Triglyceride Composition of Almond Seed Oil (*Terminalia catappa*) Grown in Nigeria using GC-MS and $^1$H-NMR Spectroscopy

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Authors’ contributions

This work was carried out in collaboration among all authors. Authors ASS and LIO designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors EED and SRA managed the analyses of the study. While authors FTA and AAY managed the literature searches data and produced the initial draft. All authors read and approved the final manuscripts.

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ABSTRACT

Almond (*Terminalia catappa*) seeds are rich in oil; however, their study has received limited attention, with researches focused mainly on their health potentials. The present study assesses the composition of the fatty acid (FA) components present in the almond seed oils extracted using soxhlet apparatus and analysed by $^1$H-NMR (Proton Nuclear Magnetic Resonance) and Gas Chromatography Mass Spectrometry (GC-MS). Generally, there was significant agreement between the results from the $^1$H-NMR and GC-MS analyses, however, $^1$H NMR gave more reliable and reproducible results. The GC-MS and $^1$H NMR results revealed that the oils contained oleic acid (>18 %), linoleic (>28%) linolenic acid (≤0.03 %) and saturated fatty acids (>44 %).

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1. INTRODUCTION

Global interest in the nutritional composition of many tropical fruits and seeds that are underutilized has increased with the tropical Almond (Terminalia catappa) being one of such. Almond has been introduced, and frequently naturalized, in many tropical parts of the world including Brazil, the Caribbean, and East Africa and it is a large tropical tree in the Leadwood tree family, Combretaceae (Combretum) [1]. This is recently receiving much interest due to its many attributed beneficial effects including its ability to lower cholesterol especially low-density lipoprotein (LDL) cholesterol without diminishing the availability of high-density lipoprotein (HDL) which is beneficial [2]. Almonds are the most widely consumed fruit and rank first in treelike nut production [3].

Almond oil can be extracted for food flavourings and the cosmetics industry [4]. It contains antioxidants such as terpenoids, triterpenoids, proanthocyanidins, flavonoids and phenolic compounds. Almond extract helps to slow down oxidative processes in food products and have great potential to become food preservation additives and dietary/nutraceutical supplements [5]. Consumption of almond has been reported to lower the risk of colon cancer because of the presence of one almond lipid-associated component [6]. Almond is considered nutritive for the brain and nervous system. It is said to induce high intellectual level and longevity [7].

Even though Almond nut oil is reported as a possible source of nutritional oil [8], more work is required to confirm its safety for consumption. This is so because edible oils need to have more than 95 to 99% of the total lipids in their triacylglycerols (TAG) makeup. The TAG composition of an oil is a very difficult to define because of the large number of individual FAs, with different degrees of unsaturation and chain lengths, and position on the glycerol molecules [9]. Nonetheless, every type of oil has a unique TAG profile which determines the nature of its physicochemical and nutritional composition, and so provides information on the oil’s quality.

Several methods and techniques have been employed and reported in literature for the qualitative and quantitative determination of TAGs in oils [9-11]. Most of them have focused on chromatographic methods including gas/liquid chromatography, high performance chromatography, thin-layer chromatography and supercritical fluid chromatography. Gas chromatography (GC) techniques however remains the most common, giving reproducible results for fatty acid composition [12-15]. In this method, lipids are converted to methyl esters before being analysed. However, one drawback to this is that the method is destructive involving the hydrolysis of the triacylglycerols and methylation of the free fatty acids before analysis [16], while being labour intensive and time consuming. In light of this, Nuclear magnetic resonance (NMR) has been proposed on the other hand as it, allows direct determination of samples without derivatization [17,18]. The advantages offered by 1H nuclear magnetic resonance (1H-NMR) including a rapid, simultaneous, and non-destructive study of oils has brought it to prominence in recent times [19-22]. This study assesses the use of 1H-NMR spectroscopy to quantify the fatty acid composition in Almond seed oil; and verified using GC-MS of fatty acid methyl esters.

2. EXPERIMENTAL

2.1 Sample Collection

Fully matured almond seeds were collected from the Nigerian Stored Products Research Institute, Ilorin, Kwara State, Nigeria. The seeds were removed from the fruits which had been dried for one week before they were further sun-dried for five days and pulverized using an analytical mill (IKA® A 10 basic Analytical mill).

2.2 Extraction Procedure

100 g of the seed powder was extracted using a Soxhlet apparatus for seven hours with hexane. The filtered extract was then put in a rotary evaporator at 40°C to remove solvent till a light golden yellow oil sample was obtained and dried in Air oven at 103°C for 30 minutes [23].

2.3 GC-MS Analysis

A GC chromatography (model:6890N, Agilent technologies network) coupled to an inert XL EI/CI Mass Selective Detector (MSD) (5975B, Agilent technologies Inc., Palo Alto, CA) was used to determine the fatty acid methyl esters (FAME) of the seed oil according to the method.
described by Thoss et al. [24]. Helium was used as the carrier gas at a 1 ml/min flow rate while a non-polar ZB-Semivolatiles (30 m, 0.25 mm ID, 0.25 µm film thickness) capillary column was used for the GC at a stable temperature of 250°C. FAME content of the oil sample were matched against a Supelco 37 component standard FAME mix.

2.4 NMR Analysis

A Bruker AV400 spectrophotometer with CDCl$_3$ as solvent was used to record $^1$H and $^{13}$C-NMR spectra with TMS as internal standard. MNova software was used to process the spectra.

3. RESULTS AND DISCUSSION

3.1 Oil Yield

The oil yield of the extracted almond seed to be 52.09 ± 2.45% (w/w) oil. This is similar to those of palm kernel (50%), melon seed (51%) and Siberian apricot (51.15%), but much higher than those of cotton (15%), tigernut (16%) and soybean (17%) [25-28].

Fig. 1 shows the GC spectrum of the seed oil and FAME standard while Table 1 gives the percentage composition of the fatty acids from GC-MS analysis.

The composition of fatty acids in the seed oil ranged from 12:0 to 20:0 (Table 1). A total of 11 fatty acids including saturated, monounsaturated and polyunsaturated fatty acids were identified. Saturated acids accounted for 44.94 % and included lauric (12:0), myristic (14:0), pentadecanoic (15:0), palmitic (16:0), margaric (17:0), stearic (18:0) and arachidic (20:0) acids; with palmitic acid being the highest (25.48 %). The monounsaturated acids which accounted for 22.75 % had oleic acid as the highest ((18.02 %). Others included palmitoleic (16:1), oleic (18:1) and elaidic (18:1) acids. Linoleic acid (18:2) which accounted for 32.31% was the only polyunsaturated fatty acid identified.

![Fatty acid methyl ester chromatogram against FAME standard](image-url)
Table 1. Fatty acid methyl ester composition of Almond seed oil

| Lipid number | Systematic name         | Trivial name         | Almond oil |
|--------------|-------------------------|----------------------|------------|
| C – 12:0     | Dodecanoic acid         | Lauric acid          | 0.01       |
| C – 14:0     | Tetradecanoic acid      | Myristic acid        | 0.18       |
| C – 15:0     | Pentadecanoic acid      | Pentadecylic acid    | 0.19       |
| C – 16:0     | Hexadecanoic acid       | Palmitic acid        | 25.48      |
| C – 16:1     | Cis-9-hexadecenoic acid | Palmitoleic acid     | 1.76       |
| C – 17:0     | Cis-10-Heptadecanoic acid | Margaric acid     | 5.14       |
| C – 18:0     | Octadecanoic acid       | Stearic acid         | 13.11      |
| C – 18:1     | Cis-9-octadecenoic acid | Oleic acid           | 18.02      |
| tri C – 18:1 | Tr-9-octadecenoic acid  | Elaidic acid         | 2.97       |
| C – 18:2     | 9,12-Octadecadienoic acid | Linoleic acid     | 32.31      |
| C – 20:0     | Eicosanoic acid         | Arachidic acid       | 0.83       |

Reports for sweet almond by Giwa and Ogunbona (11.10%) [29] showed lower percentage saturated fatty acids than those in the present study (44.94 %); however Sarkai et al. [30] reported higher values (64.87 %) for Almond seed. The palmitic acid content (25.48 %) was lower to the 52.4 % and 53.06 % reported by Sarkai et al. [30] for Almond seed and Chaves et al. [31] for Pachira glabra respectively. The differences in yield of the fatty acids might be due to locations, agronomic practices and environmental factors.

Unsaturated fatty acids (55.06 %) obtained in this study was lower than the those reported by Giwa and Ogunbona (88.9 %) [29] for Sweet Almond. Giwa and Ogunbona [29] also reported lower (70.0 %) monounsaturated fatty acid value than the present study (22.75 %). Linoleic acid was the predominant polyunsaturated fatty acid (32.31 %). This is important as linoleic acid helps in preventing distinct heart vascular diseases [32]. These differences in yield of the fatty acids might be due to locations, agronomic practices and environmental factors.

The quality and use of edible vegetable oils is determined by the composition and amount of unsaturated fatty acids. Linoleic acid is an essential fatty acid and its presence is highly desirable. The higher the amount of linoleic acid in relation to oleic acid, the better the oil quality and the less the formation of bad cholesterol [33].

Nutritionally the proportion of saturated to unsaturated fatty acids in edible oils and fats is very important during consumption. High levels of saturated fatty acids are desirable to increase oil stability. However, saturated fatty acids (SFA) become nutritionally undesirable, because they are considered to increase the concentration of low-density lipoprotein (LDL). This affects the ratio of LDL to HDL and promoting vascular smooth muscle proliferation [31,34]. The ratio of UFA/SFA for Almond seed oil is 1.23 which can be considered for low risk of cardiovascular complications [34]. Again, the relationship between saturated (S) and polynsaturated (P) fatty acid (FA) content is an important parameter for determination of the nutritional value of oils which is expressed as P/S index. Oils and fats with a P/S index > 1 are considered to have nutritional value. Several studies indicate that a higher P/S index means a smaller deposition of lipids in the body. The P/S index of Almond seed oil was 0.72 which can lead to lower deposition of lipids in the body.

However, the edibility of the Almond seed oil, and indeed the whole seed we be suitable and preferable due to amount of unsaturated fatty acid present inside the oil.

Fig. 2 gives the \(^1\)H NMR spectrum. Table 2 also gives the proton resonances of major triacylglycerols (TG) present in the oil; and assigned according to literature [19-21,35-39].

The qualitative analysis given in Table 2 indicates the presence of linolenic, linoleic, oleic and saturated fatty acid functional groups in the oil sample. Certain peaks were measured and integrated to determine the quantity of these fatty acids in the oil sample (Fig. 2).
Fig. 2. $^1$H NMR spectrum of Almond seed oil.

Table 2. Assignment of chemical shifts for the $^1$H-NMR spectrum of almond 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            | $-\text{CH}_2-\text{C} CO_2$     | Acyl chain                  | m            | 1.51-1.61            |
| 4            | $-\text{CH}_2-\text{CO}_2-$      | Methylene carboxylic acid   | m            | 1.97-2.06            |
| 5            | $-\text{C}-\text{CH}_2-\text{C}=$ | Allylic methylene hydrogen  | m            | 2.25-2.38            |
| 6            | $-\text{C}=\text{C}-\text{CH}_2-\text{C}=$ | Bisallylic methylene hydrogen | t            | 2.74-2.78            |
| 7            | $-\text{C}=\text{C}-\text{CH}_2-\text{C}=\text{C}-\text{CH}_2-\text{C}=$ | Bisallylic methylene hydrogen | t            | 2.78-2.80            |
| 8            | $-\text{C}-\text{CH}_2-\text{O}-\text{C}-\text{O}=$ | Glycerol hydrogen dd |   | 4.09-4.14            |
| 9            | $-\text{C}-\text{CH}_2-\text{O}-\text{C}=$ | Glycerol hydrogen dd |   | 4.24-4.34            |
| 10           | $\text{CH}(-\text{C}-\text{O}-\text{C}=$ | Glycerol hydrogen m        |   | 5.21-5.26            |
| 11           | $\text{C}=$ | Olefinic hydrogen signal     | m            | 5.30-5.36            |

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

The characteristic chemical shifts of vinylic protons ($^1\text{H}_v$) were used to determine the ratio of saturated to unsaturated fatty acids; bisallylic protons ($^1\text{H}_b$, $^1\text{H}_t$) were used to distinguish the properties of the polyunsaturated fatty acids, while the tertiary proton in the glycerol moiety ($^1\text{H}_g$) elucidated the structure.
Enhancement of resolution however, allowed for setting a relationship between the integral values of the protons.

Vynilic hydrogens integral \( (H_v) = 2A + 4B + 6C \)

Linoleic bisallylic integral \( (H_d) = 2B \)

Linolenic bisallylic integral \( (H_t) = 4C \)

\( A + B + C + D = 3 \) [37]

Where \( A, B, C \) and \( D \) are the amount of oleic, linoleic, linolenic and saturated acids present in the triglycerol.

Following the proton spectrum in Fig. 2, the tertiary proton peak of the glycerol moiety \( (\delta 5.26 - 5.21 \text{ ppm}) \) was integrated as 100, culminating to an integral value of 453.52 for vinyl proton \( (\delta 5.36 - 5.30 \text{ ppm}) \), 133.40 for the bisallylic protons \( (\delta 2.78 - 2.74 \text{ ppm}) \) for linoleic acids and 0.35 for the bisallylic protons \( (\delta 2.80 - 2.78 \text{ ppm}) \) for linolenic acids.

Using the proportion \( (A:B:C:D) \) of each type of fatty acid present in the triglyceride of the oil (oleic, linoleic, linolenic and saturated), the equation gives \( [\text{vynilic } H_v = 453.52 = 2A + 4B + 6C]; \) \( [\text{bisallylic } H_d = 4C = 0.35] \) and \( [\text{bisallylic } H_t = 2B = 133.40] \). Consequently, the values for the fatty acids in the oil are: \( A = 31.04 \% \), \( B = 22.23 \% \), \( C = 0.03 \% \), and \( D = 46.70 \% \).

Literature data [37,39] was used to assign the \(^{13}\text{C}\) NMR chemical shifts with Fig. 4 showing the obtained spectrum. Just as seen the spectrum of other vegetable oils, Almond seed oil showed signals in the methylene and methyl \( (10 - 35 \text{ ppm}) \), glycerol \( (60 - 79 \text{ ppm}) \), olefinic \( (127 - 131 \text{ ppm}) \) and carbonyl \( (172.81 - 173.25 \text{ ppm}) \) regions the \(^{13}\text{C}\) NMR spectrum. Signals obtained in the spectrum at 173.3 and 172.9 ppm with a chemical shift difference of 0.40 ppm represent the saturated 1,3 triglycerides and 2- positions of oleyl, linoleyl esters. Those signals seen at the olefinic carbon regions confirmed unsaturation of the oil; and is the region where linoleyl and linolenyl chains can easily be detected. The presence of oleic esters are indicated by the signals at 130.10 ppm and 128.90 ppm, jointly with 130.00 ppm, 128.20 ppm and 128.00 ppm from linoleic esters, but no peaks corresponded to linolenic acid. \(^{13}\text{C}\) is limited in the analysis of TG due to its low gyromagnetic ratio and its very low natural abundance which is confirmed by these results. The mono-, di-, and triglycerols glycerol carbons resonate in the spectral region \( 60 - 79 \text{ ppm} \) with the signals at 68.90 and 62.00 ppm indicated by those of triacylglycerol in the seed oil. The C-16 carbon that appears at chemical shifts of 31.90 and 31.70 ppm corresponds with saturated, oleyl, linoleyl chains resonating in this region whereas C-8 – C-15 resonates between 25.20 ppm to 29.80 ppm. Again, C\(\text{O}2\) and C\(\text{O}1\) carbon regions were found at 22.9 ppm to 14.1 ppm. The C\(\text{O}2\) of all acyl chains resonated at 22.9 ppm and C\(\text{O}1\) at 14.1 ppm for all acyl chains. All the signals were assigned based on literature reports [37,39]. Qualitative analysis of the Almond seed oil using the \(^{13}\text{C}\) NMR spectroscopy indicated the presence of saturated and unsaturated fatty acids in the seed oil.

A comparison between the fatty acid composition of Almond seed oil obtained using GC-MS and NMR is presented in Table 3.
Fig. 4. $^{13}$C-NMR spectrum of almond seed oil

Table 3. Comparative fatty acid composition of almond seed oil by GC-MS and $^1$H NMR

| Fatty acid | GC-MS (%) | $^1$H NMR (%) |
|------------|-----------|---------------|
| Oleic      | 18.02     | 23.53         |
| Linoleic   | 32.31     | 28.9          |
| Linolenic  | 0.00      | 0.03          |
| Saturated  | 44.53     | 47.54         |

The results showed that compounds from $^1$H-NMR and GC-MS analysis of the FAMEs reasonably agree with each other.

4. CONCLUSION

The study revealed that the oil from seeds of Almond grown in Nigeria contains high unsaturated fatty acids and has linoleic acid as the predominant unsaturated fatty acid with little or linolenic acid methyl ester. The percentage oil yield from Almond seed promotes it for commercial exploitation. With a major study of any possible health hazards, it is reasonable to recommend that Almond seed oil should be used for food purposes owing to the level of unsaturated fatty acids present in the oil.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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