, Chrysophyllum albidum, Sphenostylis stenocarpa, Cyperus esculentus,*

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Moringa oleifera, Citrus senensis, Canarium schwenfurthii, Dacryodes edulis, Elaeis guineensis, Cucumeropsis mannii, Citrullus lanatus, Parkia biglobosa, Anacardium occidentale and Irvingia gabonensis. The seeds and their source plants were identified by Dr. David O. Ekhuemelo of the Department of Forest Products and Production, Joseph Sarwuan Tarka University, Makurdi.

Extraction

The samples were prepared and extracted according to earlier described procedures[5,6] with little modifications. Each seed sample was weighed after the husk was removed. The weighed samples were dried under shade at ambient temperature. Samples were weighed every 24 h until a consistent mass was observed for three consecutive times. The dried samples were pulverized with a porcelain mortar and pestle and stored in paper bags. Each of the pulverized samples (200 g) was macerated separately at room temperature in hexane (500 cm^3) by soaking in the solvent contained in glass jars and stirring intermittently for 24 h, after which it was decanted to obtain a hexane extract. The solvent was removed on a rotary evaporator (at 40 ℃) to obtain the extracted oil. The process was repeated twice on the extracted material. The extracted oils were transferred into pre-weighed sample bottles and stored for further experiments. Percentage (%) yield of the oil was calculated as $\frac{mass\ of\ extracted\ oil}{mass\ of\ sample}$ x 100 and presented is presented in Table 1.

NMR analysis

^{1}H and 2D NMR analyses were performed on a Bruker DRX 400 spectrophotometer (400 MHz for proton and 125 MHz for 13C) in DMSO.

Results and Discussion

NMR analysis

The ^{1}H NMR spectrum of the oil samples showed at least nine characteristic signals due to protons of triglycerides and olefinic and aliphatic protons on the fatty acid chains. The samples showed two oxymethylene signals at 4.12 (2H, dd, *J* = 11.9, 6.0 Hz, H-1) and 4.28 (2H, dd, *J* = 11.9, 4.3 Hz, H-3) ppm (signal c-1 and c-2, Figure 1) while the glycerol oxymethine (signal b, Figure 1) was observed at 5.25 (1H, ddd, *J* = 10.2, 6.1, 4.4 Hz, H-2). The signals for the olefinic protons on the fatty acid chains appeared at 5.32 ppm (signal a, Figure 1). Triglycerides containing fatty acids with two or more double bonds showed signals at 2.75 (2H, t, *J* = 6.3 Hz, H-11') ppm (signal d, Figure 1) due to bisallylic protons while the allylic protons were observed at 1.99 (2H, q, *J* = 6.6 Hz, H-4', H-14')[4,5,8]. The signals for protons attached to the three α-acyl carbon atoms of the triglycerides appeared at 2.29 (2H, td, *J* = 7.5, 2.1 Hz, H-2') while the β-acyl protons appeared at 1.59 (2H, tq, *J* = 10.5, 7.0, 5.3 Hz, H-3') ppm. The ^{1}H NMR spectra of each oil sample showed a signal between 1.99 – 2.03 (signal f, Figure 1). This signal is due to the methylene protons attached to the allylic carbons of triglycerides. The allylic methylene protons are present in both mono- and polyunsaturated fatty acids. The presence of this signal in the NMR spectra of the samples imply that each of them contain some amount of unsaturated fatty acids. Signals for the terminal methyl protons of the fatty acid chains appeared at 0.86 ppm. (signal i, Figure 1). This signal is characteristic of all fatty acid chains, whether saturated or unsaturated.[7] Signals for the methylene protons of the fatty acid chains appear at 1.27 ppm.[7] This signal in the triglycerides appeared at the same position (signal j, Figure 1). Using the ^{13}C and 2D NMR spectra for a typical triglyceride **5** (Figure 2) from *Cyperus esculentus* (Supplementary data 1) and a typical non triglyceride from *Citrullus lanatus* (Supplementary data 2), the structural features of the oils were confirmed as follows: For the triglyceride, the ^{13}C spectrum showed a total of 15 carbon signals with a bunch between δ_C 28.5 and 30.0 ppm. The other signals were for three carbonyls at 173.1 (2 x C=O, C-1 and C-3) and 172.7 (C=O, C-2) while the glycerol carbons appeared at 68.9 ppm (C-2) and 62.0 (C-1 and C-3). Where there is unsaturation in the fatty acid chain, the olefinic carbons appeared at 129.9 and 129.6 ppm. The methylene of the allylic carbon was observed at 27.2 ppm, the bisallylic at 33.6 ppm. The α-acyl methylene (C-2') appeared at 34.0 ppm while the β-acyl methylene (C-3') was at 24.9 ppm. The terminal methyl of the fatty acid chain was observed at 14.0 ppm while the rest of the signals were for the methylene chains on either side of the double bonds.

Table 1: Percentage Yield of Oil

S/N	Plant Name	Mass of sample (g)	Mass of extracted oil (g)	Percentage yield
1	*Abrus precatorius*	150.0	0.2	0.1
2	*Albizia lebbeck*	251.5	1.5	0.6
3	*Anacardium occidentale*	35.3	3.2	9.1
4	*Annona muricata*	200	33.4	16.7
5	*Canarium schweinfurthii*	33.9	3.6	10.6
6	*Chrysophylium albidum*	68.6	2.4	3.5
7	*Citrullus lanatus*	85.1	6.4	7.5
8	*Citrus senensis*	100.7	20.7	20.6
9	*Cucumeropsis mannii*	125.4	11.7	9.33
10	*Cyperus esculentus*	130.1	4.3	3.3
11	*Dacryodes edulis*	51.2	5.7	11.1
12	*Delonia regia*	200.8	1	0.5
13	*Elaeis guineensis*	85.1	18.6	21.9
14	*Irvingia gabonensis*	33.9	7.5	22.1
15	*Moringa oleifera*	131	17.2	13.1
16	*Parkia biglobosa*	67.4	2.3	3.4
17	*Phoenix dectylifera*	200.4	1.3	0.6
18	*Sphenostylis stenocarpa*	211.4	1.1	0.5
19	*Terminalia catapa*	58.6	11.1	18.9
20	*Citrullus colocynthis*	33.9	1.6	4.7

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

The COSY spectrum showed correlations between the glycerol protons (H-1, H-2 and H-3) and between the bisallylic and allylic to the olefinic protons. The HMBC spectrum showed correlations from the glycerol protons (H1-3) to the carbonyl carbons (C1'-C3') respectively. The allylic and bisallylic protons showed correlations to the olefinic carbon atoms while the glycerol H-1 and H-3 had correlations to C-2 and H-2 to C-1 and C-3. Thus, the chemical shift assignments for the protons and carbon atoms in the triglycerides were made based on the 2D correlations and are given in Table 2.

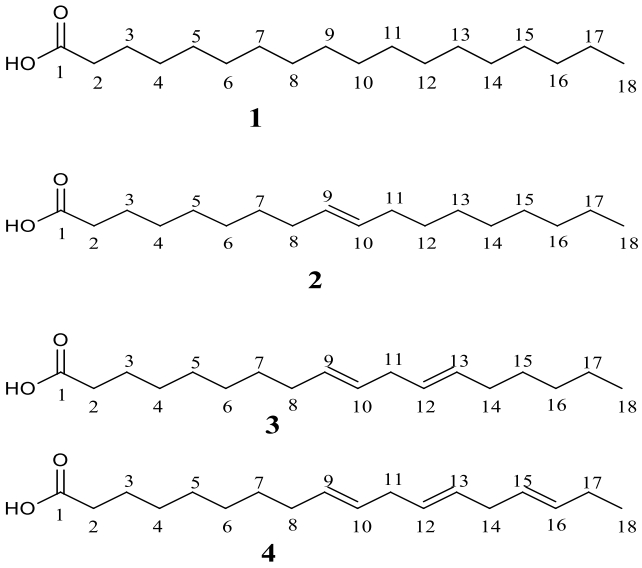
The proton spectra for the non-triglycerides were similar except for the absence of the glycerol protons. A typical non triglyceride oil sample obtained from *Citrullus lunatus* was used to characterize these oils based on its 2D NMR spectra. The proton spectrum was similar to those of the triglycerides with all the chemical shifts identical except for the absence of the signals for the glycerol moiety at δ_H 4.12 and 5.25 ppm. Its ^{13}C spectrum showed only one carbonyl signal for the carboxylic acid carbon at 180.4 ppm indicating an unesterified carbonyl compared to the carbonyl in the triglycerides which appeared at δ_C 172.7 and 173.1 ppm. The bisallylic carbons appeared at 25.6 and the allylic carbons at 27.5 ppm. The 2D correlations were identical to those of the fatty acid chains in the triglycerides and they confirmed the fatty acids to contain mixtures of saturated and unsaturated fatty acid chains.

Determination of relative unsaturation

In order to calculate the relative percentage composition of polyunsaturated (PUFA), monounsaturated (MUFA) and saturated (SFA), the integration values of the bisallylic (**d**), the α-acyl (**e**) and the allylic protons (**f**) as depicted in Figure 3 were used according to a method by[4,5]. The relative amount of PUFA was calculated using the formula PUFA $= \frac{d}{e}$, MUFA $= \frac{f}{2d} -$ PUFA. The sum of MUFA and PUFA gives the relative total unsaturation of the oils and the relative amount of SFA was calculated by $1 - \frac{f}{2d}$. Using these formulas, the estimated percentage amounts of PUFA, MUFA and SFA for each of the oils are given in Table 3

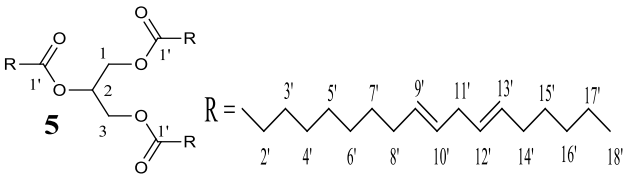
Comparison between oils from ripe and unripe kernel of Elaeis guineensis

^1H NMR spectra of oils from ripe and unripe palm kernel were the same (Figure 4). This indicates that ripening does not affect the nature of oils from palm kernel.



1 **2** **3** **4**

Figure 1: Common fatty acids from plant seed oils



5

R = acyl fatty acid.

Figure 2: Major triglyceride characterized by NMR

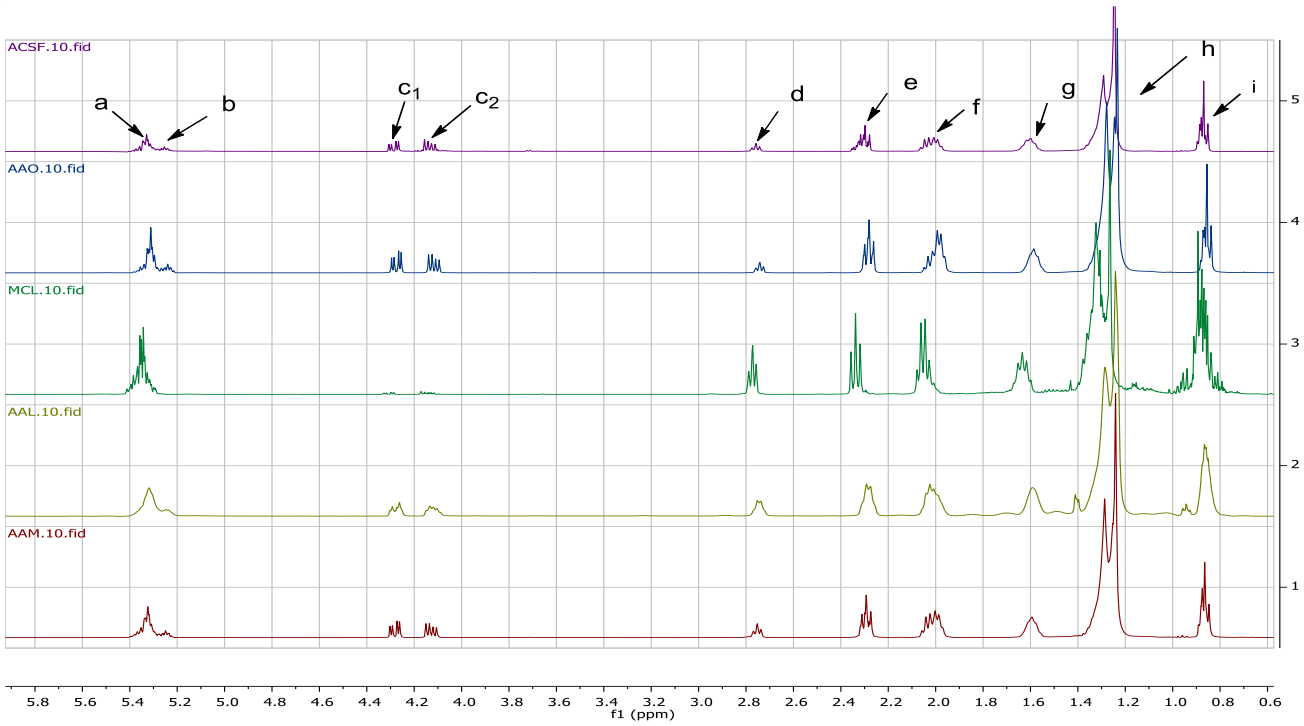


Figure 3: Proton NMR spectra of *Canarium schweinfurthii (5), Anacardium occidentale (4), Citrullus lanatus (3), Albizia lebbeck (2) and Annona muricata (1)* seed oils with characteristic signals

Notes: a = olefinic protons, b = glycerol oxymethine protons, c_1 and c_2 = glycerol oxymethylene protons, H-1 and H-3, d = bisallylic protons, f = α-acyl protons, e = allylic protons, g = β – acyl protons, h = methylene chain, i = terminal methyl protons on the fatty acid chains

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Table 2: ^{1}H and ^{13}C chemical shifts for triglyceride and non-triglyceride

Triglyceride			Non-triglyceride		
Position	**^{1}H (δ ppm) (mult, *J* in Hz)**	**^{13}C (δ ppm)**	**Position**	**^{1}H (δ ppm)**	**^{13}C (δ ppm)**
1	4.28 (dd, 11.9, 6.0)	62.1 (CH_2)	1		180.6 (C)
2	5.24 (ddd, 10.2, 6.1, 4.4)	68.9 (CH)	2	2.35	34.3 (CH_2)
3	4.12 (dd, 11.9, 4.3)	62.1 (CH_2)	3	1.64	24.8 (CH_2)
1′	-	173.1 (C)	4		29.3 (CH_2)
2′	2.29 (td, 7.51, 2.1)	34.0 (CH_2)	5		34.3 (CH_2)
3′	1.59 (tq, 10.5, 7.0, 5.3)	24.9 (CH_2)	6		29.5 (CH_2)
4′	1.99 (q, 6.6)	27.2 (CH_2)	7		31.7 (CH_2)
			8		25.8 (CH_2)
9′	5.32 (td, 7.27, 6.16, 4.22)	129.6 (CH)	9	5.36	130.1 (CH)
10′	5.32 (td, 7.27, 6.16, 4.22)	129.8 (CH)	10	5.34	128.2 (CH)
11′	2.75 (t, 6.3)	33.7 (CH_2)	11		
12′	5.32 (td, 7.27, 6.16, 4.22)	129.6 (CH)	12	5.34	128.0 (CH)
13′	5.32 (td, 7.27, 6.16, 4.22)	129.8 (CH)	13	5.36	130.3 (CH)
14′	1.99 (q, 6.6)	27.2 (CH_2)	14		27.3 (CH_2)
15′	1.26	29.4 (CH_2)	15		
16′	1.23	32.0 (CH_2)	16		31.7 (CH_2)
17′	1.25	22.6 (CH_2)	17		22.8 (CH_2)
18′	0.86	14.2 (CH_3)	18	0.89	14.1 (CH_3)

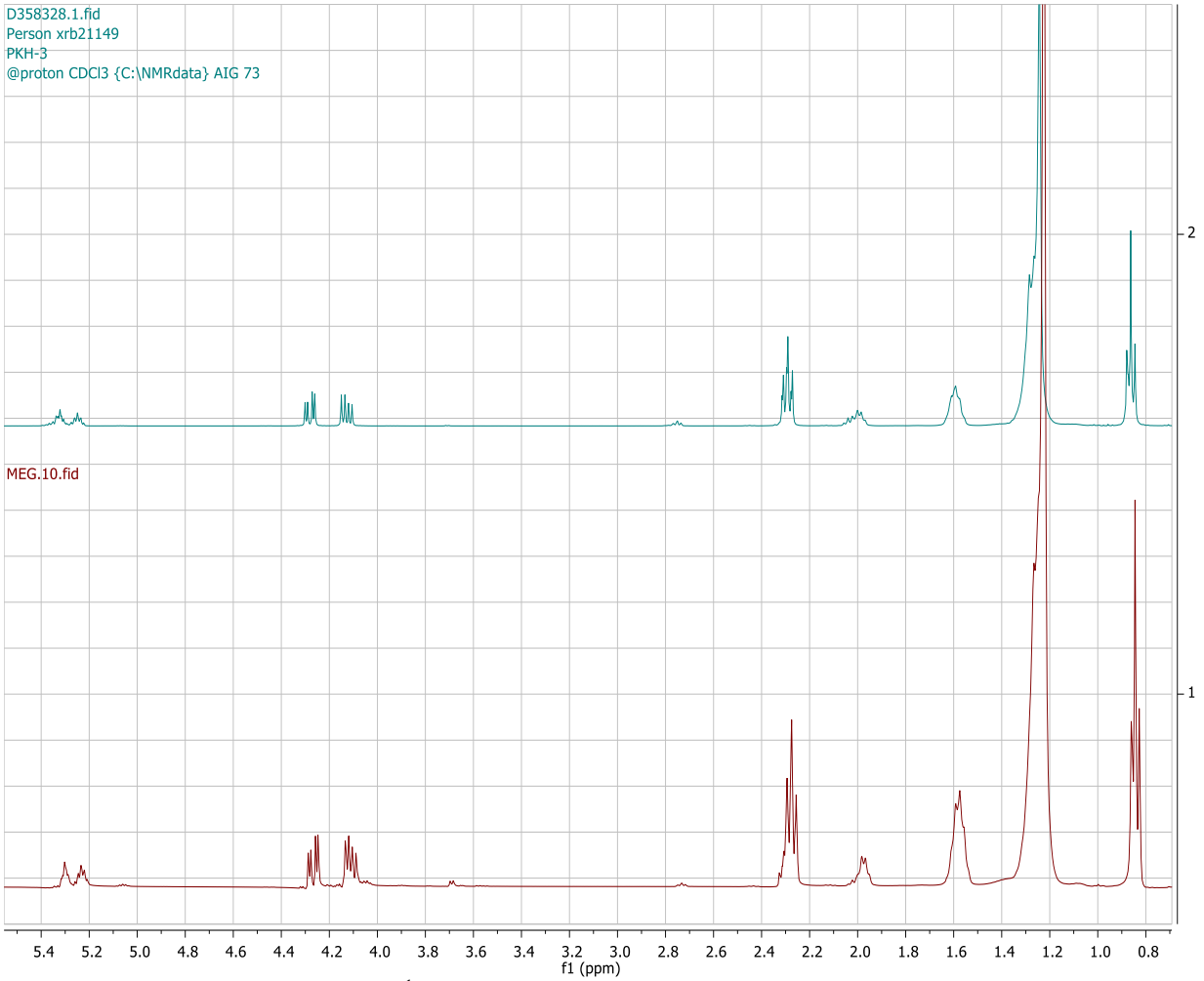


Figure 4: Stacked ^{1}H NMR spectra of oils from ripe (2) and unripe (1) palm kernel

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Table 2: ^{1}H and ^{13}C chemical shifts for triglyceride and non-triglyceride

Triglyceride			Non-triglyceride		
Position	**^{1}H (δ ppm) (mult, *J* in Hz)**	**^{13}C (δ ppm)**	**Position**	**^{1}H (δ ppm)**	**^{13}C (δ ppm)**
1	4.28 (dd, 11.9, 6.0)	62.1 (CH_2)	1		180.6 (C)
2	5.24 (ddd, 10.2, 6.1, 4.4)	68.9 (CH)	2	2.35	34.3 (CH_2)
3	4.12 (dd, 11.9, 4.3)	62.1 (CH_2)	3	1.64	24.8 (CH_2)
1′	-	173.1 (C)	4		29.3 (CH_2)
2′	2.29 (td, 7.51, 2.1)	34.0 (CH_2)	5		34.3 (CH_2)
3′	1.59 (tq, 10.5, 7.0, 5.3)	24.9 (CH_2)	6		29.5 (CH_2)
4′	1.99 (q, 6.6)	27.2 (CH_2)	7		31.7 (CH_2)
			8		25.8 (CH_2)
9′	5.32 (td, 7.27, 6.16, 4.22)	129.6 (CH)	9	5.36	130.1 (CH)
10′	5.32 (td, 7.27, 6.16, 4.22)	129.8 (CH)	10	5.34	128.2 (CH)
11′	2.75 (t, 6.3)	33.7 (CH_2)	11		
12′	5.32 (td, 7.27, 6.16, 4.22)	129.6 (CH)	12	5.34	128.0 (CH)
13′	5.32 (td, 7.27, 6.16, 4.22)	129.8 (CH)	13	5.36	130.3 (CH)
14′	1.99 (q, 6.6)	27.2 (CH_2)	14		27.3 (CH_2)
15′	1.26	29.4 (CH_2)	15		
16′	1.23	32.0 (CH_2)	16		31.7 (CH_2)
17′	1.25	22.6 (CH_2)	17		22.8 (CH_2)
18′	0.86	14.2 (CH_3)	18	0.89	14.1 (CH_3)

Conclusion

A study of twenty (20) seed oils using proton and 2D NMR found that the oil from *Irvingia gabonensis* contained mainly saturated fatty acids (100 %). Similarly, oil from *Elaeis guineensis* and *Phoenix dactylifera* contain high amounts (82.0 % and 53.0 %) of saturated fatty acids and so may be good for use as cooking oils. The rest of the seed oils studied contained majorly unsaturated fatty acids and can thus be used as drying oils. There is no difference between the oil from ripe and unripe palm kernel, hence they can be used for the same purposes as ripening does not alter the oil composition.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

References

1. Rameshkumar KB, Hisham A. Latha PG. Chemistry and Therapeutic Potential of Chaulmoogra Oil. In J.N. Govil (ed): Recent Progress in Medicinal Plants. Fixed Oils and Fats of Pharmaceutical Importance. Studium Press, LLC, USA. 2011. Vol. 33.
2. Patel V R, Dumancas, G G, Viswanath, L C, Maples R, Subong B J. Castor Oil: Properties, Uses, and Optimization of Processing Parameters in Commercial Production. Lip Insights 2016; 9: 1–12.
3. Selaimiaa R, Beghiela A, Oumeddourb R. The synthesis of biodiesel from vegetable oil; Procedia - Soc Behav Sci. 2015; 195: 1633 – 1638.
4. Thoss V, Murphy PJ, Marriott R, Wilson T. Triacylglycerol composition of British bluebell (*Hyacinthoides non-scripta*) seed oil. RSC Adv. 2012; 2:5314–5322.
5. Ibekwe NN and Igoli JO. Fatty Acid Composition of Triglycerides in Seed Oils from Three Nigerian Plants by ^{1}H-NMR Spectroscopy. J Phy Ther. 2018; 17(1): 127 – 135
6. Alexandri E, Ahmed R, Siddiqui H, Choudhary MI, Tsiafoulis CG, Gerothanassis IP. High Resolution NMR Spectroscopy as a Structural and Analytical Tool for Unsaturated Lipids in Solution. Mol. 2017; 22: 1663.
7. Guillen MD and Ruiz A. Edible oils: discrimination by ^{1}H nuclear magnetic resonance. J Sci Food Agric. 2003; 83:338–346.
8. Pietro ME, Mannu A, Mele A. NMR Determination of Free Fatty Acids in Vegetable Oils. Processes. 2020; 8(410): 1-15.
9. Waterman E and Lockwood B. Active components and clinical applications of olive oil. Altern Med Rev. 2007; 12(4):331-342.