



Determination of Triacylglycerol Composition of *Trichilia emetica* Seed Oil using GC-MS and $^1\text{H-NMR}$ Spectroscopy

A. Usman¹, N.L. Usman², A.U. Itodo³, S.S. Audu⁴, M. Haruna²

¹Basic Science Unit, Faculty of Agriculture, Nasarawa State University Keffi. PMB 135 Lafia.

²Department of Science, Nasarawa State Polytechnic Lafia.

³Department of Chemistry, University of Agriculture Makurdi, Benue State.

⁴Department of Chemistry, Nasarawa State University, Keffi.

Corresponding email: ausman2015@yahoo.com

Received: June, 2016

Accepted: August, 2016

Abstract

Trichilia emetica seed oil was analysed for their fatty acid profiles using GC-MS and $^1\text{H-nuclear}$ magnetic resonance spectroscopy ($^1\text{H-NMR}$). In the $^1\text{H-NMR}$ spectrum, integrating the values of the signals of acyl, allylic and bis-allylic protons, the unsaturated fatty acid: monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA) was quantitated. The relative percentage of saturated fatty acid (SFA) was determined by subtracting the relative amount of the total unsaturation from the total amount of fatty acid present in the oil. The procedure was verified by using GC-MS to determine the fatty acid methyl esters in the oil sample. The percentage triacylglycerol conversion to its corresponding methyl esters was determined by $^1\text{H NMR}$ and was calculated to be 94%. A total of six FAMES were identified in the seed oil by the NIST library match software and the fragmentation pattern data of GC/MS. The results obtained from the two methods were 55 % saturated (C14:0, C16:0, C18:0), 35% monounsaturated (C18:1) and 10 % polyunsaturated (C18:2 and C18:3). Generally, the $^1\text{H-NMR}$ results were in good agreement with gas chromatographic (GC) analyses. In conclusion, the $^1\text{H-NMR}$ -based procedures is fast as it does not require the oil to be derivatized and can be applied to neat *T. emetica* seed oil.

Keywords: Triacylglycerol; Transesterification; Fatty acid methyl esters; Saturated fatty acid; Analysis.

1.0 INTRODUCTION

Fatty acids are the main constituent of vegetable oils, animal fats and their derivatives. The fatty acid profiles of oils and fats determined their application for physiological and industrial uses (Knothe and Kenar, 2004). Hence, different

techniques have been developed to analyze these profiles.

Gas chromatography (GC) is the common method used for determining the fatty acid composition of vegetable oils, animal fats and their derivatives. This technique normally

involved the conversion of oils or fats into their corresponding methyl esters before the analysis. And the method has been used by several authors to report the fatty acid composition of *T. emetica* seed oil (Engelter and Wehmeyer, 1970; Khumalo *et al.*, 2002; Grundy and Campbell, 2007; Vermak *et al.*, 2011) The nuclear magnetic resonance (NMR) Spectroscopic methods, on the other hand, allows the determination of a large number of components with very little or without the need to derivatized or manipulate the oil samples (Hidalgo and Zamora., 2003; Knothe and Kenar, 2004). The NMR spectra are obtained within a short time and it contained great amount of information that can be employed as a substitute to many classical analytical procedures. The different signals present in the NMR spectra provide two kind of information: the chemical shifts, which is of qualitative value and the relative intensities which are quantitative in nature (Hidalgo and Zamora., 2003; Vlahov, 1999), the combination of this information, easily result in oil or fat been characterised. In fact, by using quantitative conditions, the results obtained by NMR are similar to GC (Wollenberg, 1990). NMR has been used for structural characterisation, as well as for authentication and quality assessment (Jie and Mustafa, 1997; Vlahov *et al.*, 2001).

¹H NMR is one of the spectroscopic techniques that have emerged as a valuable alternative for the qualitative and quantitative determination of food quality. Johnson and Shoolery in 1962, were the first to use this techniques to determine the degree of unsaturation and the average molecular weight in fat, and it has since expanded to include determination of iodine value (Miyake *et al.*, 1998), identification of vegetable oils as well as identifying individual vegetable oils in a mixture (Fauhl *et al.*, 2000; Guillen and Ruiz, 2003). The use of ¹H NMR to quantitate individual fatty acid in lipids is difficult, due to the strong overlapping peaks in aliphatic region, but quantitating the degree of saturation and unsaturation can be achieved using appropriate peaks in the spectrum (Lie Ken Jie and Lam, 1995; Thoss *et al.*, 2012).

This study describes the use of ¹H-NMR spectroscopy to quantified saturated and

unsaturated fatty acid composition in *T. emetica* seed oil. The result obtained, was verified using GC-MS of fatty acid methyl esters.

2.0 EXPERIMENTAL

2.1 Preparation of sample

T. emetica seeds used in this study were collected from Kumasi-Ghana. The seeds were manually removed from the husks, spread out to dry at ambient temperature and stored in plastic container at room temperature until the beginning of the experiment.

2.2 Extraction of Seed Oil

T. emetica seeds (100 g) were flaked two times using Eschenfelder Seed and Grain Flaker 1200. The flaked seeds were stirred in n-hexane ($7 \times 200 \text{ cm}^3$) at room temperature for 1 hour each. The extracts were combined, dried over MgSO₄ and then filtered under gravity. The n-Hexane was removed by rotary evaporator at 40 °C to give golden yellow brown oil. The oil obtained was stored at room temperature until further analysis.

2.3 Trans-esterification of the Oils

The method of (Thoss *et al.*, 2012) was used with modification. *Trichilia emetica* seeds oil (5g) was dissolved in sodium methoxide (0.5M, 40 ml) and heated under reflux for 30 minutes to produce fatty acid methyl esters (FAMES). Deionised water (100 ml) was then added, and the solution extracted with n- hexane (100 ml \times 3). The hexane fractions were combined, dried over MgSO₄, filtered under gravity and the solvent removed using rotary evaporator at 40°C. The trans-esterified samples were diluted (1:1000) with hexane and analysed by gas chromatography-mass spectrometry (GC- MS).

2.4 GC-MS Method

The method of (Thoss *et al.*, 2012) was used with modification. The fatty acid methyl esters (FAMES) contents of *T. emetica* seed oil were determined using Thermoquest Finnigan Trace GC 2000, with a Chrompack silica fused DB-5 column (L 25 m \times ID 0.32 mm \times DF 0.25 μ m). The carrier gas was nitrogen with a flow rate of 1.5 ml/min. The initial oven temperature was 150°C (held for 2 min), ramped at 5°C min⁻¹ up to 250°C (held for 10 min). The phase transfer line temperature was held at 250°C. A sample volume of 1 μ l in hexane was injected using a

Figure 1. ^1H NMR spectrum of crude *T. emetica* seed oil.

3.2 H-NMR of transesterified *T. emetica* Seed Oil

The ^1H NMR spectrum [Fig. 2] of transesterified oil sample showed two characteristic peaks, a singlet peak observed at B (δ_{H} 3.63 ppm) and a triplet of α - carbonyl methylene protons at A (δ_{H} 2.27 ppm). The presence of these distinct peaks at position A and B was used to confirm the presence of methyl esters present in the oil sample (Tariq *et al.*, 2011).

The transesterification of crude *T. emetica* seed oil to methyl esters was quantified using ^1H NMR, and this is done by integrating these two

distinct peaks at δ_{H} 3.63 and 2.27 ppm. (Tariq *et al.*, 2011). The yield of transesterification reaction was quantified using equation below;

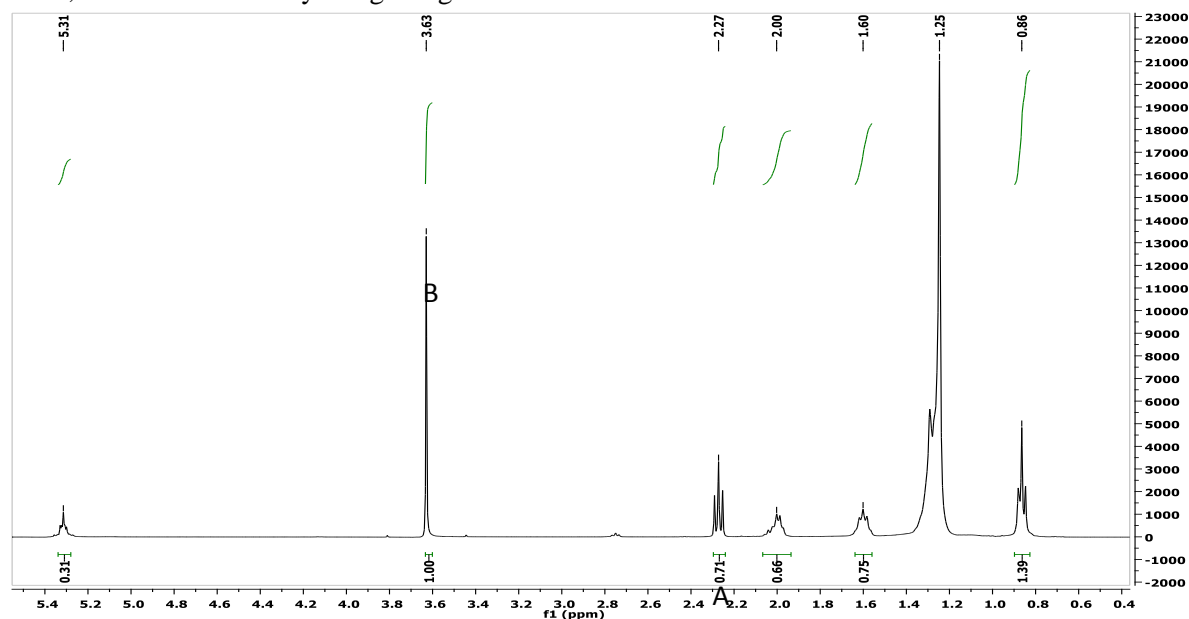
$$\text{H} (\%) = 100 \times 2\text{B} / 3\text{A}$$

Where:

H = Percentage conversion of triacylglyceride to methyl esters.

A = Integration value of α -methylene protons.

B = Integration value of the methoxy protons of the methyl esters.

Figure 2. ^1H NMR spectrum of transesterified *T. emetica* seed oil.

3.3 Extraction of *T. emetica* Seed Oil

The *T. emetica* seed oil extracted with hexane was golden yellow brown. The yield ranged from 54 to 59% by mass for dry seeds. This result is similar to 55-68% but higher than 22.90% reported by other researchers (Fupi and

Mork, 1982; Grundy and Campbell, 1993; Saka and Msonthi, 1994; Orwa *et al.*, 2009).

3.4 ^1H -NMR Analysis

The characteristic resonances in the ^1H NMR of crude *T. emetica* seed oil is similar to those reported for other seed oil [Table 1, Figure 1] (Mannina *et al.*, 1999 and Thoss *et al.*, 2012).

Table 1. Chemical shifts and assignments of the characteristic resonance in the ^1H NMR spectrum of crude

<i>T. emetica</i> seed oil		
δ (ppm)	Proton	Compound
0.86	$-\text{CH}_3$	Terminal methyl
1.24	CH_2	Methylene
1.59	$\text{CH}_2\text{-CH}_2\text{-COO}$	All acyl chains
2.00	$\text{CH}_2\text{-CH=CH}$	All unsaturated fatty acids
2.29	$\text{CH}_2\text{-COO}$	All acyl chains
2.75	$\text{C=C-CH}_2\text{C=C}$	Protons attached to bis allylic carbon
4.10-4.28	$\text{CH}_2\text{O}(\alpha)$	Glycerol (triglycerides)
5.25	$\text{CH}_2\text{O}(\beta)$	Glycerol (triglycerides)
5.32	CH=CH	Olefinic (all unsaturated fatty acids)

The relative percentage of SFA, MUFA and PUFA calculated from the integration of the acyl group at (δ_{H} 2.29), proton attached to allylic and bis-allylic carbons located at position (δ_{H} 2.75) and (δ_{H} 2.00) is shown in Table 2. And it indicated that the dominant fatty acid is SFA, followed by MUFA and then PUFA.

Table 2. Relative percentage of PUFA, MUFA, and SFA for *T. emetica* seed oil using ^1H NMR.

<i>T. emetica</i> oil	PUFA	MUFA	SFA
% composition	10	35	55

Other resonances in the spectrum, are those appearing between δ_{H} 4.10 - 4.28 ppm and 5.25 ppm were assigned for glycerol, which is one of the main components of the triacylglycerol fraction and it serve as a backbone to which fatty acid chains are attached (Fig. 3). Protons on *sn*-1, 2, and 3 on the glycerol have different environments depending on the nature of the fatty acid composition of the oil (Fig. 3). The 2 protons of the *sn*-1 and 3, assigned α -position are each split by a single proton of *sn*-2 assigned β -position thus creating a multiplet at δ_{H} 4.10 and 4.25. The single proton of the *sn*-2, was split

by four protons of the *sn*-1 and 3 yielding a multiplet at δ_{H} 5.24. The spectrum showed signals at δ_{H} 0.86 which is assigned to the terminal methyl group of the fatty acid chain. The absence of methyl signals at δ_{H} 0.93, indicated the absence of linolenic acid (Hidalgo and Zamora, 2003). The signals of the CH_{2n} chain resonate at δ_{H} 1.22. Other significant resonance is that found at δ_{H} 5.32 which is attributed to CH=CH found within all unsaturated fatty acids such as oleic and linoleic acid.

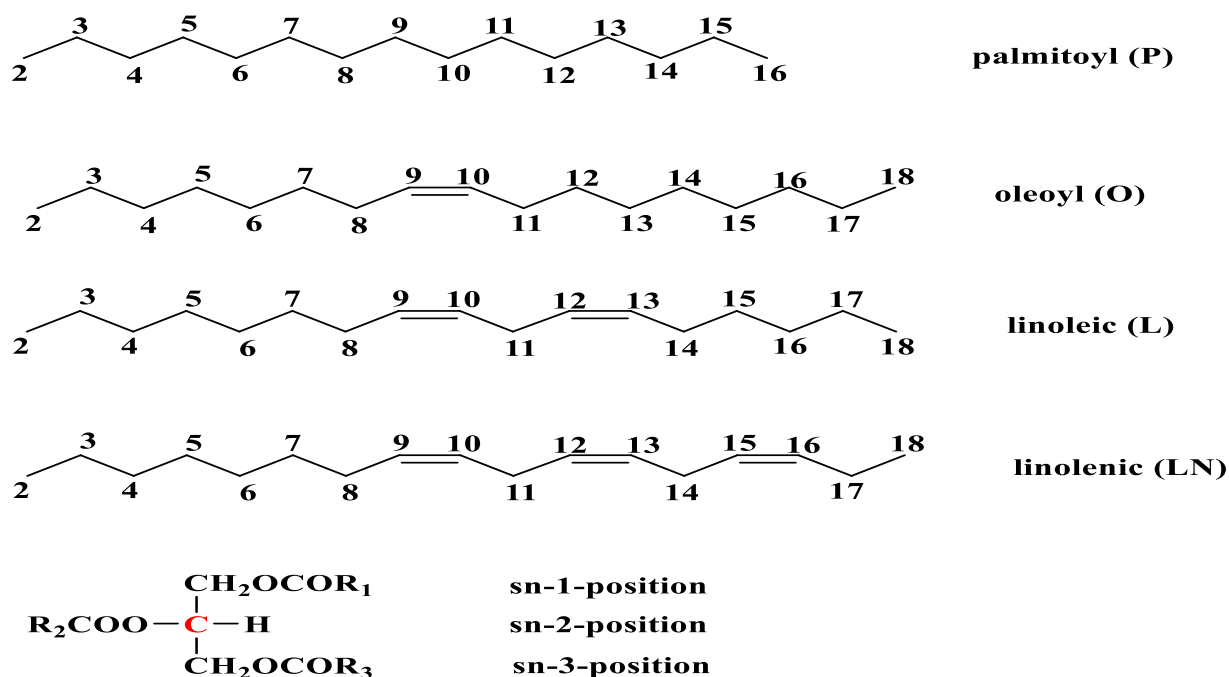


Figure 3. The structure of the model triacylglycerols.

In the ^1H NMR spectrum of transesterified oil (Fig. 2), the percentage conversion of triacylglycerides to its corresponding methyl esters was calculated using the integration values of α -methylene protons at δ_{H} 2.27 and that of methoxy protons of the methyl esters at δ_{H} 3.63. The percentage conversion is 94 %.

3.5 GC-MS analysis

GC-MS was used to analyse the fatty acid methyl ester composition of *Trichilia emetica* seed oils (Table 3). Six peaks were observed in the total ion chromatogram of the seed oil

samples [Fig 4]. Each of these peaks correspond to FAMES content of the oil samples and was identified from the NIST library match software. FAMES have characteristic fragmentation patterns due to the specific way in which the fatty acid breaks down, and also how it undergoes McLafferty rearrangement to produce new ions. The parent ion is the molecular ion, which is equal to the molecular mass and this peak is used as a reference to further characterize other peaks (Fig. 5) [Tariq *et al.*, 2011].

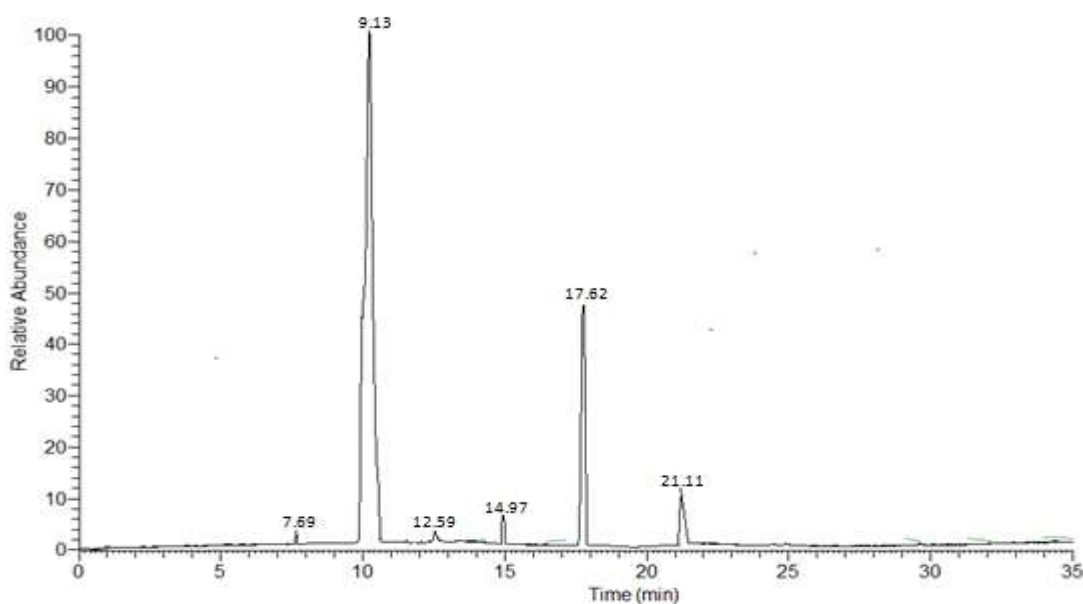


Figure 4. Total ion chromatogram of *T. emetica* seed oil showing composition of FAMES (R_t 7 – 21.11).

The mass spectra of all saturated FAMES has a base peak at m/z 74 which is due to a McLafferty rearrangement [Fig. 5]. Other peaks are those observed at m/z 227 which is due to a loss of hydrogen atom and a propyl radical $[M - 43]^+$, and also m/z 239, occurring as a result of α -cleavage of methoxy group $[M - 31]^+$ (McLafferty, 1959; Tariq *et al.*, 2011; Gunawan *et al.*, 2013). The other distinct peaks observed

for saturated fatty acid were those at m/z 87, 101, 115 and so on with a difference of 14 *amu.*, these ions were abundant in the lower mass region and were formed due to β -cleavage of carbomethoxy ion $[\text{CH}_3\text{O}_2\text{C}(\text{CH}_2)_n]^+$, where n is a positive integer and it is equal or greater than 1 (Tariq *et al.*, 2011). Similarly, palmitic acid methyl ester could be identified with peaks at m/z 270, 227 and 199 (Table 3, Fig. 6).

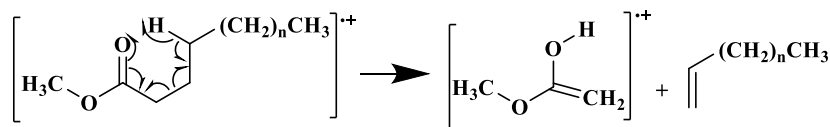


Figure 4. McLafferty Rearrangement of a Fatty Acid.

The monounsaturated FAME identified in these oil samples is oleic acid (C18:1) with a relative percentage of 33.5 %. The base peak for this FAMES is at m/z 55, other peaks are those observe at m/z 222 and 264 occurring due to loss of McLafferty ion $[M - 74]$ and a methoxy group $[M - 32]^+$ plus hydrogen ion (Gunawan *et al.*, 2013). These three distinct peaks also help to identify oleic acid methyl esters in the oil.

The polyunsaturated fatty acid found to be present in the oil samples are linoleic [C18:2] and linolenic acid [C18:3] with relative proportion of 8.9 and 0.2% respectively. This is confirmed by the characteristics base peak at m/z 67 with other prominent peaks at m/z 263 $[M - 31]^+$ and 220 $[M - 74]^+$ which is due to loss of methoxy group and McLafferty ion. At the lower mass range, peaks were also observed at

m/z 67, 81, 95, 109 (Tariq et al., 2011; Gunawan et al., 2013).

From this analysis, the presence of fatty acids containing 14, 16 and 18 carbon chain length in the seed oil was confirmed (Table 3). The saturated fatty acids found to be present were C14:0, C16:0 and C18:0, the only

monounsaturated fatty acid was C18:1 while polyunsaturated fatty acid were C18:2 and C18:3, respectively. Palmitic acid methyl ester (C16:0) was the most abundant FAME with a relative percentage of 54.7 %, followed by oleic acid methyl ester with relative percentage of 33.5 % in the oil samples.

Table 3. Relative percentage (%) of fatty acid methyl esters present in *T. emetica* oil, determined by GC-MS analysis.

Fatty acid methyl ester/ molecular weight	Characteristics MS ions	FAMEs (%)	Retention Time
Myristic (242, C14:0)	242, 199, 143	0.3±0.1	7.69
Palmitic (270, C16:0)	270, 227, 199	54.7±4.1	9.13
Stearic (298, C18:0)	298, 227, 199	2.1±0.2	21.11
Oleic (296, C18:1(ω_9))	296, 264, 222	33.5±2.3	17.62
Linoleic (294, C18:2 ($\omega_{6,9}$))	294, 263, 220	8.9±0.9	14.97
Linolenic (292, C 18:3 ($\omega_{6,9,12}$))	292, 236, 173	0.2±0.1	12.59

Where, n ± 3

These results obtained in this study differs from that reported by (Engelter and Wehmeyer, 1970; Grundy and Campbell, 1993), but similar to those reported by other researchers (Khumalo et al., 2002; Vermaak et al., 2011). The relative percentage of SFA, MUFA and PUFA determined in the oil samples using ^1H NMR agrees with that obtained using GC-MS.

The result obtained in this studies is in accord with that reported by other researchers. Consequently, the use of *T. emetica* seed oil as a

raw material in soap, candle and cosmetic industries, and also as wood oil use in upholstery (Fupi and Mork, 1982; Grundy and Campbell, 1993; Van der Vossen and Mkamilo, 2007; Orwa et al., 2009). This explains why this oil is also used as a starting material in cocoa butter production (Vermaak et al., 2011).

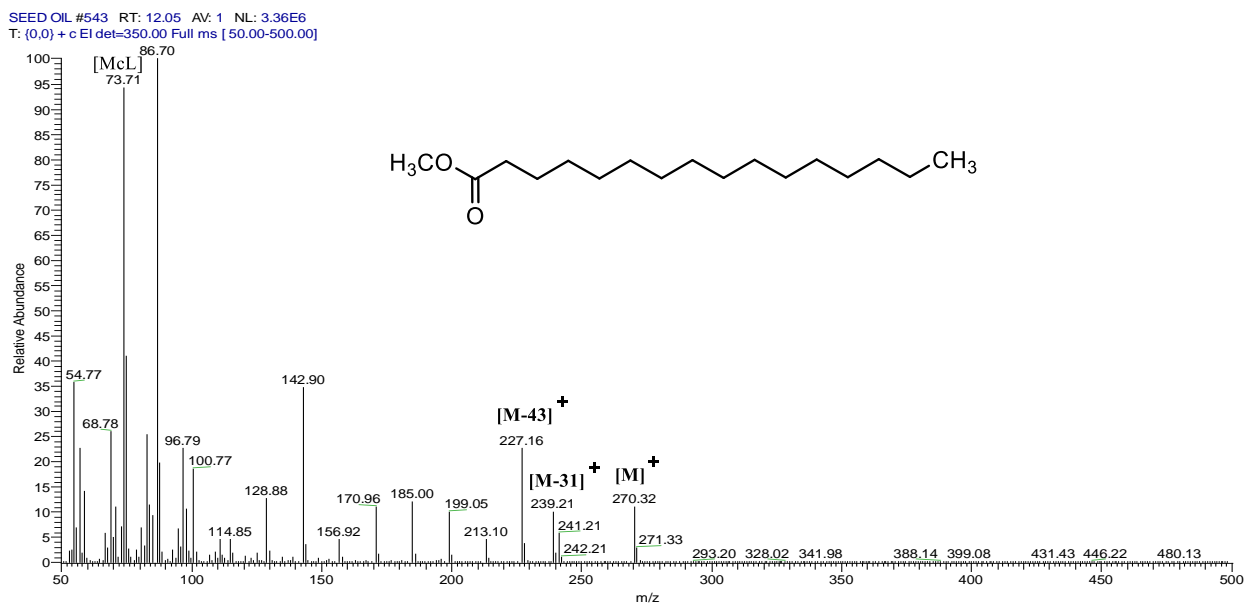


Figure 6. Mass spectrum of palmitic acid in *T. emetica* seed oil.

4.0 CONCLUSION

The fatty acid profiles of *T. emetica* seed oil was characterised using different analytical techniques. The result obtained from $^1\text{H-NMR}$ of crude *T. emetica* seed oil and GC-MS analysis of FAMES agrees with each other, and it indicated that the oil is highly saturated (>55%). It also contained more than 30 % of oleic acid methyl esters (C18:1) and had high

6.0 REFERENCES

- Engelter, C., S.A. Wehmeyer, (1957). Fatty acid composition of oils of some edible seeds of wild plants. *J. Agric. Food Chem.* 18, 25–26.
- Fauhl, C., F. Reniero, C. Guillou (2000). $^1\text{H-NMR}$ as a tool for the analysis of mixtures of virgin olive oil with oils of different botanical origin. *Magn. Reson. Chem.* 38, 436–443.

proportion of fatty acids with 18 carbon atoms. The $^1\text{H-NMR}$ -based procedures is fast and does not require the oil to be derivatized or manipulated and can be a powerful tool in the assessment oil quality and authenticity.

5.0 ACKNOWLEDGEMENT

Abdullahi Usman is thankful to the Nigerian Tertiary Education Trust Fund (TETFUND) for the sponsorship.

Fupi, P.C., Mork, W.K., (1982). Mafura Nut Oil and Meal: Processing and Purification. *JAOCs* 59, 94–98.

Gunawan, S., R. Darmawan, M. Nanda, A.D. Setiawan, H. Fansuri, (2013). Proximate composition of *Xylocarpus moluccensis* seeds and their oils. *Ind. Crops Prod.* 41, 107–112.

Guillén, M.D., A. Ruiz (2003). Edible oils: discrimination by ^1H -nuclear magnetic resonance. *J. Sci. FoodAgric.* 83, 338–346.

- Grundy, I.M., M.B. Campbell, (1993). Potential production and utilisation of oil from *Trichilia SPP.* (Meliaceae). *Econ. Bot.* 47, 148–153.
- Hidalgo, J.F., R. Zamora, (2003). Edible oil analysis by high-resolution nuclear magnetic resonance spectroscopy: recent advances and future perspectives. *Trends Food Sci. Technol.* 14, 499–506.
- Johnson, F. L. and N.J. Shoolery (1962). Determination of unsaturation and average molecular weight of natural fats by nuclear magnetic resonance. *Analytical Chemistry*, 34, 1136-1139.
- Knothe, G. and A.J. Kenar, (2004). Determination of fatty acid profile by ¹H NMR spectroscopy. *European Journal of Lipid Science and Technology.* 106, 88-99.
- Khumalo, L.M., L. Majoko, J.S. Read, I. Ncube, (2002). Characterisation of some underutilised vegetable oils and their evaluation as starting materials for lipase-catalysed production of cocoa butter equivalents. *Ind. Crops Prod.* 16, 237–244.
- Lie Ken Jie, M.S.F., J. Mustafa, (1997). High resolution nuclear magnetic resonance spectroscopy-application to fatty acids and triacylglycerols. *Lipids* 32, 1019-1034.
- Lie Ken Jie, M.S.F. and C.C. Lam. (1995a). ¹³C-NMR studies of polyunsaturated triacylglycerols of type AAA and mixed triacylglycerols containing saturated, acetylenic and ethylenic acyl groups. *Chem Phys. Lipids* 78, 1-13.
- Miyake, N., K. Yokomizo, N. Matsuzaki (1998). Rapid determination of Iodine value by ¹H-nuclear magnetic resonance spectroscopy. *J. Am. Oil Chem. Soc.* 75, 15–19.
- Mannina, L., C. Luchinat, M.C. Emanuele, A. Segre, (1999). Acyl positional distribution of glycerol tri-esters in vegetable oils: A ¹³C NMR study. *Chem. Phys. Lipids* 103, 47–55.
- McLerffaty, W.F.,(1959). Mass spectrometric analysis, molecular rearrangements. *Anal. Chem.* 31, 82–87.
- Orwa, C., Mutua, A., Kindt, R., J., R., Simons, A., (2009). *Trichilia emetica.* Agroforestry database: a tree reference and selection guide version 4.0. Available at <http://www.worldagroforestry.org/af/tree/db/>. [Accessed: January 19, 2013].
- Thoss, V., P.J. Murphy, R. Marriott, T. Wilson, (2012). Triacylglycerol composition of British bluebell (*Hyacinthoides non-scripta*) seed oil. *RSC Adv.* 2, 5314.
- Tariq, M., Ali, S., Ahmad, F., Ahmad, M., Zafar, M., Khalid, N., Khan, M.A., (2011). Identification, FT-IR, NMR (¹H and ¹³C) and GC/MS studies of fatty acid methyl esters in biodiesel from rocket seed oil. *Fuel Process. Technol.* 92, 336–341.
- Van der Vossen, H.A., Mkamilo, G.S., (2007). Vegetable oils of tropical Africa, conclusion and recommendations based on PROTA 14: “Vegetable oils”. Available on: PROTA (Plant Resources of Tropical Africa/Resources vegetales de l’Afrique tropicale), Wageningen, Netherlands <http://www.prota.co.ke/en/p.PROTA>.
- Vlahov, G., (1999). Application of NMR to the study of olive oils. *Progress in Nuclear Magnetic Resonance Spectroscopy*, 35, 341-357.
- Vlahov, G., Schiavone, C., Simone, N., (2001). Quantitative ¹³C NMR method using the DEPT pulse sequence for the determination of the geographical origin (DOP) of olive oils 35, 689–695.
- Vermaak, I G.P.P. Kamatou, B. Komane-Mofokeng, A.M. Viljoen, K. Beckett, (2011). African seed oils of commercial importance - Cosmetic applications. *South African J. Bot.* 77, 920–933.
- Wollenberg, F.K. (1990). Quantitative high resolution ¹³C nuclear magnetic resonance of the olefinic and Carbonyl carbons of edible vegetable oils. *Journal of the American Oil Chemist’ Society*, 67, 487-494.